Ranjana Patnaik · Amit Kumar Tripathi
Ashish Dwivedi *Editors*

Advancement in the Pathophysiology of Cerebral Stroke

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Chapter 1
Cerebral Stroke: An Introduction

Amit Kumar Tripathi

Abstract Stroke is the fifth leading cause of death and physical disability in the USA. Its prevention, diagnosis, and interventions are for the most part up and coming fields that give us feeling of how far medical and scientific work have progressed. Intravenous thrombolysis (IT) and endovascular thrombectomy (EVT) are evidence-based treatments for adults with a blocked blood vessel (ischemic stroke). EVT intervention is better than tpA treatment because of faster recanalization and less risk of hemorrhage especially in large artery occlusions. EVT treatment options include: Catheter-directed thrombolysis (CDT), pharmacomechanical catheter-directed thrombolysis (PCDT), percutaneous aspiration thrombectomy (PAT), vena cava filter protection, venous balloon dilatation and venous stent implantation. The feasibility, safety, and outcome of all therapies need to be assessed in adults, children, and pregnant women who have had a stroke. The significance of neuroprotective investigations in murine and stroke patients should be deliberately checked. Stem cells, nanoformulations and electromagnetic fields (EMF) are helpful emerging therapeutic interventions that contribute to the treatment of stroke patients.

Keywords Stroke · Thrombectomy · Oxygen-glucose deprivation · Stem cell · tPA

1.1 Introduction

Stroke is the fifth leading cause of physical disability and mortality occurring in around 700,000/year in the USA. [1, 2]. Currently, the incidence of stroke in India is substantially higher than that in Western nations [3]. In developing countries, stroke is main cause of death. Ischemic stroke is a permanent or temporary state where the blood supply to the brain becomes blocked, resulting in oxygen-glucose deprivation (OGD), brain damage, and cognitive dysfunction. The future of neurons undergoing ischemic injury is regulated by transcriptional and post-transcriptional events that contribute to competing cell death and survival programs [4]. The

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neurological outcomes are permanent damage, including partial paralysis and impairment in speech, comprehension, and memory. Moreover, it affects children and both men and women. The incidence of stroke increases promptly with age. The major risk factors are summarized in Table 1.1 and Fig. 1.1.

Table 1.1 Major risk factors for incidence of ischemic stroke

S.No	Factors that can control, treat, and improve	Factors that are not within your control	Additional factors that may be linked to a higher stroke risk
1	Sustained high blood pressure (hypertension)	Age	Geographic location
2	Smoking (nicotine and carbon oxide)	Stroke family history	Socioeconomic factors
3	Diabetes	Sex (gender)	Drug addiction
4	Diet with saturated and trans fat	Transient ischemic attack	Sleep habits can affect stroke
5	Physical activity		Alcohol abuse can raise the risk of stroke
6	Excess body weight and obesity		

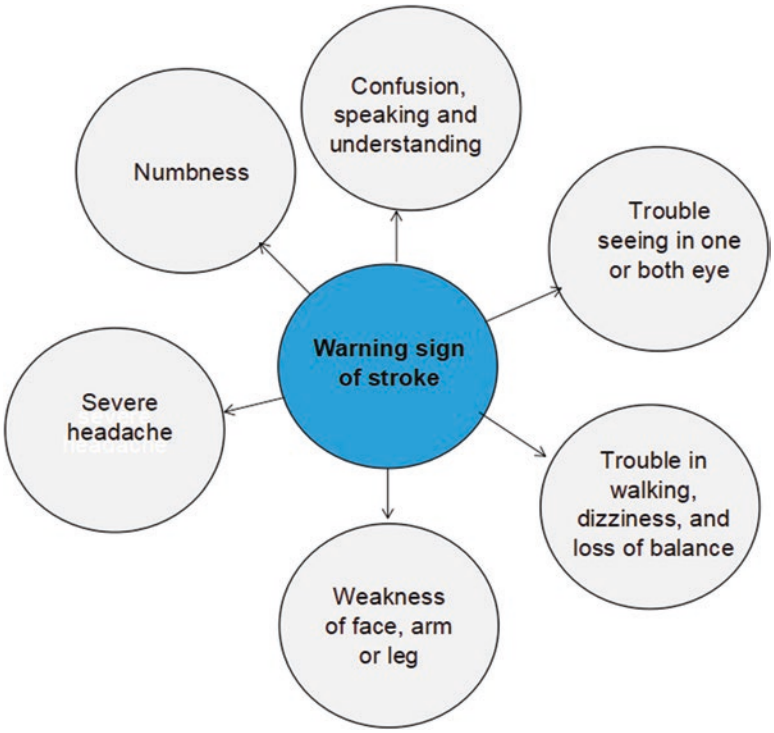

Fig. 1.1 Warning sign for cerebral stroke incidence

1.2 Types of Stroke

1.2.1 Ischemic Stroke

Ischemic stroke occurs as a result of an obstruction of the blood vessels. It is a blockage in an artery supplying to blood to the brain in a situation known as ischemic (~92%).

1.2.2 Hemorrhagic Stroke

Hemorrhagic stroke is caused by rupture of vessels and leakage of blood into the brain (~10%). There are two types of weakened blood vessel that usually cause hemorrhagic stroke: aneurysms and arteriovenous malformations. However, the most common cause of hemorrhagic stroke is uncontrolled hypertension (sustained high blood pressure).

1.2.3 Transient Ischemic Attack

Transient ischemic attack is the temporary interruption of arterial blood flow known as a mini-stroke (Table 1.1).

1.3 Stroke Pathophysiology

Ischemic stroke injury is primarily attributed to the excessive release of glutamate, overactivation of N-methyl-D-aspartate receptor (NMDAR), and increased influx of Ca^{2+} [5]. The glutamate is responsible for causing neurotoxicity via binding to the NMDAR. NMDARs play a pivotal role in excitotoxic neuronal cell death caused by ischemic stroke. However, NMDAR channel blockers remain unsuccessful for clinical stroke treatment. However, a few recent lines of research have suggested that the NMDAR-associated signaling mechanism has explored novel cell death signaling mechanisms to identify the inhibitors that block these downstream pathways without blocking the receptors. Activation of these receptors allows the entry of large amounts of Ca^{2+}. Ca^{2+} is normally used by cells as a hypercritical intracellular signaling molecule and is involved in many diverse functions, including control of cell-surface ion channels and enzyme activity. Abnormal high concentrations of Ca^{2+} lead to inappropriate activation of cascades of enzymes such as proteases, nucleases, and lipases (Fig. 1.2).

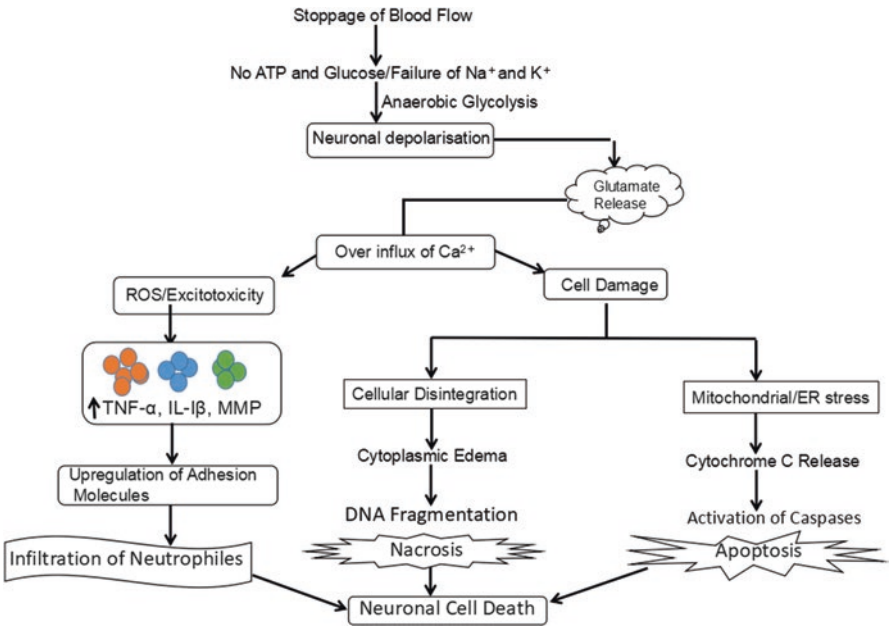

Fig. 1.2 Molecular mechanism involved in pathophysiology of cerebral stroke

1.4 Current Treatment Option for Stroke Patients

Recombinant tissue plasminogen activator (rt-PA) is a serine protease that accelerates fibrinolysis and is used for the reperfusion of the artery after acute ischemic stroke (AIS). However, its potential benefits are compromised by side-effects such as increased risk for hemorrhagic transformation, low therapeutic window, and neurotoxicity [6]. The second most promising therapy for stroke mechanical thrombectomy (MT) is physically removing the obstructing clot using specifically designed devices. This is a treatment option for large vessel ischemic stroke. MT is used when thrombolysis has failed to dissolve the clot or is contraindicated. The disadvantage of thrombectomy intervention is that it is recommended for a maximum of 6 h following stroke and challenge owing to the non-availability of this facility at all health centers. Endovascular thrombectomy (EVT) is a rapid and recommended treatment for AIS and is associated with various operative and post-operative complications such as procedural, device, and anesthetic-related problems that increase both the cost and the delay of rehabilitation [7]. Several methods of neuroimaging have been reported for the diagnosis and detection of arterial blockage in ischemic stroke patients.

1.5 Neuroprotective Agents in Preclinical and Clinical Trials

The term neuroprotection is generally used to describe the effect of various therapeutic interventions that protect the brain from neuropathological damage. It involves the inhibition of signaling mechanisms of various pathological consequences occurring during neuronal ischemia and leading to calcium entry, activation of reactive oxygen species (ROS), and neuronal cell death. The accumulated research of the pathophysiology involved in ischemic stroke has led to the screening of a large number of neuroprotective molecules for therapeutic intervention [8]. Better brain stroke therapy has two pre-requisites: (1) a clear-cut understanding of the molecular mechanism involved and (2) identification of new small molecules/therapeutics that can cross the blood–brain barrier (BBB). The large number of candidate molecules shows neuroprotective efficacy in various rodent models, but so far no clinical study has found significant statistical benefit in patients with AIS. Initial neuroprotective studies suggested that a candidate drug with no side effect was given by various staff members on the way of a hospital and generate a clinically significant outcome, as there were only benefits and no risks. The question is: why have neuroprotective molecules not been successful in a clinical trial on human stroke? There are several possible explanations why neuroprotective trials have been unable to prove an effect, in addition to the eventuality that the basic concept is wrong. The effect of neuroprotective agents on infarct and edema volume are time-dependent and treatment has often been initiated much later than in successful experimental stroke models. An inadequate dose and the slow availability of the drug in the target area may be another explanation. The future new approach of neuroprotective studies has suggested that candidate molecules should be standardized in older animals with common co-morbidity such as atherosclerosis than in younger and healthy animals. Another possibility is the need to discover a highly effective new neuroprotective agent and the synergistic mode of action of the molecules tried. In clinical trials, the highest possibility of success may be with neuroprotection involving mechanisms in experimental stroke pathophysiology, in combination with thrombolytic therapy.

1.6 Stroke-Induced BBB Disruption

Blood–brain barrier (BBB) damage is a critical event in ischemic stroke, contributing to vasogenic edema formation and deteriorated disease outcomes [9]. The BBB mainly consists of microvascular endothelial cells locked by tight junctions (TJs) and adherens junction (AJ) proteins to protect the brain from an entry of blood-borne macromolecules. The TJ consists of three integral membrane proteins, junction adhesion molecules (JAM; occludin, claudin), and cytoplasmic accessory proteins zonula occludens (ZO-1, ZO-2, ZO-3) and cingulin [10, 11]. The BBB has

been a great obstacle to drug delivery in the central nervous system (CNS). Stroke-induced BBB opening will be the focus of different stages and mechanisms of disruption in AIS and a novel therapeutic strategy to target the pathways for better outcomes of stroke.

1.7 Ischemic Stroke-Induced ER Stress

Endoplasmic reticulum (ER) stress is an essential event in the progression of ischemia/reperfusion (I/R) injury in the brain [12]. However, the ER malfunction is well recognized in various neurological disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease, but the mechanism remains unresolved. The impairment of ER function results in the incidence of cell death to protect the organism by removal of damaged cells. ER stress following protein misfolding and their accumulation in the ER lumen induces the unfolded protein response (UPR) in energy-deprived neurons of the ischemic brain. UPR is the phosphorylation of eukaryotic initiation factor 2α (eIF2α) by the activated PKR-like endoplasmic reticulum kinase (PERK) [13, 14] and cleavage of the X-box binding protein-1 (XBP-1) mRNA by the activated inositol-requiring enzyme 1 (IRE1). This helps in the translocation of cleaved XBP-1 from cytoplasm to the nucleus and regulates expression of ER chaperone genes, such as glucose-regulated protein 78 (GRP78) [15]. Furthermore, cleavage of activating transcription factor 6 (ATF6) by proteases, such as S1P and S2P in the Golgi, which regulates expression of ER stress-target genes, such as C/EBP homologous protein (CHOP) and XBP-1 [16]. A detailed explanation of ischemic stroke-induced ER stress is given in the chapter "Ischemic Stroke-Induced Endoplasmic Reticulum Stress."

1.8 The Emerging Role of mi-RNA in Stroke Pathophysiology

The etiological origins and pathological processes of ischemic stroke are mediated by a multifaceted cascade of molecular mechanisms that are in part modulated by post-transcriptional activity [17, 18]. Accumulating evidence has revealed the role of endogenously expressed and noncoding RNA molecules that function to inhibit mRNA translation, popularly known as miRNAs. miRNAs are essential regulators of post- and co-transcriptional gene modification in both the brain physiology and ischemic stroke. These are 21- to 23-oligonucleotide RNAs, which modulate the translation of messenger RNAs (mRNAs) by binding to the 3′-complementary regulatory RNA sequence, thereby causing mRNA degradation and sequestration. More than 5000 miRNAs likely to exist in humans, and each miRNA binds on average 200 mRNAs. The unique chapter describes the emerging role of miRNA in the

pathophysiology of ischemic stroke, highlighting the current progress and understanding that miRNAs alter cellular machinery in I/R brain injury.

1.9 Neuroprotective Potential of Low-Frequency Electromagnetic Field

The low-frequency electromagnetic field (LF-EMF) affects many biological processes; however, the fundamental function remains unresolved in ischemic insult. LF-EMF has the potential to modulate ROS generation followed by a decrease in the activity of antioxidant defense enzymes after I/R brain injury [19]. Exposure of LF-EMF in patients showed the decreased activity of catalase and superoxide dismutase (SOD) in hemolysate and plasma samples. The cellular and molecular signaling mechanism participating in the neuroprotective effect of LF-EMF exposure against I/R injury in rodents remains unresolved. Further studies are warranted to investigate the full neuroprotective potential of LF-EMF. An explanatory chapter is allocated to the neuroprotective potential of electromagnetic field and the emerging role of electromagnetic field in stroke.

1.10 Stem Cell Therapies for Cerebral Stroke

Preclinical trials indicate that the emerging role of stem cells, including embryonic stem cells (ESCs), pluripotent stem cells (PSCs), neural stem cells (NSCs), and mesenchymal stem cell (MSCs), might be due to cell replacement, neuroprotection, neurogenesis, angiogenesis, and regulation of the inflammation and immune response [20]. Neural stem cells (NSCs) offer a therapeutic potential to restore the lost function from ischemic stroke. Clinical studies performed using various stem cell types show encouraging results with regard to their safety and effectiveness in reducing the infarct size of stroke patients [21]. Surprisingly, stem cell-based gene therapy provides a unique potential therapeutic opportunity for stroke patients. Stem-cell therapy a emerging therapeutic intervention in stroke, a assembly was organized to spread information in both clinician and basic researcher with industry and regulatory representative to evaluate the critical issues in a subject.

1.11 Conclusion

Previous research investigation suggested that the unraveling of molecular signaling pathways and therapeutic intervention are the major goals of stroke research. The current treatment choice for stroke patients is the utilization of rt-PA instead of

numerous weaknesses, for example, neurotoxicity and hemorrhagic change. EVT is the main treatment alternative utilized when standard treatment has failed to dissolve the clot. In any case, there is a dire necessity for the investigation of new flagging pathways that assist the improvement of future remedial mediation. The emerging therapeutic interventions, stroke-induced BBB disruption, and ER stress upregulation are summarized in various chapters of the present e-book.

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References

1. Kissela, B., Broderick, J., Woo, D., Kothari, R., Miller, R., Khoury, J., Brott, T., Pancioli, A., Jauch, E., Gebel, J., & Shukla, R. (2001, June 1). Greater Cincinnati/Northern Kentucky Stroke Study: Volume of first-ever ischemic stroke among blacks in a population-based study. *Stroke, 32*(6), 1285–1290.
2. Rosamond, W. (2007). American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics – 2007 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation, 115*, e69–e171.
3. Banerjee, T. K., & Das, S. K. (2016, January). Fifty years of stroke researches in India. *Annals of Indian Academy of Neurology, 19*(1), 1.
4. Fuchs, Y., & Steller, H. (2011). Programmed cell death in animal development and disease. *Cell, 147*(4), 742–758.
5. Wu, Q. J., & Tymianski, M. (2018, December). Targeting NMDA receptors in stroke: New hope in neuroprotection. *Molecular Brain, 11*(1), 15.
6. Ribo, M., Montaner, J., Molina, C. A., Arenillas, J. F., Santamarina, E., & Alvarez-Sabín, J. (2004, December). Admission fibrinolytic profile predicts clot lysis resistance in stroke patients treated with tissue plasminogen activator. *Thrombosis and Haemostasis, 92*(06), 1146–1151.
7. Balami, J. S., White, P. M., McMeekin, P. J., Ford, G. A., & Buchan, A. M. (2017). Complications of endovascular treatment for acute ischemic stroke: Prevention and management. *International Journal of Stroke, 13*(4), 348–361.
8. Wahlgren, N. G., & Ahmed, N. (2004). Neuroprotection in cerebral ischaemia: Facts and fancies – the need for new approaches. *Cerebrovascular Diseases, 17*(Suppl 1), 153–166.
9. Zhang, H., Park, J. H., Maharjan, S., Park, J. A., Choi, K. S., Park, H., Jeong, Y., Ahn, J. H., Kim, I. H., Lee, J. C., & Cho, J. H. (2017, December). Sac-1004, a vascular leakage blocker, reduces cerebral ischemia–reperfusion injury by suppressing blood–brain barrier disruption and inflammation. *Journal of Neuroinflammation, 14*(1), 122.
10. Gumbiner, B., Lowenkopf, T., & Apatira, D. (1991, April 15). Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proceedings of the National Academy of Sciences, 88*(8), 3460–3464.
11. Haskins, J., Gu, L., Wittchen, E. S., Hibbard, J., & Stevenson, B. R. (1998, April 6). ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. *The Journal of Cell Biology., 141*(1), 199–208.

12. Zhang, H. Y., Wang, Z. G., Lu, X. H., Kong, X. X., Wu, F. Z., Lin, L., Tan, X., Ye, L. B., & Xiao, J. (2015, June 1). Endoplasmic reticulum stress: Relevance and therapeutics in central nervous system diseases. *Molecular Neurobiology, 51*(3), 1343–1352.
13. Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., & Ron, D. (2000, November 1). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Molecular Cell, 6*(5), 1099–1108.
14. Welihinda, A. A., Tirasophon, W., Green, S. R., & Kaufman, R. J. (1998, April 1). Protein serine/threonine phosphatase Ptc2p negatively regulates the unfolded-protein response by dephosphorylating Ire1p kinase. *Molecular and Cellular Biology, 18*(4), 1967–1977.
15. Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., & Ron, D. (2002, January). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature, 415*(6867), 92.
16. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., & Mori, K. (2001, December 28). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell, 107*(7), 881–891.
17. Li, G., Morris-Blanco, K. C., Lopez, M. S., Yang, T., Zhao, H., Vemuganti, R., & Luo, Y. (2018). Impact of microRNAs on ischemic stroke: From pre-to post-disease. *Progress in Neurobiology, 163–164*, 59–78.
18. Tripathi, A. K., Dwivedi, A., Pal, M. K., Rastogi, N., Gupta, P., Ali, S., BH, M. P., Kushwaha, H. N., Ray, R. S., Singh, S. K., & Duggal, S. (2014, December). Attenuated neuroprotective effect of riboflavin under UV-B irradiation via miR-203/c-Jun signaling pathway in vivo and in vitro. *Journal of Biomedical Science, 21*(1), 39.
19. Cichoń, N., Bijak, M., Miller, E., & Saluk, J. (2017, July 1). Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. *Bioelectromagnetics, 38*(5), 386–396.
20. Hao, L., Zou, Z., Tian, H., Zhang, Y., Zhou, H., & Liu, L. (2014). Stem cell-based therapies for ischemic stroke. *BioMed Research International, 2014*, 468748.
21. Reis, C., Wilkinson, M., Reis, H., Akyol, O., Gospodarev, V., Araujo, C., Chen, S., & Zhang, J. H. (2017). Look into stem cell therapy: Exploring the options for treatment of ischemic stroke. *Stem Cells International, 2017*, 3267352.

Chapter 2
Inflammation, Oxidative Stress, and Cerebral Stroke: Basic Principles

Shashi Kant Tiwari, Priyanka Mishra, and Tripathi Rajavashisth

Abstract Cerebral stroke has assorted causes, disrupting the cerebral blood flow and subsequently damaging the brain tissues in affected areas. Stroke is the third primary cause of death and disability in adults around the globe. In approximately 25–40% of cerebral stroke patients, the neurological signs are initiated during the early hours. The mechanism involved in the pathophysiology of cerebral stroke are oxidative stress and inflammation. Oxidative stress takes place when there is an impairment to the balance of antioxidant generation with reactive oxygen species (ROS) and other free radicals/oxidants. The brain is extremely vulnerable to oxidative stress owing to the high consumption of body oxygen to produce energy and free radicals, which may cause damage to the main cellular components, such as lipids, proteins, and DNA, and contributing to late-stage apoptosis and inflammation. Inflammation plays significant role in the pathogenesis of cerebral stroke and associated brain damage. Experimentally and clinically, ischemic brain injury takes place because of the initiation of severe and extended inflammatory progression. These processes include activation of brain microglial cells, production of pro-inflammatory mediators (cytokines and chemokines), and infiltration of numerous inflammatory cells such as neutrophils, T-cells, monocyte/macrophages, and natural killer cells into the ischemic brain regions, which initiates neuronal injuries and cell death mechanisms. In this chapter, we focus on the cellular and molecular evidence for oxidative stress and inflammation in cerebral stroke. In addition, we highlight certain current findings and knowledge of the neuroprotective strategies that target oxidative stress/inflammation and their implications in the pathogenesis of cerebral stroke.

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Keywords Antioxidants · Inflammation · Reactive oxygen species · Ischemic stroke · Cerebral stroke · Reactive nitrogen species · Oxidative stress

Abbreviations

BBB	Blood–brain barrier
NO	Nitric oxide
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TNF	Tumor necrosis factor

2.1 Introduction

In this chapter, we introduce the basic biology of oxidative stress and inflammatory response during ischemic stroke. We also highlight the developmental remedies for cerebral stroke/ischemia. Stroke is the third leading cause of mortality and the most frequent cause of chronic disability in adults and imposes a significant health and financial burden on societies worldwide [1, 2]. Stroke is defined as a pathological condition in which an impairment in blood flow in the cerebral part of the brain due to the immediate occlusion of blood vessels by embolism or thrombus, which results in a sudden loss of glucose and oxygen in the brain regions [3, 4]. Strokes are mainly categorized into two types: ischemic and hemorrhagic. Nearly 87% of strokes are categorized as ischemic, whereas others are hemorrhagic. Ischemic strokes are caused by disruption of the blood flow in the brain, whereas hemorrhagic strokes result from an abnormal vasculature or because of the rupture of a blood vessel. The onset of cerebral ischemia activates the pathological paths of the ischemic cascade and finally causes irrevocable neuronal damage in the ischemic core [5]. However, there are other tissues of the brain surrounding the ischemic core known as the penumbra, where the cerebral blood flow is restored. Thus, the ischemic penumbra is defined as the region of the brain that is damaged but not fully dysfunctional. Ischemic conditions initiate a cascade of cell death mechanisms, including the activation of oxidative stress, excitotoxicity, and inflammatory pathways, which can ultimately result in irreversible damage or dysfunction of the affected brain tissues [6]. Thus, neuronal injury due to ischemia primarily occurs by hypoxia, disruption of the blood flow, adenosine triphosphate (ATP) exhaustion, and consequent re-oxygenation of the ischemic brain by reperfusion. These mechanisms contribute to the death of neurons through apoptosis or necrosis. Other mechanisms predominantly involved in the pathogenesis of stroke are reactive oxygen species (ROS) and inflammatory cytokines [7]. Cerebral stroke causes various deficits such as difficulty with memory, language, and movements, and with paralysis (Fig. 2.1).

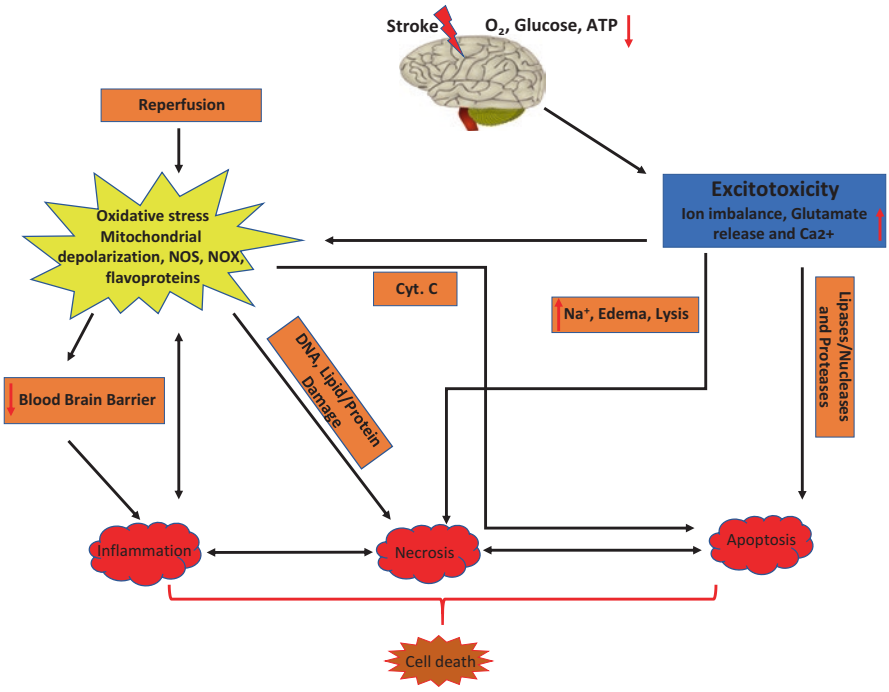

Fig. 2.1 Schematic of the ischemic stroke cascade during cerebral damage: hypo-perfusion due to ischemic stroke in the brain area initiates a cascade of events. Apoptosis and the death of brain cells take place because of excitotoxicity, micro-vascular injury, blood–brain barrier (BBB) dysfunction, oxidative stress, and inflammation under post-ischemic conditions

This chapter mainly focuses on the cellular and molecular parts of the generation of ROS and nitrogen species and their features in the pathogenesis of ischemic stroke. Next, the mechanisms of oxidative stress and post-ischemic inflammatory-related neuronal cell death, in addition to specific targeted therapies for neuroprotection, which target oxidative stress/inflammation, and their further implications in the pathogenesis of stroke are discussed.

2.2 Inflammation

Stroke is considered to be a devastating cerebrovascular incident that causes deaths and permanent disability worldwide [1]. Although various mechanisms are involved in the pathogenesis of cerebral stroke, increasing evidence suggests that inflammation might play an important role in the pathogenic progression of stroke and central nervous system (CNS) injury [7, 8]. Clinically, systemic inflammatory responses are involved in the vulnerability of the patients to stroke and their

consequent prognosis [9, 10]. Stroke patients with systemic inflammation display clinically poorer consequences [11, 12, 13]. Post-ischemic inflammatory responses are involved the activation of resident microglial cells and the infiltration of circulating inflammatory cells such as monocytes/macrophages, neutrophils, and T-cells, in the ischemic brain part, as confirmed by animal models [14–17] and in stroke patients [18–21]. During the early phases of stroke, the immune system contributes to the expansion of the infarct size and involves the penumbra area. However, inhibition of inflammatory processes reduces infarct size and improves CNS defects in stroke models [8]. An inflammatory response exerts both deleterious and protective effects, depending on the pathophysiological conditions [22, 23].

2.3 Inflammatory Role of Cytokines and Chemokines During Cerebral Stroke

During both the acute and the reparative phase of ischemic stroke, proinflammatory mediators such as cytokines and chemokines are released, and excessive ROS creation and induction of matrix metalloproteinase exacerbate the tissue injury [14, 15, 24]. Cytokines belong to a small group of glycoproteins whose levels are enhanced in the brain in numerous disease, including stroke. Cytokines enhance the expression of cell adhesion molecules. In the brain, cytokines are released by immune cells, microglia, neurons, and astrocytes [25]. In addition, other peripheral cells such as mononuclear phagocytes, T lymphocytes, natural killer (NK) cells, and polymorphonuclear leukocytes secrete cytokines that might contribute to inflammation during cerebral stroke [26].

Owing to ischemia, microglia are activated and transformed into phagocytes and release various cytotoxic and cytoprotective molecules. Microglia also contribute neuroprotection by releasing neurotrophic factors, such as brain-derived neurotrophic factor and insulin-like growth factor I, and cytokines (TGF-β and IL-10). Furthermore, activated microglial cells releasing several proinflammatory cytokines such as IL-1β, tumor necrosis factor (TNF)α, and IL-6, along with other potential cytotoxic molecules such as nitric oxide (NO), ROS, and prostanoids appear to exacerbate cerebral injury [23, 27, 28]. Similarly, astrocytes secrete inflammatory molecules such as cytokines, chemokines, and NO, and contribute to the inflammatory damage to ischemic tissues [29].

Chemokines (monocyte chemoattractant protein 1 [MCP-1], macrophage inflammatory protein-1α [MIP-1α]) and fractalkine comprise a group of cytokines that direct the relocation of blood-borne inflammatory cells (neutrophils and macrophages) close to the chemokine. Thus, chemokines play complex roles and function as vital cytokines in cerebral ischemia. Induction of CXC and CC chemokines during cerebral stroke causes extensive infiltration of leukocytes and neutrophils, which may cause increased cerebral edema in the ischemic region [30]. In an ischemic animal model, the level of chemokines has been found to increase, whereas inhibition of mice without chemokine receptor CCR2 show protection against ischemia–reperfusion injury [31–33] (Fig. 2.2).

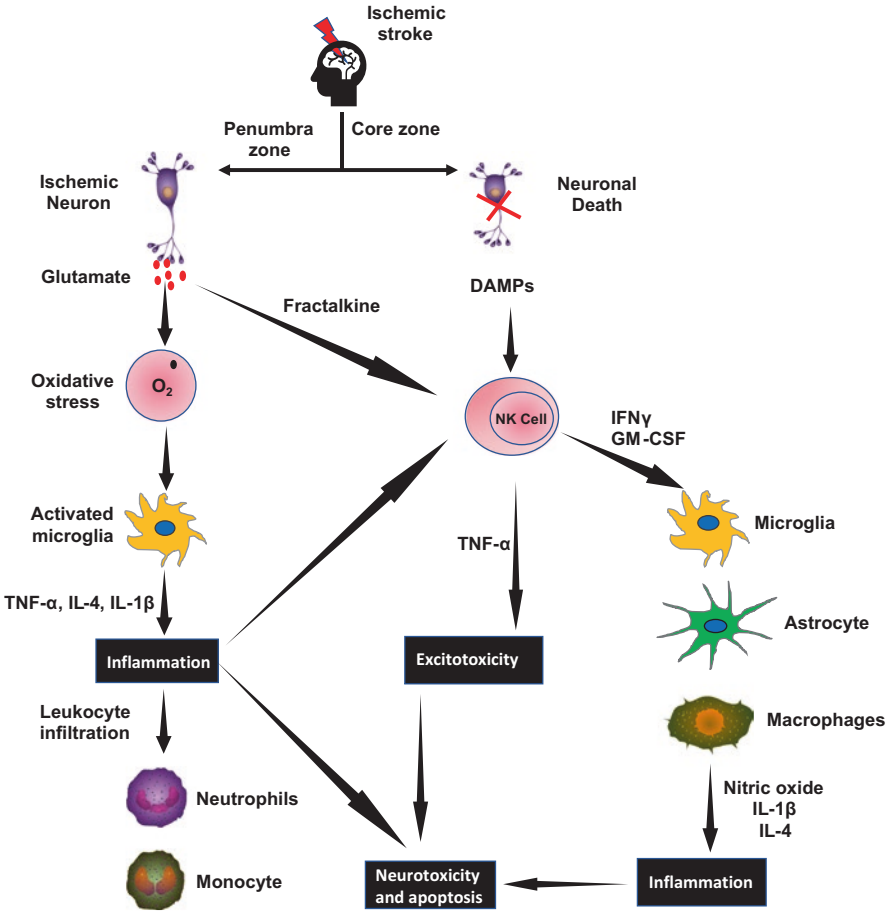

Fig. 2.2 Schematic representation showing post-ischemic inflammatory response: representative scheme showing the effects of cerebral ischemia on inflammatory cytokines and immune cells and their impact on neurotoxicity. The cerebral ischemic condition is divided into two categories: one with major reperfusion defined as the ischemic core and another with minimal reperfusion defined as the extent of the penumbra. Cerebral ischemia leads to a decrease in blood supply in the penumbra zone that elicits a complex cascade such as oxidative stress and excitotoxicity in the ischemic neurons. These ischemic events cause activations of microglia and astrocytes that secrete proinflammatory cytokines and chemokines such as TNFα, IL-6, and IL-1β. These cytokines may enhance inflammation by the infiltration and recruitment of monocytes, neutrophils, and T-cells into brain lesions. In core zone ischemia, neurons endure permanent damage and cause the release of damage-associated molecular patterns (DAMPs). These DAMPs are recognized by toll-like receptors, which are expressed by immune cells such as NK cells. Fraktaline released from ischemic neurons during the early stage of a stroke recruits NK cells into the ischemic zone. These NK cells disturb ischemic neurons by discharging mainly TNFα, which increases glutamate release and causes neuronal excitotoxicity and hyperactivity. In addition, NK cells also discharge IFN-γ and GM-CSF, which trigger macrophages, microglia, and astrocytes, which in turn secrete inflammatory intermediaries such as IL-1β and IL-6. Thus, inflammation and excitotoxicity induced by NK cells and microglia result in neurotoxicity and the apoptotic death of cells, and increase damage to the ischemic brain

2.4 Oxidative Stress

Oxidative stress is a condition that occurs when the physiological imbalance between oxidants and antioxidants takes place and the consequent excessive production of ROS causes enormous damage to an organism. ROS play a role in both normal physiological processes and are also occupied in various disease conditions, whereby they facilitate damage to cellular components such as lipids, membranes, proteins, and DNA. Oxidative stress is involved in the pathogenesis of various neurological conditions including stroke. Oxidative stress contributes to ischemic cell death through the formation of ROS/RNS via various cellular damage pathways, such as mitochondrial inhibition, reperfusion injury, Ca^{2+} overload, and inflammation [34]. There is substantial evidence to indicate that ROS generated during acute ischemic stroke causes tissue damage in the brain [35]. The major target of oxidative stress is the cerebral vasculature and plays a dangerous role in the pathogenesis of ischemic brain damage following a cerebral stroke. During ischemia, primary ROS generates superoxide ($O2^{-}$) and its derivative hydrogen peroxide is the source of hydroxyl radical (OH). These radicals induce vasodilatation through the opening of the potassium channels and impaired vascular reactivity, breakdown of the BBB and focal destructive lesions in the ischemic stroke animal model. However, ROS are also involved in normal physiobiological processes such as cell signaling, immune defense, and induction of mitogenesis [36]. NO is a lipid and water-soluble free radical that is produced from L-arginine by means of nitric oxide synthases (NOS). Cerebral stroke causes an enhancement of NOS type I and III activity in neurons and the vascular endothelium respectively. Later, enhanced NOS type II (iNOS) activity takes place in a variety of cells, including infiltrating neutrophils and glia. Thus, free radicals are observed as a central therapeutic target for refining the consequences of cerebral stroke (Fig. 2.3).

2.5 Treatment Strategy for Stroke

The prevalance and frequency of stroke increases with life expectancy. Stroke is considered one of the major causes of mortality and morbidity and accounts for around 80–85% of all cases. Ischemic stroke is characterized by the interruption of the cerebral blood flow and lack of oxygen and energy depletion, which activates a cascade of actions such as oxidative stress, inflammatory responses, glutamate excitotoxicity, and apoptosis, which result in a profound brain injury in the affected region. The following treatment strategy are commonly utilised for the treatment of stroke:

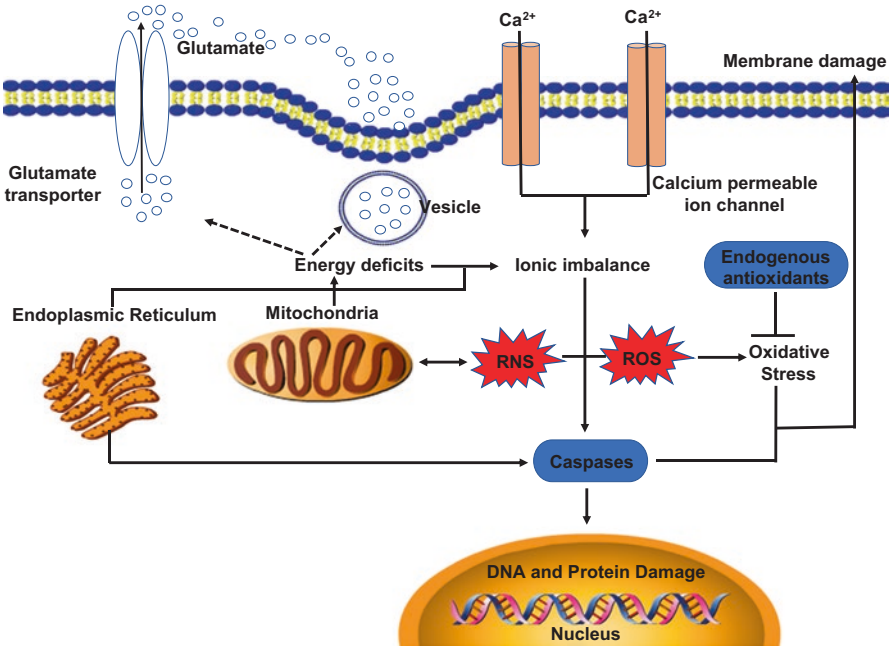

Fig. 2.3 Main pathways associated with ischemic cell death: extensive interaction and overlap occur among various mediators of cell damage and death. Post-ischemic onset causes loss of energy, which leads to mitochondrial dysfunction and the generation of reactive nitrogen species (RNS) and reactive oxygen species (ROS). In addition, energy depletion causes excitotoxic glutamate efflux, ionic imbalance, and a build-up of intracellular calcium ion. Downstream signaling includes the free radical damage to membrane lipids, cellular proteins, and DNA. Further, calcium-activated proteases and caspase cascade alter a wide array of homeostatic and cytoskeletal proteins

2.5.1 Targeting Antioxidant Enzyme as a Therapeutic Strategy for Ischemic Stroke

Oxidative stress due to cerebral ischemic stroke causes damage to the neurons and glia in the brain. ROS are also implicated in calcium dysregulation in brain cell types and the incursion of activated immune cells causes stroke-induced neurodegeneration. Thus, it is important to recognize antioxidant-based drugs that can augment neural cell survival and improve retrieval after stroke. By means of the increasing expression of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD), families of neural cells can protect against stroke. Cellular therapy such as neural stem cells and human umbilical cord blood cells employs neuroprotective effects via the release of pro-survival factors that trigger PI3K/Akt signaling mechanisms to upregulate these antioxidant enzymes. In addition, leukemia inhibitory factor enhances the expression of metallothionein III and peroxiredoxin IV in glia and increases the expression of SOD3 in neurons [37].

However, several compounds that also have antioxidant properties, including ebselen [38] and resveratrol [39], have been verified to reduce stroke-mediated brain damage in animal models. Similarly, hemorrhagic stroke is a common and severe neurological disorder and is associated with high rates of mortality and morbidity [6, 30]. The pathways involved in hemorrhagic stroke include endoplasmic reticulum stress, neuronal apoptosis and necrosis, inflammation, and autophagy can be modulated for therapeutic purposes [40]. In addition, oxidative DNA and protein damage following stroke is typically associated with epigenetic changes such as DNA methylation, histone modification, and microRNAs in experimental stroke in animal and cell models. Thus, an understanding of the epigenetic regulatory network upon oxidative stress in the vascular neural network may provide effective antioxidant approaches to treating stroke [41].

2.5.2 Regulation of Microglial Activation in Stroke

Microglia are considered as the resident immune cells in the brain that regularly monitor the brain's micro-environment under various conditions. Under ischemic conditions, the microglia become activated and produce both harmful and neuroprotective mediators. Thus, the balance between these two counteracting mediators regulates the fate of damaged neurons. Microglial activation is characterized by either classic (M1), which secrete proinflammatory cytokines (TNFα, IL-23, IL-1β, IL-12, etc.) and exacerbate neuronal injury, or the alternative (M2), which encourages anti-inflammatory responses that are reparative. There are various regulators of microglia/macrophage activation that can be utilized to diminish the detrimental effects or maximize the defensive part in the context of ischemic stroke [42].

2.5.3 Targeting the Cholinergic Anti-inflammatory Pathway

It is well established that inflammation plays a key role in the pathophysiology of stroke and targeting inflammatory pathways and mediators after stroke are investigated as promising therapeutic targets. The cholinergic anti-inflammatory path (CHAIP) is a biological mechanism through which the CNS regulates inflammation and immune response. The major components of the CHAIP are the spleen, the vagus nerve, and the α7 nicotinic acetylcholine receptor (α7 nAChR). Targeting CHAIP is a promising method of immunomodulation that diminishes inflammation [43].

In addition, a drug could be developed based on using chemokines as diagnostic or prognostic biomarkers in ischemic stroke [44]. Connexin and pannexin channels are promising therapeutic targets in cerebral stroke-mediated cell death mechanisms. Connexin hemichannels may contribute most of the ATP release owing to ischemia and during reperfusion [6].

References

1. Donnan, G. A., Fisher, M., Macleod, M., & Davis, S. M. (2008). Stroke. *Lancet, 371*(9624), 1612–1623.
2. Lo, E. H., Dalkara, T., & Moskowitz, M. A. (2003). Mechanisms, challenges and opportunities in stroke. *Nature Reviews. Neuroscience, 4*(5), 399–415.
3. Kriz, J., & Lalancette-Hebert, M. (2009). Inflammation, plasticity and real-time imaging after cerebral ischemia. *Acta Neuropathologica, 117*(5), 497–509.
4. Lakhan, S. E., Kirchgessner, A., & Hofer, M. (2009). Inflammatory mechanisms in ischemic stroke: Therapeutic approaches. *Journal of Translational Medicine, 7*, 97.
5. Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences, 22*(9), 391–397.
6. Kim, Y., Davidson, J. O., Green, C. R., Nicholson, L. F., O''Carroll, S. J., & Zhang, J. (2017). Connexins and pannexins in cerebral ischemia. *Biochimica et Biophysica Acta, 1860*(1), 224–236.
7. Muir, K. W., Tyrrell, P., Sattar, N., & Warburton, E. (2007). Inflammation and ischaemic stroke. *Current Opinion in Neurology, 20*(3), 334–342.
8. Yilmaz, G., & Granger, D. N. (2008). Cell adhesion molecules and ischemic stroke. *Neurological Research, 30*(8), 783–793.
9. Emsley, H. C., & Hopkins, S. J. (2008). Acute ischaemic stroke and infection: Recent and emerging concepts. *Lancet Neurology, 7*(4), 341–353.
10. McColl, B. W., Allan, S. M., & Rothwell, N. J. (2009). Systemic infection, inflammation and acute ischemic stroke. *Neuroscience, 158*(3), 1049–1061.
11. Baird, T. A., Parsons, M. W., Barber, P. A., Butcher, K. S., Desmond, P. M., Tress, B. M., Colman, P. G., Jerums, G., Chambers, B. R., & Davis, S. M. (2002). The influence of diabetes mellitus and hyperglycaemia on stroke incidence and outcome. *Journal of Clinical Neuroscience, 9*(6), 618–626.
12. Elkind, M. S., Cheng, J., Rundek, T., Boden-Albala, B., & Sacco, R. L. (2004). Leukocyte count predicts outcome after ischemic stroke: The Northern Manhattan Stroke Study. *Journal of Stroke and Cerebrovascular Diseases, 13*(5), 220–227.
13. McColl, B. W., Rothwell, N. J., & Allan, S. M. (2007). Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. *The Journal of Neuroscience, 27*(16), 4403–4412.
14. Amantea, D., Nappi, G., Bernardi, G., Bagetta, G., & Corasaniti, M. T. (2009). Post-ischemic brain damage: Pathophysiology and role of inflammatory mediators. *The FEBS Journal, 276*(1), 13–26.
15. Kriz, J. (2006). Inflammation in ischemic brain injury: Timing is important. *Critical Reviews in Neurobiology, 18*(1–2), 145–157.
16. Schilling, M., Besselmann, M., Leonhard, C., Mueller, M., Ringelstein, E. B., & Kiefer, R. (2003). Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: A study in green fluorescent protein transgenic bone marrow chimeric mice. *Experimental Neurology, 183*(1), 25–33.
17. Tanaka, R., Komine-Kobayashi, M., Mochizuki, H., Yamada, M., Furuya, T., Migita, M., Shimada, T., Mizuno, Y., & Urabe, T. (2003). Migration of enhanced green fluorescent protein expressing bone marrow-derived microglia/macrophage into the mouse brain following permanent focal ischemia. *Neuroscience, 117*(3), 531–539.
18. Buck, B. H., Liebeskind, D. S., Saver, J. L., Bang, O. Y., Yun, S. W., Starkman, S., Ali, L. K., Kim, D., Villablanca, J. P., Salamon, N., Razinia, T., & Ovbiagele, B. (2008). Early neutrophilia is associated with volume of ischemic tissue in acute stroke. *Stroke, 39*(2), 355–360.
19. Gerhard, A., Neumaier, B., Elitok, E., Glatting, G., Ries, V., Tomczak, R., Ludolph, A. C., & Reske, S. N. (2000). In vivo imaging of activated microglia using [11C]PK11195 and positron emission tomography in patients after ischemic stroke. *Neuroreport, 11*(13), 2957–2960.

20. Lindsberg, P. J., Carpen, O., Paetau, A., Karjalainen-Lindsberg, M. L., & Kaste, M. (1996). Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. *Circulation, 94*(5), 939–945.
21. Price, C. J., Menon, D. K., Peters, A. M., Ballinger, J. R., Barber, R. W., Balan, K. K., Lynch, A., Xuereb, J. H., Fryer, T., Guadagno, J. V., & Warburton, E. A. (2004). Cerebral neutrophil recruitment, histology, and outcome in acute ischemic stroke: An imaging-based study. *Stroke, 35*(7), 1659–1664.
22. Vidale, S., Consoli, A., Arnaboldi, M., & Consoli, D. (2017). Postischemic inflammation in acute stroke. *Journal of Clinical Neurology, 13*(1), 1–9.
23. Zhu, Y., Yang, G. Y., Ahlemeyer, B., Pang, L., Che, X. M., Culmsee, C., Klumpp, S., & Krieglstein, J. (2002). Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. *The Journal of Neuroscience, 22*(10), 3898–3909.
24. Bonaventura, A., Liberale, L., Vecchie, A., Casula, M., Carbone, F., Dallegri, F., & Montecucco, F. (2016). Update on inflammatory biomarkers and treatments in ischemic stroke. *International Journal of Molecular Sciences, 17*(12).
25. Barone, F. C., & Feuerstein, G. Z. (1999). Inflammatory mediators and stroke: New opportunities for novel therapeutics. *Journal of Cerebral Blood Flow and Metabolism, 19*(8), 819–834.
26. Ferrarese, C., Mascarucci, P., Zoia, C., Cavarretta, R., Frigo, M., Begni, B., Sarinella, F., Frattola, L., & De Simoni, M. G. (1999). Increased cytokine release from peripheral blood cells after acute stroke. *Journal of Cerebral Blood Flow and Metabolism, 19*(9), 1004–1009.
27. Lucas, S. M., Rothwell, N. J., & Gibson, R. M. (2006). The role of inflammation in CNS injury and disease. *British Journal of Pharmacology, 147*(Suppl 1), S232–S240.
28. Spera, P. A., Ellison, J. A., Feuerstein, G. Z., & Barone, F. C. (1998). IL-10 reduces rat brain injury following focal stroke. *Neuroscience Letters, 251*(3), 189–192.
29. Swanson, R. A., Ying, W., & Kauppinen, T. M. (2004). Astrocyte influences on ischemic neuronal death. *Current Molecular Medicine, 4*(2), 193–205.
30. Kim, J. S., Gautam, S. C., Chopp, M., Zaloga, C., Jones, M. L., Ward, P. A., & Welch, K. M. (1995). Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. *Journal of Neuroimmunology, 56*(2), 127–134.
31. Dimitrijevic, O. B., Stamatovic, S. M., Keep, R. F., & Andjelkovic, A. V. (2007). Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. *Stroke, 38*(4), 1345–1353.
32. Hughes, P. M., Allegrini, P. R., Rudin, M., Perry, V. H., Mir, A. K., & Wiessner, C. (2002). Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. *Journal of Cerebral Blood Flow and Metabolism, 22*(3), 308–317.
33. Soriano, S. G., Amaravadi, L. S., Wang, Y. F., Zhou, H., Yu, G. X., Tonra, J. R., Fairchild-Huntress, V., Fang, Q., Dunmore, J. H., Huszar, D., & Pan, Y. (2002). Mice deficient in fractalkine are less susceptible to cerebral ischemia-reperfusion injury. *Journal of Neuroimmunology, 125*(1–2), 59–65.
34. Coyle, J. T., & Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science, 262*(5134), 689–695.
35. Cuzzocrea, S., Riley, D. P., Caputi, A. P., & Salvemini, D. (2001). Antioxidant therapy: A new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. *Pharmacological Reviews, 53*(1), 135–159.
36. Allen, C. L., & Bayraktutan, U. (2009). Oxidative stress and its role in the pathogenesis of ischaemic stroke. *International Journal of Stroke, 4*(6), 461–470.
37. Davis, S. M., & Pennypacker, K. R. (2017). Targeting antioxidant enzyme expression as a therapeutic strategy for ischemic stroke. *Neurochemistry International, 107*, 23–32.
38. Yamagata, K., Ichinose, S., Miyashita, A., & Tagami, M. (2008). Protective effects of ebselen, a seleno-organic antioxidant on neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rat. *Neuroscience, 153*(2), 428–435.
39. Ozkan, O. V., Yuzbasioglu, M. F., Ciralik, H., Kurutas, E. B., Yonden, Z., Aydin, M., Bulbuloglu, E., Semerci, E., Goksu, M., Atli, Y., Bakan, V., & Duran, N. (2009). Resveratrol, a natural

antioxidant, attenuates intestinal ischemia/reperfusion injury in rats. *The Tohoku Journal of Experimental Medicine, 218*(3), 251–258.
40. Duan, X., Wen, Z., Shen, H., Shen, M., & Chen, G. (2016). Intracerebral hemorrhage, oxidative stress, and antioxidant therapy. *Oxidative Medicine and Cellular Longevity, 2016*, 1203285.
41. Zhao, H., Han, Z., Ji, X., & Luo, Y. (2016). Epigenetic regulation of oxidative stress in ischemic stroke. *Aging and Disease, 7*(3), 295–306.
42. Zhao, S. C., Ma, L. S., Chu, Z. H., Xu, H., Wu, W. Q., & Liu, F. (2017). Regulation of microglial activation in stroke. *Acta Pharmacologica Sinica, 38*(4), 445–458.
43. Duris, K., Lipkova, J., & Jurajda, M. (2017). Cholinergic anti-inflammatory pathway and stroke. *Current Drug Delivery, 14*(4), 449–457.
44. Chen, C., Chu, S. F., Liu, D. D., Zhang, Z., Kong, L. L., Zhou, X., & Chen, N. H. (2018). Chemokines play complex roles in cerebral ischemia. *Neurochemistry International, 112*, 146–158.

Chapter 3
Stroke Induced Blood-Brain Barrier Disruption

Amit Kumar Tripathi, Nirav Dhanesha, and Santosh Kumar

Abstract Blood-brain barrier (BBB) is highly ultra-synchronized, structural and biochemical barrier between the peripheral blood circulation and the central nervous system (CNS) coordinate entry of blood-borne entities into the CNS. BBB anatomy is comprised of microvascular endothelium, pericytes, astrocytes and neuronal cells that constitute a neurovascular unit (NVU), participating a crucial role in proper functioning of the CNS. Every cell of NVU forms an indispensable contribution to BBB integrity. BBB functions are mainly controlled by tight and adherens junctional (TJ and AJ) protein complexes. These restrictive angioarchitectures at BBB reduce the paracellular diffusion of molecules, whereas carrier proteins determine which substance can cross the transcellular barrier. Under normal condition, BBB prevents extravasation of blood-borne cell, solute, ions and molecules. However, its disruption can lead to change in paracellular and transcellular permeability and extravasation of leukocytes into brain tissue, contributing oedema formation in neuropathological disorder including brain stroke. This chapter emphasized recently gained information on BBB anatomy and its neuropathogenetic alteration in an ischemic cerebral injury.

Keywords Ischemic stroke · BBB · Endothelium · Neurovascular unit · Occludin

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Abbreviations

AJ	Adherens junction
BBB	Blood-brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
JAM	Junctional adhesion molecule
MAGUK	Membrane-associated guanylate kinase
MS	Multiple sclerosis
NMO	Neuromyelitis optica
NPSLE	Neuropsychiatric systemic lupus erythematosus
NVU	Neurovascular unit
TBI	Traumatic brain injury
TEMPOL	4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl
TJ	Tight junction

3.1 Introduction

The first experimental proof for presence of BBB was studied by Paul Ehrlich in 1885, who discovered that water-soluble dye coloured all body organ except the brain and spinal cord after intravenous administration in rats [1]. Further his fellow Lewandowsky concluded "Brain capillaries must hold back certain molecules" from entering brain in 1900. The next experiment was done by Ehrlich research scholar Edwin E. Goldmann who demonstrated that trypan blue injection given into the cerebrospinal fluid (CSF) stained the brain but not body organ tissue [2–4]. They have given first evidence that there is the existence of barrier between brain and blood flow. Also electron microscopic studies corroborated the existence of barrier by localization of endothelial cells (ECs) by Reese and Karnovsky [5]. With this experimental finding it has been chosen that term Blut-Hirn-Schranke was given to BBB. Afterwards, Stern established the presence of a special filter at blood-brain interface that protects the brain from harmful toxic substance called BBB (earlier known as haematoencephalic barrier). But our understanding of the BBB has developed from a physico-biochemical barrier separating the brain from peripheral blood circulation. It is the dynamic and metabolic interface that bidirectionally regulates trafficking of fluid, solutes and blood cells.

The research has been extended with a concept of a neurovascular unit comprises with endothelial cells, astrocytes, pericytes, neuronal cells and other components. BBBs play as site-specific crosstalk of CNS and blood-borne cells and provide fundamental regulation of normal brain internal environment and function. BBB dysfunction, referring loss of structural integrity and normal functions, is also a prominent pathological feature of many neurological disorders, including stroke [3].

Stroke is the fifth leading cause of physical disability and cognitive impairment in the USA. Ischemic injury reports for ~87% of all stroke in the USA [5]. Intensive basic and clinical research has revealed multiple risk factors and many deciphered mechanisms in ischemic brain injury. However the current therapy for acute ischemic stroke remains largely focused on recombinant tissue plasminogen. BBB disruption during and after the stroke leads to ischemic brain injury progression. The comorbid conditions such as hypertension and hyperglycaemia exacerbate BBB disruption after stroke. The stroke induced BBB disruption is well known characterised in comorbid condition of hypertension and hyperglycemia.

3.2 BBB Anatomy

The current understanding of BBB anatomy was described in the twentieth century. The structural component of BBB includes the endothelial cell (EC), tight junction (TJ), pericytes, astrocytes and extracellular matrix components that form neurovascular unit (NVU) which contributes BBB formation [6, 7] (Fig. 3.1). Three barrier layers provide separation of the blood and brain tissue: (1) specific endothelial cells separating blood and brain, (2) the blood and CSF barrier with choroid plexus secreting cerebrospinal fluid (CSF) into cerebral hemisphere ventricles and (3) arachnoid epithelium separating blood from subarachnoid CSF. BBB has another function as the carrier with ectoenzymes, receptors and transporters, controlling the movement of molecules, ions and solute from one side of BBB to the other. The main function of BBB is to serve as a selective diffusion barrier having cell-cell junctions. Anatomically, the ECs of a capillary lumen of BBB are different from those in the periphery by increased mitochondrial content [9], a lack of fenestrations [10], negligible pinocytotic activity [11] and the presence of TJ proteins [12].

Pericytes can be granular or filamentous subtypes [13]. Although not much information is known about pericytes, the presence of contractile proteins suggests that they may regulate capillary blood flow [14]. Pericytes have ability to migrate

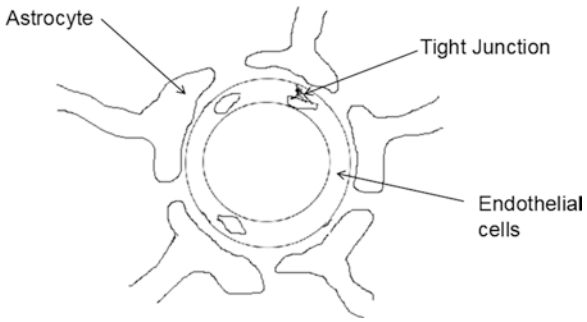

Fig. 3.1 Diagrammatic illustration of BBB angioarchitecture [8]

away from brain microvessels during hypoxia [15] and traumatic brain injury (TBI) [16]; both conditions can enhance BBB permeability. Occludin which is a vital component of BBB TJ starts getting expressed upon induction with angiopoietin which is derived from pericyte [17]. Astrocytes induce and maintain the BBB and form the glia limitans [18]. It has been hypothesized that astrocytes coordinate regulation of cerebral microvascular permeability [19], via dynamic Ca^{+2} signalling between astrocytes and endothelium [20, 21]. Astrocyte destruction is shown to be associated with BBB disruption [22]. During haemorrhage, TBI or ischemia, BBB damage brings about pathological alteration in cerebral blood flow and perfusion pressure [8, 23]. It has also been reported that BBB opening may be a specific event instead of anatomical damage [24]. Anatomical confirmation has been found for direct innervation of the microvascular endothelium and associated astrocytic processes by noradrenergic [25, 26], serotonergic [27], cholinergic [28, 29] and GABAergic [30] neurons, as well as others [31, 32]. Besides reacting with the neurons, pericytes and astrocytes, cerebral microvascular endothelium also interacts with the extracellular matrix of basal lamina. A disrupted extracellular matrix has been demonstrated to be associated with increased BBB permeability in pathological states [33]. It has been proposed that laminin and other matrix proteins may interact with endothelial integrin receptors to form an anchor-like situation where endothelium rests [34]. These matrix proteins can also influence the expression of endothelial TJ proteins, showing that these proteins are very likely involved in the maintenance of TJ [35, 36].

3.3 BBB Junctional Complexes

The space between endothelial cells of NVU contains specialized junctional complexes such as tight junctions (Claudin, occludin, junctional adhesion molecules and ZO-1, ZO-2 and ZO-3) and adherens junctions (VE-Cadherin and nectin).

Role of tight junction protein in BBB

TJ	Functional Role in BBB
Occludin	Regulate paracellular permeability
Claudin-1	Tighten BBB sealing
Claudin-5	Regulate size-specific opening of BBB for small molecules less than 800 Da
JAMs	Regulate junctional tightness and trans-endothelial migration of lymphocytes
ZO-1, ZO-2 and ZO-3	Scaffold protein

3.3.1 Adherens Junctions (AJs)

The primary structure of AJ consists of vascular endothelial-cadherin, a calcium-mediated protein molecule that contributes cellular attachments. This cellular attachment is homophilic and exits between extracellular protein domains present on cells [37, 38]. AJs are present almost everywhere in the blood vessel and help in attachment of ECs to each other. AJs play an important role in contact inhibition during endothelial cell growth and the regulation of paracellular permeability to migrating leukocytes and solutes [39, 40]. And also, they are useful for a meaningful organization of new vessel formation. A recent few lines of research has demonstrated many molecular component of AJ such as integral membrane and intracellular proteins.

3.3.2 Tight Junctions (TJs)

Tight junctions are well-developed between neighbouring endothelial cells of blood vessels in the CNS and play an important role in maintaining BBB. TJs are composed of integral and cytoplasmic proteins associated with actin microfilament which allow this complex to form a tight junctional attachment. TJs participate as major functional role for maintaining barrier within membrane. These are present at the top portion of the junctional complex formed by union of the outer layer of plasma membranes from adjacent cells [19, 37, 38, 41]. TJs participate in regulating the paracellular permeability and maintaining cellular polarity. And, also, the migration of molecules from the lumen to the tissue parenchyma is limited by the TJ proteins [42–44].

3.3.3 Gap Junctions

The stabilization of AJ complex is initiated by binding of the cytoplasmic tail of VE-cadherin to catenin and plakoglobin, which in turn binds to the actin cytoskeleton via actinin, catenin and vinculin, thus stabilizing the AJ complex [45–49]. Although disruption of AJ at the BBB can lead to increased permeability [50], it is primarily the TJ that confers the low paracellular permeability and high electrical resistance [51]. AJ and TJ are responsible for controlling permeability across the endothelium, whereas gap junctions help in intercellular signalling [39].

At molecular level TJ is composed majorly of junctional adhesion molecule (JAM)-1 [52], occludins [53] and claudins [54]. Among these three claudins are believed to play the major role in TJ formation. Although the claudin superfamily consists of 24 members, claudin-1 and claudin-5 have been shown to be expressed in the endothelium of the brain capillaries [55–57]. Primary seal of the TJ is formed by the claudins [58], whereas an increased electrical resistance across the barrier and decreased paracellular permeability is imparted by the occludins [59]. Occludin

is overexpressed in ECs of the brain capillaries, and its presence is associated with enhanced barrier properties of the BBB.

3.3.3.1 Junctional Adhesion Molecule (JAM)

Junctional adhesion molecules (JAMs) are the transmembrane protein present in the TJ and play an important role in brain metastasis [60]. It is categorized into JAM-A, JAM-B, JAM-C and JAM-4. All of the JAMs are present on plasma membrane of ECs, and JAM-A is highly expressed in cerebral vessels. JAM-A is a 40 kDa member of the IgG superfamily and is believed to mediate the early attachment of adjacent cell membranes through homophilic interactions. It is composed of a single membrane-spanning chain with a large extracellular domain [61]. Related proteins JAM-B and JAM-C are related to JAM-A and are found in lymphatic cells and endothelial tissues but are not present in epithelia [62]. In addition to their developmental roles, JAMs may regulate the trans-endothelial migration of leukocytes [63], but their function in the mature BBB is mostly unknown.

3.3.3.2 Occludin

Occludin was a novel integral protein identified in chick liver by freeze fracture microscopy [63]. Occludin, a phosphoprotein (molecular wt 60–65 kDa), consists of four integral domains, two extracellular domains and three cytoplasmic domains. Occludin forms a homophilic dimer with other endothelial cells. The two extracellular loops of occludin and claudin originating from neighbouring cells form the paracellular barrier of TJ. Occludin can regulate paracellular permeability [59] and increases electrical resistance in tissues [64]. The effect of C-terminal truncated occludin function trigger to increased paracellular permeability of BBB for low molecular weight molecules [65]. Claudins recruit the occludin in fibroblast and assembled into heteropolymers and form intramembranous strands. These strands possess oscillating channels passing the specific diffusion of ions and water-soluble molecules [66]. Some studies suggest that claudins and occludins form the extracellular component of TJs and are both required for formation of the BBB [67], whereas contradictory results of artificial alteration in gene expression experiments suggest that occludin is not required for the TJ formation. However, some studies reported downregulated occludin expression is associated with dissociated BBB functionality in pathological condition [68, 69].

3.3.3.3 Claudins

The molecular size of claudins (molecular size 20–24 KDa) is smaller as compared to occludins. Claudins are the group of the superfamily proteins, consisting of 18 homologous proteins in humans. It is reported in mammals as claudin-1 (sealing BBB), claudin-3 (unknown), claudin-5 (size-specific opening of BBB for less than 800 Da small molecules) and claudin-12 (unknown) which are present in CNS endothelial cells [69, 70]. All of the claudins have similar molecular folding and sequence homology [71]. The carboxylic terminal of claudin associates with zonula occludins (ZO-1, ZO-2, ZO-3) and MAGUK proteins to make tight association between endothelial cells. However, claudin-5 is a hallmark of BBB and plays a pivotal functional role in the early level of CNS blood vessel development [72]. And, also, claudin-5 is ubiquitously expressed in the endothelial cells of all brain regions. But recent lines of report suggest no alteration in the morphology of blood vessel and brain oedema formation in claudin-5-deficient mice [73]. Experimentally increased expression of claudin protein in extracellular matrix and collagen synthesizing cell can stimulate cellular assemblage and formation of TJ-like strands. So it is presumed that claudin is responsible for the development of primary sealing of TJ, whereas occludin acts as the additional support structure [74].

3.3.4 Membrane-Associated Guanylate Kinase (MAGUK)-Like Proteins

Membrane-associated guanylate kinase (MAGUK) homolog family proteins consist of multiple postsynaptic density protein-95 (PSD-95)/discs-large/ZO-1 binding domains, an Src homolog-3 (SH3) domain and a guanylate kinase (GK)-like domain [75]. MAGUK proteins (ZO-1, ZO-2 and ZO-3) appear to be involved in the coordination and clustering of protein complexes to the cell membrane [62]. ZO-1 is a 220 kDa phosphoprotein positively expressed in endothelial and epithelial cells and associated with the TJ [76, 77]. Interaction between ZO-1 and TJ is important for the stability and function of later molecule, since ZO-1 dissociation from the junctional complex is reported to increase permeability [80–82]. There remains a possibility of ZO-1 acting as a signalling molecule and communicating the internal cell conditions to TJ and vice versa. Nuclear localization of ZO-1 is observed due to proliferation and injury [83], depletion of Ca^{2+} [84] and as a response to nicotine [85], whereas it co-localizes with transcription factors [86] and G-proteins [87]. ZO-2, a 160 kDa phosphoprotein and high-sequence homolog of ZO-1, co-precipitates along with ZO-1 [88]. Though the exact functional properties of ZO-2 are not clear till date, it has been elucidated that similar to ZO-1, ZO-2 also shows binding with various transcription factors and both structural constituents of TJ [89]. Similar to ZO-1, ZO-2 also manifests nuclear localization during stress and proliferation [78, 79]. ZO-2 localization in in vitro-cultured brain microvessel endothelial cells is reported to take place along the membranes at cell-cell contacts [82],

but it is much more diffusedly distributed in cerebral microvessel fragments [85]. ZO-2 might functionally replace the ZO-1 and is able to facilitate formation of morphologically normal TJ in epithelial cell cultures which lack ZO-1 [90]. The presence of ZO-2 is also reported in non-TJ-containing tissues [78, 79]. ZO-3 is another homolog of 130 kDa, which is present in some TJ-containing tissues but is not reported to be present in BBB till date [91].

3.3.5 Accessory Proteins

Among the other accessory proteins present in BBB, cingulin, AF-6 and 7H6 require special mention. Cingulin is 140–160 kDa protein associated with ZOs, myosin and JAM-1 and is known to mediate cytoskeleton-TJ interactions [61]. AF-6 is another ZO-1 interacting protein with two Ras-associating domains and weighs 180 kDa. AF-6/ZO-1 interaction is inhibited by Ras activation, thus indicating that af-6/ZO-1 complex disruption might be critical in TJ modulation via Ras-mediated signalling pathways [92]. 7H6 has a molecular mass of 155 kDa, and it shows reversible dissociation from TJ complex under ATP depletion [93]. It has been also established that multiple pathways can simultaneously modulate the responses of TJ to various stimuli [94]. Self-interaction between TJ proteins and the interaction of TJ and other molecules of intracellular signalling pathways have also been studied extensively [95].

3.4 Calcium Modulation of TJ and TJ Proteins

Calcium is critical for regulating the cell to cell TJ interaction [96, 97]. Experimental results suggested that epithelial cells are present in media with low calcium result AJ dissociation mediated by protein kinase A (PKA) leading to a distribution of E-cadherin and ZO-1 [40]. The low calcium level changes confirmation of E-cadherin, removing the binding sites and interrupting cell-cell adhesion. Likewise, loss of ZO-1, ZO-2, and occludin from the cell membrane, related with increment in paracellular permeability in cultured epithelial cells in Ca^{2+}-inadequate media. [11]. Protein kinase C (PKC) is involved in Ca^{2+}-dependent regulation of TJ, and its over-activation can decrease deleterious effect of low extracellular Ca^{2+} [98]. Intracellular Ca2+ stores play an important role in TJ regulation, since both abnormally high [99] and abnormally low [100] concentrations of intracellular Ca2+ can disrupt the TJ. Along with PKC, Ca2+ release or influx from the intracellular stores leads to activation of kinase signalling cascades, further activating the transcription factors, like cAMP response element-binding (CREB) protein, nuclear factor-B, c-fos, etc., thus regulating the TJ expression [40, 101].

3.5 Phosphorylation: A Novel Regulatory Mechanism of TJ Proteins

Earlier reports suggest that tight junction permeability was regulated by mechanisms involving tyrosine phosphorylation of AJ and TJ proteins [102, 103]. Serine phosphorylation regulates the subcellular localization of occludin [104], and threonine phosphorylation of occludin is more associated with the TJ interaction following disruption [105–107]. Initial evidence suggested the junctional BBB permeability was correlated with the phosphate content of ZO-1 [108], although subsequent studies have indicated that phosphorylation of ZO-1 is critical to its membrane targeting during the development of TJ [109]. The phosphorylation of TJ proteins, for example, occludin and ZO-1 demonstrated change in an epithelial cell resulting loosening of BB [110]. In conclusion, phosphorylation of different sites of serine, tyrosine and threonine residues has distinguished structural and functional consequences.

3.6 Impairment of BBB Integrity in Neuropathological Disorder

BBB is the most critical structural and functional shielding device against various neurological disorders. Extended BBB permeability is an outcome of neuropathological disorder, such as brain stroke, MS, TBI, CNS inflammation, epilepsy, Parkinson disease and Alzheimer disease [111–114]. The basic mechanism for BBB integrity impairment in MS involves various pleiotropic effects of cytokine and chemokine [113–115].

3.6.1 Alteration of BBB Integrity in Stroke Injury

Cerebral stroke is an arterial blockage resulting in Oxygen Glucose Deprivation (OGD), contributes to neuronal cell death and is associated with increased microvasculature permeability [8, 116, 117]. BBB opening during stroke leads to brain oedema formation (Fig. 3.2). The brain oedema is divided into two types:

1. Cytotoxic intracellular oedema is developed after cerebral ischemia and metabolic disorder. The basic mechanisms for cytotoxic intracellular oedema are (1) abnormal Na^+ export in ATP-depleted neurons, (2) activated Na^+-permeability or (3) increased Na^+-mediated membrane pumps.
2. Vasogenic extracellular oedema is developed by BBB opening during various pathogeneses and infections. The pathogenic mechanisms are (1) TJ protein degradation for BBB opening in acute conditions or (2) development of less-developed blood vessels in chronic conditions.

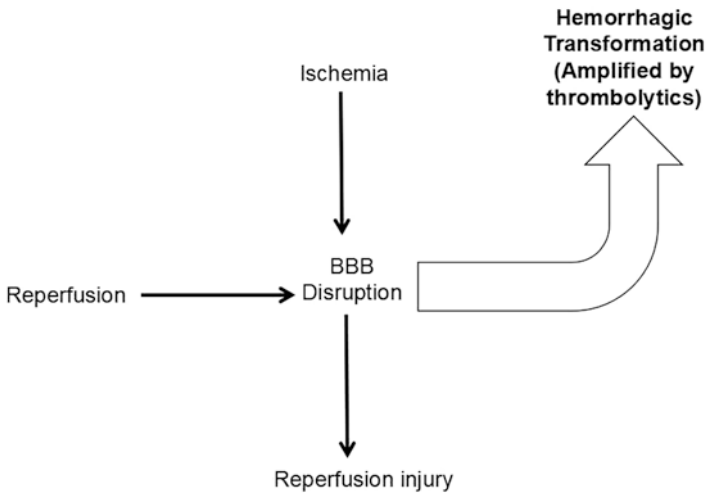

Fig. 3.2 Schematic representation of blood-brain barrier (BBB) changes in acute ischemic stroke. Thrombolytics can amplify the risk of haemorrhagic transformation secondary to reperfusion injury

Cytotoxic oedema formation few minutes after ischemic onset followed by a relatively late onset of vasogenic oedema is correlated to BBB breakdown and its opening. Na^+-K^+-$2Cl^-$ cotransporter is present in BBB endothelial cells and is stimulated by factors in such way that increased secretion of NaCl and water in the brain, contributing to oedema formation. Other reports suggested that increased luminal activity of Na^+-K^+-$2Cl^-$ cotransporter in BBB-NVU containing ECs contributes to brain oedema [117]. Furthermore, inhibition of Na^+-K^+-$2Cl^-$ cotransporter decreases oedema and infarct volume in a rodent model of transient focal cerebral ischemia [117]. TJs are dynamic protein complexes made up of occludins, claudin (1, 3, 5), membrane-associated guanylate kinase-like proteins (ZO-1, ZO-2 and ZO-3) and junctional adhesion molecules (JAMs) which contribute BBB permeability. The ROS generation resulting oxidative stress alters the molecular expression and functional organization of TJ proteins leading to the increased paracellular leakage at BBB. The molecular rearrangement of TJ proteins across the BBB following ischemia facilitates the significant flow of vascular fluid across the microvascular BBB [118].

BBB disturbances have been associated with hypoxic situations that happen with ischemic stroke [50, 82, 118]. Hypoxic and posthypoxic reoxygenation in cerebromicrovasculature was performed using primary BBMEC. The results corroborated that hypoxia-induced molecular alteration in paracellular BBB permeability may be due to change in TJs protein expression. Also, decreased occludin expression is associated with elevated BBB permeability and its trafficking continuously from TJ protein complexes due to ischemic injury. TEMPOL, a ROS scavenging antioxidant that can easily cross BBB, inhibits the occludin relocalization at BBB during inflammatory pain [119, 120]. TEMPOL is a key modulator of disulphide bond

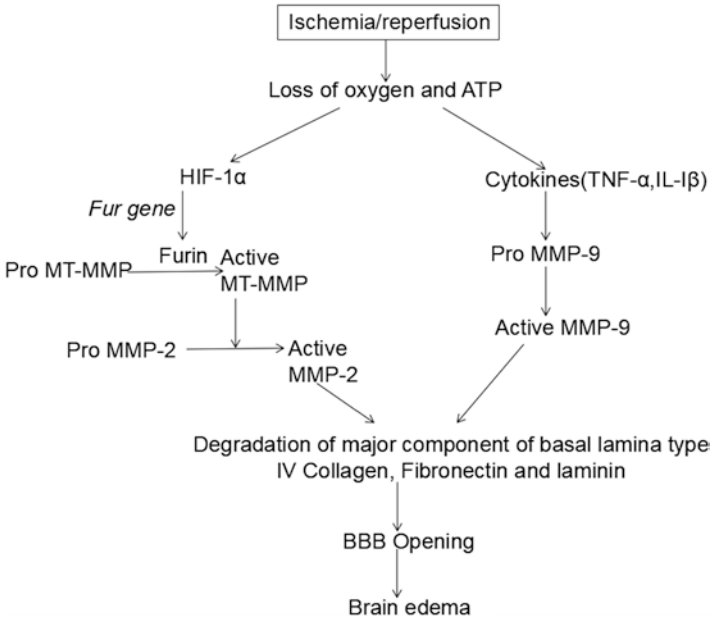

Fig. 3.3 Schematic illustration of BBB opening after ischemia/reperfusion injury

formation in occludin oligomeric assemblies and inhibition of occludin oligomeric assemblies. However, the previous report suggested that increase BBB permeability during stroke involves TJ modification following transcellular transport pathways [121] (Fig. 3.3).

The enhanced BBB permeability has been observed at different time points after ischemic insult in middle cerebral artery occlusion, an experimental stroke rodent model. However, BBB opening in stroke patients has been reported only during early reperfusion. The regional BBB opening has been observed in focal ischemic stroke rodent model. Also, there is a difference in BBB opening between an ipsilateral and contralateral region of the brain. The depletion of astrocytes in both necrotic umbra and apoptotic penumbra region contributes to enhance BBB splitting in ischemic brain injury. And, an association of pericytes with endothelial cells is the important functional integration, and loss of this association may lead to leakage of BBB resulting oedema development. Pharmacological interventions provide cytoprotective mechanism for NVU within penumbra that stop worsening of brain tissue damage during cerebral I/R injury. The exposure of pro-inflammatory cytokines (IFN-$^{\gamma}$, TNF-α and IL-1β) on endothelium regulated the BBB integrity [113]. BBB opening is well accepted as an early consequence in ischemic stroke patients with magnetic resonance imaging (MRI) [114].

3.7 Evaluation of BBB Disruption in Rodent Ischemic Stroke

BBB integrity impairments are the critical issue in many neurodegenerative diseases. Evans blue (EB) is an intravital dye that binds to albumin and can therefore be used to estimate extravasation of albumin-EB complexes across BBB. EB-protein complexes can be estimated by various methods such as spectrophotometric absorbance at 610 nm and also ultrahigh-performance liquid chromatography (UHPLC). EB in ischemic brain tissue could give validated explanation for novel therapeutic interventions. The above protocol can be performed in any laboratory without need of any specific instruments and produces high quality reproducible data [122].

3.8 Quantitative Evaluation of BBB in Ischemic Stroke Using Dynamic Contrast-Enhanced (DCE) MRI

Dynamic contrast-enhanced (DCE) MRI enables the measurement of BBB permeability by analysing the temporal enhancement pattern after contrast agent (CA) administration. The obtained signal is used to generate intensity curve, which is related to vessel permeability and surface area [123].

3.9 Conclusion

The BBB disruption is the result of various CNS disorders including stroke, and their repair is a great task in the coming future. Biphasic BBB disruption can facilitate dynamic maintenance and restoration of the neurovascular niche components. However, we do not know the key molecular mediators that lead to BBB recovery or those that lead to BBB disruption. From the repair aspect, we have mounting evidence of neovascularization mechanisms occurring after stroke. Additionally, several reports show either an early absence or delayed neovascularization or even a disappearance of vasculature around the site of injury after stroke.

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References

1. Lewandowsky, M. (1900). Zur lehre von der cerebrospinalflussigkeit. *Zeitschrift für Klinische Medizin, 40*(480), 19.

2. Goldmann, E. E. (1909). Diessere und innere Sekretion des gesunden und kranken Organismus m Lichte der 'vitalen Farbung'. *Beiträge zur Klinischen Chirurgie, 64*, 192–265.
3. Goldmann, E. E. (1913). Vitalfarbung am Zentralnervensystem. *Abhandlungan Preussischen Akademii Wissenschaften Physics-Mathematics, 1*, 1–60.
4. Zlokovic, B. V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron, 57*, 178–201.
5. Reese, T. S., & Karnovsky, M. J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *The Journal of Cell Biology, 34*(1), 207–217.
6. Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., Das, S. R., de Ferranti, S., Despres, J. P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Isasi, C. R., Jimenez, M. C., Judd, S. E., Kissela, B. M., Lichtman, J. H., Lisabeth, L. D., Liu, S., Mackey, R. H., Magid, D. J., McGuire, D. K., Mohler, E. R., 3rd, Moy, C. S., Muntner, P., Mussolino, M. E., Nasir, K., Neumar, R. W., Nichol, G., Palaniappan, L., Pandey, D. K., Reeves, M. J., Rodriguez, C. J., Rosamond, W., Sorlie, P. D., Stein, J., Towfighi, A., Turan, T. N., Virani, S. S., Woo, D., Yeh, R. W., Turner, M. B., American Heart Association Statistic, C, & Stroke Statistics, S. (2016). Heart disease and stroke statistics-2016 update: A report from the American Heart Association. *Circulation, 133*, e38–e360.
7. Keaney, J., & Campbell, M. (2015). The dynamic blood-brain barrier. *The FEBS Journal, 282*, 4067–4079.
8. Petty, M. A., & Wettstein, J. G. (2001). Elements of cerebral microvascular ischaemia. *Brain Research Reviews, 36*(1), 23–34.
9. Oldendorf, W. H., Cornford, M. E., & Brown, W. J. (1977). The large apparent work capability of the blood-brain barrier: A study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Annals of Neurology, 1*(5), 409–417.
10. Fenstermacher, J., Gross, P., Sposito, N., Acuff, V., Pettersen, S., & Gruber, K. (1988). Structural and functional variations in capillary systems within the brain. *Annals of the New York Academy of Sciences, 529*(1), 21–30.
11. Sedlakova, R., Shivers, R. R., & Del Maestro, R. F. (1999). Ultrastructure of the blood-brain barrier in the rabbit. *Journal of Submicroscopic Cytology and Pathology, 31*(1), 149–161.
12. Klingler, C., Kniesel, U., Bamforth, S. D., Wolburg, H., Engelhardt, B., & Risau, W. (2000). Disruption of epithelial tight junctions is prevented by cyclic nucleotide-dependent protein kinase inhibitors. *Histochemistry and Cell Biology, 113*(5), 349–361.
13. Tagami, M., Nara, Y., Kubota, A., Fujino, H., & Yamori, Y. (1990). Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. *Stroke, 21*(7), 1064–1071.
14. Bandopadhyay, R., Orte, C., Lawrenson, J. G., Reid, A. R., De Silva, S., & Allt, G. (2001). Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *Journal of Neurocytology, 30*(1), 35–44.
15. Gonul, E., Duz, B., Kahraman, S., Kayali, H., Kubar, A., & Timurkaynak, E. (2002). Early pericyte response to brain hypoxia in cats: An ultrastructural study. *Microvascular Research, 64*(1), 116–119.
16. Dore-Duffy, P., Owen, C., Balabanov, R., Murphy, S., Beaumont, T., & Rafols, J. A. (2000). Pericyte migration from the vascular wall in response to traumatic brain injury. *Microvascular Research, 60*(1), 55–69.
17. Hori, S., Ohtsuki, S., Hosoya, K. I., Nakashima, E., & Terasaki, T. (2004). A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *Journal of Neurochemistry, 89*(2), 503–513.
18. Sofroniew, M. V. (2015). Astrocyte barriers to neurotoxic inflammation. Nature reviews. *Neuroscience, 16*(5), 249.
19. Ballabh, P., Braun, A., & Nedergaard, M. (2004). The blood–brain barrier: An overview: Structure, regulation, and clinical implications. *Neurobiology of Disease, 16*(1), 1–13.
20. Braet, K., Paemeleire, K., D'herde, K., Sanderson, M. J., & Leybaert, L. (2001). Astrocyte–endothelial cell calcium signals conveyed by two signalling pathways. *European Journal of Neuroscience, 13*(1), 79–91.

21. Zonta, M., Angulo, M. C., Gobbo, S., Rosengarten, B., Hossmann, K. A., Pozzan, T., & Carmignoto, G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nature Neuroscience, 6*(1), 43–50.
22. Asgari, N., Berg, C. T., Mørch, M., Khorooshi, R. M., & Owens, T. (2016). Cerebrospinal fluid aquaporin-4-immunoglobulin G induces blood brain barrier breakdown. *Annals of Clinical and Translational Neurology*.
23. Hatashita, S., & Hoff, J. T. (1990). Brain edema and cerebrovascular permeability during cerebral ischemia in rats. *Stroke, 21*(4), 582–588.
24. Lee, E. J., Hung, Y. C., & Lee, M. Y. (1999). Early alterations in cerebral hemodynamics, brain metabolism, and blood-brain barrier permeability in experimental intracerebral hemorrhage. *Journal of Neurosurgery, 91*(6), 1013–1019.
25. Ben-Menachem, E., Johansson, B. B., & Svensson, T. H. (1982). Increased vulnerability of the blood-brain barrier to acute hypertension following depletion of brain noradrenaline. *Journal of Neural Transmission, 53*(2), 159–167.
26. Cohen, Z., Molinatti, G., & Hamel, E. (1997). Astroglial and vascular interactions of noradrenaline terminals in the rat cerebral cortex. *Journal of Cerebral Blood Flow & Metabolism, 17*(8), 894–904.
27. Cohen, Z. V. I., Bonvento, G., Lacombe, P., & Hamel, E. (1996). Serotonin in the regulation of brain microcirculation. *Progress in Neurobiology, 50*(4), 335–362.
28. Vaucher, E., & Hamel, E. (1995). Cholinergic basal forebrain neurons project to cortical microvessels in the rat: Electron microscopic study with anterogradely transported *Phaseolus vulgaris* leucoagglutinin and choline acetyltransferase immunocytochemistry. *Journal of Neuroscience, 15*(11), 7427–7441.
29. Tong, X. K., & Hamel, E. (1999). Regional cholinergic denervation of cortical microvessels and nitric oxide synthase-containing neurons in Alzheimer's disease. *Neuroscience, 92*(1), 163–175.
30. Vaucher, E., Tong, X. K., Cholet, N., Lantin, S., & Hamel, E. (2000). GABA neurons provide a rich input to microvessels but not nitric oxide neurons in the rat cerebral cortex: A means for direct regulation of local cerebral blood flow. *Journal of Comparative Neurology, 421*(2), 161–171.
31. Kobayashi, H., Magnoni, M. S., Govoni, S., Izumi, F., Wada, A., & Trabucchi, M. (1985). Neuronal control of brain microvessel function. *Experientia, 41*(4), 427–434.
32. Rosenberg, G. A., Estrada, E., Kelley, R. O., & Kornfeld, M. (1993). Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neuroscience Letters, 160*(1), 117–119.
33. Rascher, G., Fischmann, A., Kröger, S., Duffner, F., Grote, E. H., & Wolburg, H. (2002). Extracellular matrix and the blood-brain barrier in glioblastoma multiforme: Spatial segregation of tenascin and agrin. *Acta Neuropathologica, 104*(1), 85–91.
34. Hynes, R. O. (1992). Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell, 69*(1), 11–25.
35. Tilling, T., Korte, D., Hoheisel, D., & Galla, H. J. (1998). Basement membrane proteins influence brain capillary endothelial barrier function in vitro. *Journal of Neurochemistry, 71*(3), 1151–1157.
36. Savettieri, G., Di Liegro, I., Catania, C., Licata, L., Pitarresi, G. L., D'agostino, S., Schiera, G., De Caro, V., Giandalia, G., Giannola, L. I., & Cestelli, A. (2000). Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport, 11*(5), 1081–1084.
37. Schulze, C., & Firth, J. A. (1993). Immunohistochemical localization of adherens junction components in blood-brain barrier microvessels of the rat. *Journal of Cell Science, 104*(3), 773–782.
38. Wolburg, H., & Lippoldt, A. (2002). Tight junctions of the blood–brain barrier: Development, composition and regulation. *Vascular Pharmacology, 38*(6), 323–337.
39. Bazzoni, G., & Dejana, E. (2004). Endothelial cell-to-cell junctions: Molecular organization and role in vascular homeostasis. *Physiological Reviews, 84*(3), 869–901.

40. Brown, R. C., & Davis, T. P. (2002). Calcium modulation of adherens and tight junction function. *Stroke, 33*(6), 1706–1711.
41. Vorbrodt, A. W., & Dobrogowska, D. H. (2003). Molecular anatomy of intercellular junctions in brain endothelial and epithelial barriers: Electron microscopist's view. *Brain Research Reviews, 42*(3), 221–242.
42. Van Meer, G., & Simons, K. (1986). The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *The EMBO Journal, 5*, 1455–1464.
43. Nag, S. (2003). Morphology and molecular properties of cellular components of normal cerebral vessels. *Methods in Molecular Medicine, 89*, 3–36.
44. Del Maschio, A., De Luigi, A., Martin-Padura, I., Brockhaus, M., Bartfai, T., Fruscella, P., et al. (1999). Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhibited by an antibody to junctional adhesion molecule (JAM). *The Journal of Experimental Medicine, 190*, 1351–1356.
45. Tao-Cheng, J. H., Nagy, Z., & Brightman, M. W. (1987). Tight junctions of brain endothelium in vitro are enhanced by astroglia. *Journal of Neuroscience, 7*(10), 3293–3299.
46. Kojima, T., Yamamoto, T., Murata, M., Chiba, H., Kokai, Y., & Sawada, N. (2003). Regulation of the blood–biliary barrier: Interaction between gap and tight junctions in hepatocytes. *Medical Electron Microscopy, 36*(3), 157–164.
47. Simard, M., Arcuino, G., Takano, T., Liu, Q. S., & Nedergaard, M. (2003). Signaling at the gliovascular interface. *Journal of Neuroscience, 23*(27), 9254–9262.
48. Lampugnani, M. G., Corada, M., Caveda, L., Breviario, F., Ayalon, O., Geiger, B., et al. (1995). The molecular organization of endothelial cell to cell junctions: Differential association of plakoglobin, beta-catenin, and alpha-catenin with vascular endothelial cadherin (VE-cadherin). *The Journal of Cell Biology, 129*, 203–217.
49. Watabe-Uchida, M., Uchida, N., Imamura, Y., Nagafuchi, A., Fujimoto, K., Uemura, T., et al. (1998). alpha-Catenin-vinculin interaction functions to organize the apical junctional complex in epithelial cells. *Journal of Cellular Biochemistry, 142*, 847–857.
50. Abbruscato, T. J., & Davis, T. P. (1998). Protein expression of brain endothelial cell E-cadherin after hypoxia/aglycemia: Influence of astrocyte contact. *Brain Research, 842*, 277–286.
51. Farquhar, M. G., & Palade, G. E. (1963). Junctional complexes in various epithelia. *The Journal of Cell Biology, 17*, 375–412.
52. Furuse, M., Hirase, T., Itoh, M., Nagafuchi, A., Yonemura, S., Tsukita, S., et al. (1993). Occludin: A novel integral membrane protein localizing at tight junctions. *Journal of Cellular Biochemistry, 123*, 1777–1788.
53. Furuse, M., Fujita, K., Hiiragi, T., Fujimoto, K., & Tsukita, S. (1998). Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *The Journal of Cell Biology, 141*, 1539.
54. Morita, K., Sasaki, H., Fujimoto, K., Furuse, M., & Tsukita, S. (1999). Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis. *The Journal of Cell Biology, 145*, 579–588.
55. Liebner, S., Fischmann, A., Rascher, G., Duffner, F., Grote, E.-H., Kalbacher, H., et al. (2000). Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathologica, 100*, 323–331.
56. Liebner, S., Kniesel, U., Kalbacher, H., & Wolburg, H. (2000). Correlation of tight junction morphology with the expression of tight junction proteins in blood-brain barrier endothelial cells. *European Journal of Cell Biology, 79*, 707–717.
57. Lippoldt, A., Kniesel, U., Liebner, S., Kalbacher, H., Kirsch, T., Wolburg, H., et al. (2000). Structural alterations of tight junctions are associated with loss of polarity in stroke-prone spontaneously hypertensive rat blood-brain barrier endothelial cells. *Brain Research, 885*, 251–261.

58. Hirase, T., Staddon, J. M., Saitou, M., Ando-Akatsuka, Y., Itoh, M., Furuse, M., et al. (1997). Occludin as a possible determinant of tight junction permeability in endothelial cells. *Journal of Cell Science, 110*(Pt 14), 1603–1613.
59. Dejana, E., Lampugnani, M. G., Martinez-Estrada, O., & Bazzoni, G. (2000). The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability. *The International Journal of Developmental Biology, 44*, 743–748.
60. Jia, W., Martin, T. A., Zhang, G., & Jiang, W. G. (2013). Junctional adhesion molecules in cerebral endothelial tight junction and brain metastasis. *Anticancer Research, 33*(6), 2353–2359.
61. Bauer, H. C., & Traweger Auer, H. (2004). 1-proteins of the tight junction in the blood-brain barrier. In *Blood-spinal cord brain barriers health disease* (pp. 1–10). San Diego: Academic.
62. Ando-Akatsuka, Y., Saitou, M., Hirase, T., Kishi, M., Sakakibara, A., Itoh, M., et al. (1996). Interspecies diversity of the occludin sequence: cDNA cloning of human, mouse, dog, and rat-kangaroo homologues. *The Journal of Cell Biology, 133*, 43–47.
63. Furuse, M., Sasaki, H., & Tsukita, S. (1999). Manner of interaction of heterogeneous claudin species within and between tight junction strands. *The Journal of Cell Biology, 147*, 891–903.
64. McCarthy, K. M., Skare, I. B., Stankewich, M. C., Furuse, M., Tsukita, S., Rogers, R. A., et al. (1996). Occludin is a functional component of the tight junction. *Journal of Cell Science, 109*(Pt 9), 2287–2298.
65. Balda, M. S., Whitney, J. A., Flores, C., Gonzalez, S., Cereijido, M., & Matter, K. (1996). Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *The Journal of Cell Biology, 134*, 1031–1049.
66. Matter, K., & Balda, M. S. (2003). Signalling to and from tight junctions. *Nature Reviews. Molecular Cell Biology* 2003*, 4*, 225–236.
67. Sonoda, N., Furuse, M., Sasaki, H., Yonemura, S., Katahira, J., Horiguchi, Y., et al. (1999). *Clostridium perfringens* enterotoxin fragment removes specific claudins from tight junction strands: Evidence for direct involvement of claudins in tight junction barrier. *The Journal of Cell Biology, 147*, 195–204.
68. Bolton, S. J., Anthony, D. C., & Perry, V. H. (1998). Loss of the tight junction proteins occludin and zonula occludens-1 from cerebral vascular endothelium during neutrophil-induced blood-brain barrier breakdown in vivo. *Neuroscience, 86*, 1245–1257.
69. Huber, J. D., Hau, V. S., Borg, L., Campos, C. R., Egleton, R. D., & Davis, T. P. (2002). Blood-brain barrier tight junctions are altered during a 72-h exposure to lambda-carrageenan-induced inflammatory pain. *American Journal of Physiology. Heart and Circulatory Physiology, 283*, H1531–H1537. https://doi.org/10.1152/ajpheart.00027.2002.
70. Brown, R. C., & Davis, T. P. (2005). Hypoxia/aglycemia alters expression of occludin and actin in brain endothelial cells. *Biochemical and Biophysical Research Communications, 327*, 1114–1123.
71. Heiskala, M., Peterson, P. A., & Yang, Y. (2001). The roles of claudin superfamily proteins in paracellular transport. *Traffic, 2*, 93–98.
72. Tam, S. J., & Watts, R. J. (2010). Connecting vascular and nervous system development: Angiogenesis and the blood-brain barrier. *Annual Review of Neuroscience, 33*, 379–408.
73. Nitta, T., Hata, M., Gotoh, S., Seo, Y., Sasaki, H., Hashimoto, N., Furuse, M., & Tsukita, S. (2003). Size-selective loosening of the blood-brain barrier in claudin-5–deficient mice. *The Journal of Cell Biology, 161*(3), 653–660.
74. Kubota, K., Furuse, M., Sasaki, H., Sonoda, N., Fujita, K., Nagafuchi, A., et al. (1999). Ca(2+)-independent cell-adhesion activity of claudins, a family of integral membrane proteins localized at tight junctions. *Current Biology: CB, 9*, 1035–1038.
75. Shin, H., Hsueh, Y. P., Yang, F. C., Kim, E., & Sheng, M. (2000). An intramolecular interaction between Src homology 3 domain and guanylate kinase-like domain required for channel clustering by postsynaptic density-95/SAP90. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 20*, 3580–3587.

76. Stevenson, B. R., Siliciano, J. D., Mooseker, M. S., & Goodenough, D. A. (1986). Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. *The Journal of Cell Biology, 103*, 755–766.
77. Howarth, A. G., Hughes, M. R., & Stevenson, B. R. (1992). Detection of the tight junction-associated protein ZO-1 in astrocytes and other nonepithelial cell types. *The American Journal of Physiology, 262*, C461–C469.
78. Islas, S., Vega, J., Ponce, L., & Gonzalez-Mariscal, L. (2002). Nuclear localization of the tight junction protein ZO-2 in epithelial cells. *Experimental Cell Research, 274*, 138–148. https://doi.org/10.1006/excr.2001.5457.
79. Traweger, A., Fuchs, R., Krizbai, I. A., Weiger, T. M., Bauer, H.-C., & Bauer, H. (2003). The tight junction protein ZO-2 localizes to the nucleus and interacts with the heterogeneous nuclear ribonucleoprotein scaffold attachment factor-B. *The Journal of Biological Chemistry, 278*, 2692–2700.
80. Abbruscato, T. J., Lopez, S. P., Mark, K. S., Hawkins, B. T., & Davis, T. P. (2002). Nicotine and cotinine modulate cerebral microvascular permeability and protein expression of ZO-1 through nicotinic acetylcholine receptors expressed on brain endothelial cells. *Journal of Pharmaceutical Sciences, 91*, 2525–2538. https://doi.org/10.1002/jps.10256.
81. Fischer, S., Wobben, M., Marti, H. H., Renz, D., & Schaper, W. (2002). Hypoxia-induced hyperpermeability in brain microvessel endothelial cells involves. *Microvascular Research, 63*, 70–80.
82. Mark, K. S., & Davis, T. P. (2002). Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *American Journal of Physiology - Heart and Circulatory Physiology, 282*, H1485.
83. Gottardi, C. J., Arpin, M., Fanning, A. S., & Louvard, D. (1996). The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. *Proceedings of the National Academy of Sciences, 93*, 10779–10784.
84. Riesen, F. K., Rothen-Rutishauser, B., & Wunderli-Allenspach, H. (2002). A ZO1-GFP fusion protein to study the dynamics of tight junctions in living cells. *Histochemistry and Cell Biology, 117*, 307–315.
85. Hawkins, B. T., Abbruscato, T. J., Egleton, R. D., Brown, R. C., Huber, J. D., Campos, C. R., et al. (2004). Nicotine increases in vivo blood-brain barrier permeability and alters cerebral microvascular tight junction protein distribution. *Brain Research, 1027*, 48–58.
86. Balda, M. S., & Matter, K. (2000). The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. *The EMBO Journal, 19*, 2024.
87. Meyer, T. N., Schwesinger, C., & Denker, B. M. (2002). Zonula occludens-1 is a scaffolding protein for signaling molecules: Gα12 directly binds to the src homology 3 domain and regulates paracellular permeability in epithelial cells. *The Journal of Biological Chemistry, 277*, 24855–24858.
88. Gumbiner, B., Lowenkopf, T., & Apatira, D. (1991). Identification of a 160-kDa polypeptide that binds to the tight junction protein ZO-1. *Proceedings of the National Academy of Sciences, 88*, 3460–3464.
89. Betanzos, A., Huerta, M., Lopez-Bayghen, E., Azuara, E., Amerena, J., & Gonzalez-Mariscal, L. (2004). The tight junction protein ZO-2 associates with Jun, Fos and C/EBP transcription factors in epithelial cells. *Experimental Cell Research, 292*, 51–66.
90. Umeda, K., Matsui, T., Nakayama, M., Furuse, K., Sasaki, H., Furuse, M., et al. (2004). Establishment and characterization of cultured epithelial cells lacking expression of ZO-1. *The Journal of Biological Chemistry, 279*, 44785–44794.
91. Inoko, A., Itoh, M., Tamura, A., Matsuda, M., Furuse, M., & Tsukita, S. (2003). Expression and distribution of ZO-3, a tight junction MAGUK protein, in mouse tissues. *Genes to Cells: Devoted to Molecular & Cellular Mechanisms, 8*, 837–845.
92. Yamamoto, T., Harada, N., Kawano, Y., Taya, S., & Kaibuchi, K. (1999). In vivo interaction of AF-6 with activated Ras and ZO-1. *Biochemical and Biophysical Research Communications, 259*, 103–107.

93. Zhong, Y., Enomoto, K., Tobioka, H., Konishi, Y., Satoh, M., & Mori, M. (1994). Sequential decrease in tight junctions as revealed by 7H6 tight junction-associated protein during rat hepatocarcinogenesis. *Japanese Journal of Cancer Research, 85*, 351–356.
94. Fischer, S., Wiesnet, M., Marti, H. H., Renz, D., & Schaper, W. (2004). Simultaneous activation of several second messengers in hypoxia-induced hyperpermeability of brain derived endothelial cells. *Journal of Cellular Physiology, 198*(3), 359–369.
95. Thomas, F. C., Sheth, B., Eckert, J. J., Bazzoni, G., Dejana, E., & Fleming, T. P. (2004). Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo. *Journal of Cell Science, 117*(23), 5599–5608.
96. Nagy, Z., Szabo, M., & Hüttner, I. (1985). Blood-brain barrier impairment by low pH buffer perfusion via the internal carotid artery in rat. *Acta Neuropathologica, 68*(2), 160–163.
97. Abbott, N. J., & Revest, P. A. (1991). Control of brain endothelial permeability. *Cerebrovascular and Brain Metabolism Reviews, 3*(1), 39–72.
98. Balda, M. S., Gonzalez-Mariscal, L., Matter, K., Cereijido, M., & Anderson, J. M. (1993). Assembly of the tight junction: The role of diacylglycerol. *The Journal of Cell Biology, 123*(2), 293–302.
99. Stewart, M. P., Cabanas, C., & Hogg, N. (1996). T cell adhesion to intercellular adhesion molecule-1 (ICAM-1) is controlled by cell spreading and the activation of integrin LFA-1. *The Journal of Immunology, 156*(5), 1810–1817.
100. Pun, P. B., Lu, J. I., & Moochhala, S. (2009). Involvement of ROS in BBB dysfunction. *Free Radical Research, 43*(4), 348–364.
101. Hawkins, B. T., & Davis, T. P. (2005). The blood-brain barrier/neurovascular unit in health and disease. *Pharmacological Reviews, 57*(2), 173–185.
102. Staddon, J. M., Herrenknecht, K., Smales, C., & Rubin, L. L. (1995). Evidence that tyrosine phosphorylation may increase tight junction permeability. *Journal of Cell Science, 108*(2), 609–619.
103. Morita, K., Sakakibara, A., Kitayama, S., Kumagai, K., Tanne, K., & Dohi, T. (2002). Pituitary adenylate cyclase-activating polypeptide induces a sustained increase in intracellular free Ca2+ concentration and catecholamine release by activating Ca2+ influx via receptor-stimulated Ca2+ entry, independent of store-operated Ca2+ channels, and voltage-dependent Ca2+ channels in bovine adrenal medullary chromaffin cells. *Journal of Pharmacology and Experimental Therapeutics, 302*(3), 972–982.
104. Brown, R. C., Morris, A. P., & O'Neil, R. G. (2007). Tight junction protein expression and barrier properties of immortalized mouse brain microvessel endothelial cells. *Brain Research, 1130*, 17–30.
105. Farshori, P., & Kachar, B. (1999). Redistribution and phosphorylation of occludin during opening and resealing of tight junctions in cultured epithelial cells. *The Journal of Membrane Biology, 170*(2), 147–156.
106. Tsukamoto, T., & Nigam, S. K. (1999). Role of tyrosine phosphorylation in the reassembly of occludin and other tight junction proteins. *American Journal of Physiology-Renal Physiology, 276*(5), F737–F750.
107. Ishizaki, T., Chiba, H., Kojima, T., Fujibe, M., Soma, T., Miyajima, H., Nagasawa, K., Wada, I., & Sawada, N. (2003). Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood–brain-barrier endothelial cells via protein kinase A-dependent and-independent pathways. *Experimental Cell Research, 290*(2), 275–288.
108. Stevenson, M. H., & Gray, R. (1989). Effect of irradiation dose, storage time and temperature on the ESR signal in irradiated chicken bone. *Journal of the Science of Food and Agriculture, 48*(3), 269–274.
109. Kurihara, H. I., Anderson, J. M., & Farquhar, M. G. (1995). Increased Tyr phosphorylation of ZO-1 during modification of tight junctions between glomerular foot processes. *American Journal of Physiology-Renal Physiology, 268*(3), F514–F524.

110. Sakakibara, A., Furuse, M., Saitou, M., Ando-Akatsuka, Y., & Tsukita, S. (1997). Possible involvement of phosphorylation of occludin in tight junction formation. *The Journal of Cell Biology, 137*(6), 1393–1401.
111. Ilzecka, J. (1996). The structure and function of blood-brain barrier in ischaemic brain stroke process. In *Annales Universitatis Mariae Curie-Sklodowska. Sectio D: Medicina* (Vol. 51, pp. 123–127). Lublin: Uniwersytet Marii Curie-Skłodowskiej.
112. Morganti-Kossmann, M. C., Rancan, M., Stahel, P. F., & Kossmann, T. (2002). Inflammatory response in acute traumatic brain injury: A double-edged sword. *Current Opinion in Critical Care, 8*(2), 101–105.
113. Minagar, A., & Alexander, J. S. (2003). Blood-brain barrier disruption in multiple sclerosis. *Multiple Sclerosis Journal, 9*(6), 540–549.
114. Floris, S., Blezer, E. L., Schreibelt, G., Döpp, E., Van der Pol, S. M., Schadee-Eestermans, I. L., Nicolay, K., Dijkstra, C. D., & De Vries, H. E. (2004). Blood–brain barrier permeability and monocyte infiltration in experimental allergic encephalomyelitis: A quantitative MRI study. *Brain, 127*(3), 616–627.
115. Wardlaw, J. M., Sandercock, P. A., Dennis, M. S., & Starr, J. (2003). Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? *Stroke, 34*(3), 806–812.
116. Del Zoppo, G. J., & Hallenbeck, J. M. (2000). Advances in the vascular pathophysiology of ischemic stroke. *Thrombosis Research, 98*(3), 73–81.
117. O'Donnell, M. E., Lam, T. I., Tran, L., & Anderson, S. E. (2004). The role of the blood-brain barrier Na-K-2Cl cotransporter in stroke. In *Cell volume and signaling* (pp. 67–75). Boston: Springer.
118. Heo, J. H., Han, S. W., & Lee, S. K. (2005). Free radicals as triggers of brain edema formation after stroke. *Free Radical Biology & Medicine, 39*(1), 51–70.
119. Lochhead, J. J., McCaffrey, G., Sanchez-Covarrubias, L., Finch, J. D., DeMarco, K. M., Quigley, C. E., Davis, T. P., & Ronaldson, P. T. (2011). Tempol modulates changes in xenobiotic permeability and occludin oligomeric assemblies at the blood-brain barrier during inflammatory pain. *American Journal of Physiology-Heart and Circulatory Physiology, 302*(3), H582–H593.
120. Cuzzocrea, S., McDonald, M. C., Mazzon, E., Siriwardena, D., Costantino, G., Fulia, F., Cucinotta, G., Gitto, E., Cordaro, S., Barberi, I., & De Sarro, A. (2000). Effects of tempol, a membrane-permeable radical scavenger, in a gerbil model of brain injury. *Brain Research, 875*(1–2), 96–106.
121. Stamatovic, S. M., Keep, R. F., & Andjelkovic, A. V. (2008). Brain endothelial cell-cell junctions: How to "open" the blood brain barrier. *Current Neuropharmacology, 6*(3), 179–192.
122. Panahpour, H., Farhoudi, M., Omidi, Y., & Mahmoudi, J. (2018). An in vivo assessment of blood-brain barrier disruption in a rat model of ischemic stroke. *Journal of Visualized Experiments*, (133).
123. Gordon, Y., Partovi, S., Müller-Eschner, M., Amarteifio, E., Bäuerle, T., Weber, M. A., Kauczor, H. U., & Rengier, F. (2014). Dynamic contrast-enhanced magnetic resonance imaging: Fundamentals and application to the evaluation of the peripheral perfusion. *Cardiovascular Diagnosis and Therapy, 4*(2), 147.

Chapter 4
Ischemic Stroke-Induced Endoplasmic Reticulum Stress

Namrata Rastogi and Vikas Kumar Srivastava

Abstract Endoplasmic reticulum (ER) stress is a complex cellular mechanism that is induced by the accumulation of misfolded proteins under various stress stimuli. ER stress is implicated in various pathological conditions, including cerebral ischemia. In cerebral ischemia, ER stress is presumed to be an early event and pro-survival. However, its precise involvement in ischemia/reperfusion (IR) injury is still contentious and under investigation. Mechanistically, ER stress and its associated unfolded protein response is presumed to be a defensive mechanism, which, when it becomes chronic, has fatal effects on neuronal survival and outcomes of stroke patients. Recent investigations have presented interesting contributions of autophagy to cerebral ER stress. As an innate process, autophagy is a defensive process for neuronal cell survival, but when triggered by chronic ER stress, it becomes destructive and induces cell death. Moreover, other factors, such as small non-coding microRNAs (miRs), have also been shown to regulate both of these processes through their gene expression regulatory properties. However, it is still very important to understand the interrelationship between ER stress and autophagy in IR injury following cerebral stroke so as to define its therapeutic significance. Furthermore, we need to approach combined therapies using ER stress and autophagy inhibitors with the intent to improve current treatments for cerebral stroke.

Keywords Cerebral ischemia · IR injury · ER stress · Autophagy and microRNAs

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Abbreviations

ASK1	Apoptosis signal-regulating kinase 1
ATF/CREB	Activating transcription factor/cyclic AMP response element binding protein
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
bcl-2	B-cell lymphoma-2
BIP	Binding immunoglobulin protein
CHOP	CCAAT/enhancer binding protein homologous protein
COX-2	Cyclooxygenase-2
CRE	cAMP-response element
CREB	cAMP-response element binding factor
ER	Endoplasmic reticulum
ERSE	ER stress-response elements
GADD34	Growth arrest and DNA damage-inducible gene/protein 34
GRP78	Glucose-regulated protein 78
IR	Ischemia/reperfusion
IRE1	Inositol-requiring enzyme-1
JNK	c-Jun N-terminal kinase
MCAO	middle cerebral artery occlusion
MAPKs	Mitogen-activated protein kinases
miR	microRNA
nNOS	Neuronal NOS
NOS	Nitric oxide synthase
OGD	Oxygen and glucose deprivation
p38 MAPK	p38 mitogen-activated protein kinase
PERK	Double-stranded RNA-dependent protein kinase-like ER kinase
PSI	Protein synthesis inhibition
TRAF2	Tumor necrosis factor receptor-associated factor 2
UPR	Unfolded protein response
XBP1	X-box-binding protein 1

4.1 Introduction

Cerebral stroke is a pathological condition whereby normal blood flow to a specified part of the brain is obstructed, depriving it of nutrients and oxygen supply. As a consequence, the brain cells in the affected area succumb to death in a very small time frame, narrowing the therapeutic window [1]. Of the two major causes of cerebral stroke, ischemia accounts for more than 85% of incidences, followed by hemorrhagic stroke. In cerebral ischemia, the blood in general is interrupted by cerebral artery occlusion due to atherosclerosis resulting from the formation of a cholesterol plug [2]. Massive destruction is not caused by the ischemia, but instead results from

the restoration of normal blood flow, often termed ischemia/reperfusion (IR) injury. Studies at the molecular level have shown different pro- and anti-survival mechanisms and signaling pathways to be deregulated under conditions of IR injury, among which endoplasmic reticulum (ER) stress is significant [3].

The ER is a versatile and dynamic cellular organelle that is profoundly involved in the gluconeogenesis, the synthesis of protein lipids, and the proper folding of nascent proteins in the cell. The extended tubular structure of ER forms an important secretory network of recycling and trafficking of proteins within and outside the cells. It is also involved in the biogenesis of autophagosomes, peroxisomes, and protein transport vesicles. ER is also the biggest reservoir of Ca^{+} ions in the cells, which is an important secondary messenger to several signaling pathways. To ensure the fidelity of the system, ER also has a quality control checkpoint where proteins that have been misfolded are eliminated by the ER-associated protein degradation system through ubiquitination and proteasomal digestion. The protein processing in ER is a sensitive process and is affected by various stress stimuli including IR injury [4]. The perturbations in the normal functioning of the ER protein folding process, leading to accumulation of unfolded protein in the ER lumen, results in what is commonly known as ER stress. As it is evident in ER stress, cells activate combat mechanisms to reciprocate the protein folding stress situation and ensure normal protein functionality, i.e., the unfolded protein response (UPR). The UPR, upon sensing an unfolded protein load, signals the nucleus to stall further protein synthesis and activate cellular chaperones to clear the misfolded or unfolded proteins by triggering their degradation. If the ER stress in the cells is irreversible and beyond repair, the UPR leads to the elimination of these cells through apoptosis. ER stress and its complementary UPR have been studied in various physiological and pathological conditions and have therefore emerged as a pathway of great importance in drug discovery. After many years of thorough research it has been proposed that ER stress also plays a detrimental role in cerebral IR injury, whereby its inhibition can significantly improve fatal ischemia outcomes. In this chapter, we provide an extensive and updated overview of the mechanistic aspects of ER stress in cerebral ischemia and describe the current therapeutic approaches.

4.2 ER Stress, UPR, and Ischemia

Early investigations linking ER stress to cerebral ischemia were based on the observation that both processes lead to protein synthesis inhibition (PSI) [5]. Although for ER stress, it was evident that it is the depletion in the ER Ca^{2+} levels that causes a translation block, speculation was still unanswered with regard to IR injury [6]. Later studies showed that transient brain ischemia shunts the translation machinery at an early stage through inhibition of a very important initiation factor, eIF2, and the disaggregation of polyribosomes, events that are similar under conditions of ER stress [7]. These other exploratory studies also showed that there is significant

modulation of the expression of genes involved in the UPR, indicating that ischemia impairs ER function.

4.2.1 Components of UPR in Ischemia

Over several years of research, UPR is now considered to be a group of complex signaling networks that work in a well-coordinated manner to lessen the devastating effects of ER stress in cells. The UPR has three main branches: (i) activation and translocation of activating transcription factor (ATF) 6; (ii) autophosphorylation-mediated activation of inositol-requiring enzyme-1 (IRE1) and X-box binding protein-1 (XBP1) mRNA cleavage; and (iii) protein kinase RNA-like ER kinase (PERK)-mediated inhibition of eukaryotic initiation factor-2 (eIF2α) [8]. However, apart from these major players, there are other proteins such as glucose-related protein 78 (GRP78), CCAAT/enhancer binding protein homologous protein (CHOP), caspase 12, and c-Jun N-terminal kinase (JNK), which play a centralized role in UPR [9]. In this section, we discuss each of these components and their relative therapeutic potential in IR injury.

4.2.1.1 GRP78

Glucose-regulated protein 78 (GRP78) is an ER chaperone that is alternatively referred to as binding immunoglobulin protein (BIP) and as heat shock protein 5A. Accumulating evidence so far indicates that GRP78 is to neuroprotective under ER stress induction post IR injury. Under normal physiological conditions, GRP78 is known to bind to the luminal domains of all three significant ER stress-related proteins ATF6, PERK, and IRE1, and ensures proper protein homeostasis. Under protein stress, BIP binding is sequestered to the unfolded proteins in the ER lumen, thereby releasing UPR executers from its captivity [10]. Therefore, BIP accumulation in the ER lumen is considered to be the early onset marker for ER dysfunction and UPR activation. BIP ensures proper folding of the protein, otherwise the unfolded protein is further targeted toward proteasome-mediated degradation. Although BIP binding to PERK and IRE1 has been shown to inhibit their spontaneous self-dimerization and activation, BIP binding to ATF6 masks its Golgi localization signal where it is processed and activated [11]. Accumulation of BIP in the ER lumen is associated with the increased expression of pro-survival protein B-cell lymphoma (Bcl-2) protein [12]. However, one study also indicates that under severe ER stress conditions, BIP upregulates bcl-2 and activates mitochondrially mediated apoptosis rendering its precise role in ER stress debatable. Studies have shown that increased expression of BIP decreases bcl-2-associated X (BAX) and CHOP protein expression, and inhibits the interaction of bcl-2 interacting killer (BIK) with bcl-2, thereby significantly inhibiting induction of apoptosis [13, 14]. More recently, interaction of serine protease tissue-type plasminogen activator (tPA) with GRP78

located on the neuron cell surface has been shown to decrease PERK pathway activation. This decrease was associated with a reduction of the deleterious factor CHOP protein, proposing a novel molecular insight into GRP78-mediated neuroprotection [15]. Many neuroprotective agents have also been shown to work through the upregulation of GRP78 in ischemia, which again shows its therapeutic prominence in terms of cerebral ischemia.

4.2.1.2 PERK

Protein kinase R-like endoplasmic reticulum kinase (PERK) is an ER stress-sensing protein that is found on the surface of the ER membrane bound to BIP. PERK, along with IRE1, is among early sensors of UPR and is responsible for initiating PSI to lessen the ER burden by protein accumulation. Activation of PERK occurs through autophosphorylation and dimerization as soon as it becomes dissociated from BIP. The prime functional attributes of PERK is to induce the phosphorylation and inactivation of a major protein synthesis factor, eukaryotic initiation factor 2α (eIF2 α), and the activation of apoptotic signaling [16, 17]. Paradoxically, PERK-mediated phosphorylation of eIF2α further promotes translation of activating transcription factor 4 (ATF4), which has both pro-apoptotic and anti-apoptotic targets [18]. ATF4 is an activating transcription factor/cyclic AMP response element binding protein (ATF4/CREB) family of basic region-leucine zipper transcription factors that has consensus binding sites for cAMP-responsive elements (CRE). On the one hand, it forms a complex between its nuclear co-activator, the phosphorylated form of CREB1 and binds to the promoter region of GRP78, a pro-survival gene [19]. Alternatively, it increases the transcription of CHOP and growth arrest and DNA damage inducible gene/protein 34 (GADD34), which are pro-apoptotic factors in chronic ER stress and irreversible UPR [15, 16]. Phospho-PERK overexpression and inhibition have both been shown to be associated with ER-stressed neurons post-IR injury [20]. However, the religious use of PERK inhibitors in post-ischemia and the underlying mechanism is highly desired to elucidate its precise role in neuroprotection.

4.2.1.3 ATF6

Activating transcription factor 6 (ATF6) is a short-lived transcription factor and one of the important signaling proteins in ER stress. It lies in its inactivated form in the ER membrane bound to BIP. Upon sensing UPR, ATF6 becomes unbound from BIP and migrates to the Golgi apparatus, where it is sequentially cleaved by site-1 and site-2 proteases (S1P and S2P) producing an active short form of ATF6 (sATF6 or cleaved ATF6, ~50 kDa) [21, 22]. Cleaved ATF6 translocates to the nucleus and binds to ER stress response-elements (ERSE) in genes such as BIP, protein disulfide isomerase, and CHOP, enhancing their transcription [23]. The activation of CHOP by ATF6 is explicit in its involvement in ER stress-induced pro-apoptotic events, but

Table 4.1 Compounds targeting endoplasmic reticulum (ER) stress in cerebral ischemia

Compound	Targets	Mode of action
Edaravone	Free radical scavenger	Inhibits eIF2α
Dantrolene	Ryanodine receptor antagonist	Inhibits ER stress-induced apoptosis
Simvastatin	Neuroprotective agent	Increases ATF6 expression
3-bromo-7-nitroindazole	nNOS inhibitor	NO depletion
Cocaine and amphetamine-regulated transcript	Secretion of brain-derived neurotrophic factor	Inhibits ER stress
Sodium 4-phenybutyrate	Chemical chaperone	Inhibits ER stress
Parecoxib	COX-2 inhibitor	Upregulates GRP78
EGCG	Green tea polyphenols	Inhibits markers of ER stress
Monosialotetrahexosy-1 ganglioside	Ganglioside	Upregulates GRP78 and decreases CHOP
Pinocembrin (5,7-dihydroxyflavanone)	Natural flavonoid	Upregulates GRP78 and decreases CHOP and caspase 12
Nafamostat mesilate	A serine protease inhibitor	Decreases GRP78, CHOP, and eIF2α
Magnolol	Polyphenol	Downregulates p38/MAPK and CHOP

the role of ATF6 in stroke remains controversial owing to a lack of experimental evidence in this scenario. Therapeutically, some anti-ischemic compounds have been shown to accumulate cellular levels of ATF6 in favor of neuronal survival (Table 4.1). Deletion of ATF6 in experimental mice has shown impaired activation of neuroprotective astroglial cells, increased infarct volume, and cell death after middle cerebral artery occlusion (MCAO) [24]. Most recently, a functional study using conditional AFT6 knock-in animals has shown that overexpression of ATF6 not only significantly reduces the infarct volume, it consequently also improved the functional outcome 24 h after stroke [25]. These studies provide strong evidence that AFT6 may have a positive effect in IR injury. However, contradictions to these findings have also been reported with associated ATF6 deactivation. Future studies should be conducted for a more conclusive overview of this master transcription factor of the ER stress signaling pathway.

4.2.1.4 IRE1

Inositol requiring enzyme-1 (IRE1) is an ER transmembrane protein with interesting dual functional motifs. It possesses a serine-threonine kinase in addition to catalytic endoribonuclease cytosolic domains. Under conditions of ER stress, it has been observed that IRE1 is oligomerized and triggers catalytic auto-phosphorylation of its cytosolic domain, which simultaneously also activates its ribonuclease activity [26]. Apparently, the ribonuclease activity of IRE1 has gained much attention in IR injury-evoked UPR. IRE1 post-transcriptionally cleaves a 26-nucleotide-long intronic region of XBP1 mRNA, providing a frame-shift to its coding region [27]. Alternatively, spliced XBP1 mRNA translates into a 54 kDa XBP1 protein instead of the normal 33 kDa one [21]. XBP1 is a transcription factor that has been described to transcribe the expression of proteins involved in pro-survival mechanisms in UPR. Moreover, it has been observed that OGD injury transiently inactivates XBP1 protein, which results in accelerated neuronal death. Apparently, it has also been reported that IRE1 under chronic ER stress conditions binds to tumor necrosis factor receptor-associated factor 2 (TRAF2) and this association leads to the activation of JNK and caspase-12, which are pro-apoptotic factors in ER stress [28]. Neuroprotective compounds tend to either downregulate or upregulate the expression of IRE1 (Table 4.1), indicating that the extent and duration of ER stress has a strong influence on the expression of IRE1 in IR injury. Also, the use of specific inhibitors of IRE1 should be optimized with precision in cerebral ischemia.

4.3 Chronic ER Stress, UPR, and Pro-apoptotic Signaling in IR Injury

4.3.1 CHOP

The CCAAT/enhancer binding protein homologous protein (CHOP) is a transcription factor belonging to the family of growth arrest and DNA damage protein and is therefore also known as GADD153. CHOP is the critical mediator of programmed cell death, apoptosis, and is therefore designated as a hallmark for ER stress and UPR activation [29]. CHOP expression is positively regulated by the PERK-eIF2α-ATF4 signaling axis where activated ATF4 promotes the transcription of the CHOP gene. CHOP expression has also been reported to be activated by cleaved ATF6 through binding at ERSE in the CHOP promoter [7]. More recently, an alternative mechanism of CHOP activation has also been seen through IRE1 and p38 mitogen-activated protein kinase [30]. Earlier investigations using different

artery occlusion (AO) rat models defining the involvement of CHOP in IR injury showed that ER-mediated CHOP expression is one of the major causes of IR-induced neuronal cell death and CHOP−/− mice have shown less apoptotic neuron loss [31]. Moreover, ischemic pre-conditioning of the brain, which includes shorter intermittent ischemic events to prepare the brain for more severe ischemic insults, have been shown to decrease the activation of CHOP 24 h after reperfusion. Furthermore, CHOP is not only responsible for ischemic neuron cell death, but its accumulation has also been observed to induce apoptosis in the neighboring supporting astrocytes under oxygen and glucose deprivation (OGD) [32]. Systemic knockdown of CHOP has also been shown to reduce the pro-apoptotic effect of ER stress activators such as thapsigargin and Brefeldin A. Nevertheless, when considering therapeutic aspects, the down-regulation of CHOP has been found to be an important molecular event of many of the anti-ischemic compounds. All this evidence indicates that CHOP is an early but essential molecule in the pro-apoptotic signaling cascade following ischemic insult [9]. However, activation of CHOP post-ischemia is supposedly dependent on the extent of the damage caused after IR injury. Therefore, its manipulation in the context of neuronal survival still required optimization (Fig. 4.1).

4.3.2 Caspase 12

Caspase 12 is member of the interleukin-1β converting enzyme (ICE) subfamily of caspases, which are the group of proteins specialized in the execution of programmed cell death, otherwise known as apoptosis. In particular the association of

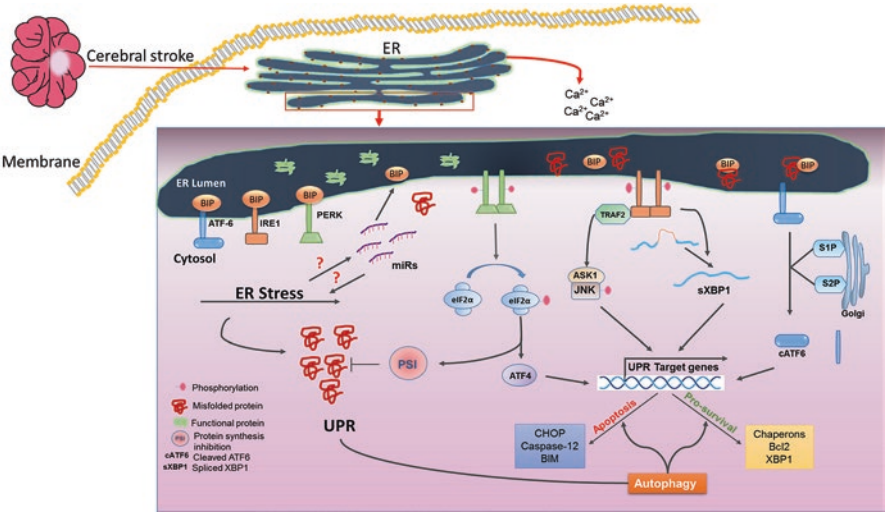

Fig. 4.1 Schematic representation of the mechanistic role of ER stress in cerebral stroke

caspase 12 is more akin to the ER stress-related apoptotic events in different cellular systems. However, some reports have shown that activation of caspase 12 is a contradictory event independent of ER stress [33, 34]. Precisely, caspase 12 is known to be bound to GRP78 on the ER membrane like other ER stress-related proteins and its dissociation is triggered by the disrupted calcium homeostasis. Caspase 12 cleavage from its proactive to active form is reported to be evident in brain injury, including temporary and permanent focal ischemia, and mice lacking caspase 12 are resistant to ER stress-induced apoptosis [35, 36]. The events following caspase-12 cleavage are traditional, including activation of caspase-9 and -3, DNA fragmentation, and eventually, apoptosis [37]. Previously, caspase-12 activation was thought to have been primarily mediated by m-calpain, a Ca^{2+}-dependent cysteine protease family member, but it has also been reported to be activated by other caspases, caspase-3/7 even more so than m-calpain [38]. Normally, pro-caspase-12 lays in an unactivated state in a complex with TRAF2. Upon any stress stimuli, it detangles itself to oligomerize to become activated. IRE1 has also been reported to activate caspase-12; however, the mechanism of how it works being associated with TRAF2 is under speculation, but IRE1 is indeed involved in the ER stress-induced activation of caspase-12 [28], although some studies have shown that it is not a prerequisite for ER stress-induced apoptosis. Furthermore, some have also suggested caspase-4 to be a surrogate to caspase-12 in humans. Owing to limited investigations in certain cell line and animal models, caspase-12 activation, however, is not yet considered to be a universal event following cerebral ischemia. However, it is still believed to possess mechanistic and therapeutic importance in prolonged IR injury.

4.3.3 JNK

c-Jun N-terminal kinase (JNK) or stress-activated protein kinases is one of the family members of mitogen-activated protein kinases (MAPKs). JNK is actively involved in responses mainly confined to the induction of cell death following various stress stimuli [39]. In ER stress, JNK activation has also been shown to evoke cell death through the activation of pro-apoptotic BH3-only protein BIM by increasing its expression in addition to phosphorylation. JNK is also known to induce death receptor-mediated apoptosis by phosphorylating c-fos and c-jun [40]. Upstream activation of JNK has been proposed by IRE1-TRAF2 complex along with the apoptosis signal-regulating kinase 1 (ASK1) signaling axis. ASK1 is an upstream regulator of stress-related kinases JNK and p38. Correlation of ASK1 with ER stress can be concluded by a study in which ASK1-deficient mice were found to be resistant to ER stress inducers and failed to activate JNK-induced apoptosis [41]. Activation of JNK is a major pro-apoptotic response of IRE1; however, other factors involved in the process are the activation of C-jun-N-terminal inhibiting kinase (JIK), the phosphorylation and dissociation of TRAF2 from caspase 12, leading to its activation, JNK-mediated upregulation of the pro-apoptotic BH3-only protein

Bim, and downregulation of the prosurvival protein Bcl2 and ultimately apoptosis. JNK also phosphorylates c-fos and c-jun to promote expression of mediator proteins of apoptosis through the death receptor pathway [40]. Interestingly, JNK inhibition through JNK inhibitor SP600125 showed protection of HT22 neuronal cells against apoptotic death by decreasing CHOP induced by ER stress inducers [42]. Therefore, JNK inhibition may provide a potential therapeutic strategy in combination with ER stress inhibitors to improve the overall outcome of stroke patients.

4.4 ER Stress and Autophagy in IR Injury

Autophagy is an evolutionary mechanism that is effectively used by cells to degrade, dissolve, and recycle their cytoplasmic entities to maintain survival [43]. Autophagy is being evolved not only to maintain the routine intracellular homeostasis, but also to safeguard cells from various stress and pathological conditions. It has been shown that mice deficient in significant executors of autophagy, autophagy-related (ATG) proteins, Atg5 and Atg7, suffered from neurodegeneration, implicating its vital role in neuronal function [44]. Autophagy in the controlled way is constructive and when in excess, it is highly destructive, leading to cell death in a non-apoptotic form, termed autosis [43], and sometimes apoptosis. However, the importance of autophagy is still undetermined in cerebral ischemia with regard to whether it is protective or not, and further, if it is destructive, then to what extent? Induction of autophagy has been observed in both types of cerebral stroke [45, 46]. As glucose deprivation is supposedly the most potent stimulus for autophagy induction because of the activation of the catabolic pathways, the addition of hypoxia may even aggravate the stimulus when considering an ischemic brain [46]. Experimental results have apparently shown that induction autophagy is somehow a defensive mechanism toward neuronal cell death following hypoxic ischemia injury. Administration of autophagy inhibitors such as 3-methyladenine and bafilomycin A1 have been shown to decrease neuronal and astroglial cell death. The cytoprotective effect of autophagy is most evident in the ischemic penumbra core regions. However, there are also some discrepancies to these findings whereby inducers of autophagy have caused more neuronal cell death in different models of ischemic injury in rodents [43]. Therefore, more studies are required for the correct placement of autophagy in cerebral ischemia so that its therapeutic potential can be evaluated.

4.5 ER Stress and miRNAs in IR Injury

MicroRNAs (miRs) are small (~22 nucleotide long) non-coding RNAs that are primarily involved in regulating cellular gene expression at the post-transcriptional level. MiRs are known to form an intricate network that involves both upstream regulators and downstream effectors of these tiny RNAs and which are capable of

modulating complex cellular pathways. ER stress is also known to be the cause and consequence of the biogenesis of differential miRs in various cellular contexts [47, 48]. However, very little information is available on how ER stress and miRs correlate in cerebral stroke, but there are certain cues that strongly represent their association. The first evidence is that cerebral ischemia itself has been shown to deregulate miR expression in different models [49]. Certain miRs were found to be deleterious and their repression improved neuronal survival after IR injury; miR-15a and miR-497 are important in this subset [50, 51]. Although some of the miRs were found to be inherently expressed as cytoprotective toward ischemia (miR-200b, miR-200c, and miR-429), some were even related to a better prognosis in stroke patients (miR-210) [52]. Mechanistically, there are many miRs that are known for regulating the expression of molecular chaperones such as the GRP78 and Bcl2 group of proteins, which are keynote players in ER stress and cerebral ischemia. MiR-181, a brain-enriched miR, is one such piece of evidence that has shown a direct link between ER stress and IR injury. Expression of miR-181 correlates inversely with the expression of GRP78 in both the core and the penumbra regions of the brain following MCAO and its inhibition provides protection toward neurons from cerebral ischemia through GRP78 upregulation [53]. Recently, two miRNAs, namely miR-7 and miR-30, have shown their contrasting role in ER stress and IR injury. miR-30a was shown to aggravate neuronal damage after OGD through upregulation of HSPA5, which was found to be a novel player in ER stress-induced apoptosis in IR injury through conjugating with caspase-12 [54]. MiR-7, on the contrary, was found to be cytoprotective and its overexpression decreased the expression of ER stress-related markers after OGD [55]. However, it has still not been discovered if and how ER stress following IR injury has an effect on miRs expression. But this evidence is sufficient for miRs to become potential therapeutic targets in IR injury-associated ER stress and evaluation of their mechanistic role is highly recommended.

4.6 Conclusion

Cerebral stroke is a serious health condition for which the only effective therapy is tPA, which has a very limited therapeutic window of only 4 h. Considering this, alternative therapies with definitive targets and broader applicability are mandatory to improve overall patient relief from cerebral stroke. The ER stress signaling pathway is a recently described molecular pathway that has many interesting targets with important roles in the pathophysiology of cerebral ischemia. Moreover, preclinical studies so far have also provided much convincing evidence that by targeting ER stress in cerebral ischemia we may achieve better therapeutic outcomes. Paradoxically, the divergent roles of the components of ER stress should also be taken into consideration before deciding the actual target for drug discovery. Signaling crosstalk between ER stress and autophagy seems promising but the timely activation and deactivation of autophagic signals following cerebral stroke

may again potentially narrow the therapeutic window of autophagy regulators in cerebral stroke. Experimenting with drug combinations using ER stress inhibitors and autophagy regulators can also provide a rational therapeutic approach that does still not attract much attention. Based on the facts detailed so far, it is important to gain a deeper understanding of ER stress and its associated events in cerebral stroke so that maximum benefits can be obtained from ER stress-based therapeutics in cerebral stroke.

References

1. Towfighi, A., & Saver, J. L. (2011, August 1). Stroke declines from third to fourth leading cause of death in the United States: Historical perspective and challenges ahead. *Stroke, 42*(8), 2351–2355.
2. Zhu, C., Wang, X., Xu, F., Bahr, B. A., Shibata, M., Uchiyama, Y., Hagberg, H., & Blomgren, K. (2005, February). The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia–ischemia. *Cell Death and Differentiation, 12*(2), 162.
3. Moskowitz, M. A., Lo, E. H., & Iadecola, C. (2010, July 29). The science of stroke: Mechanisms in search of treatments. *Neuron, 67*(2), 181–198.
4. Yoshida, H. (2007, February 1). ER stress and diseases. *The FEBS Journal, 274*(3), 630–658.
5. Paschen, W., Doutheil, J., Uto, A., & Gissel, C. (1996, October 11). Changes in endoplasmic reticulum Ca2+-ATPase mRNA levels in transient cerebral ischemia of rat: A quantitative polymerase chain reaction study. *Neuroscience Letters, 217*(1), 41–44.
6. Brostrom, C. O., & Brostrom, M. A. (1997, January 1). Regulation of translational initiation during cellular responses to stress. In *Progress in nucleic acid research and molecular biology* (Vol. 58, pp. 79–125). Cambridge, MA: Academic.
7. DeGracia, D. J., & Montie, H. L. (2004, October 1). Cerebral ischemia and the unfolded protein response. *Journal of Neurochemistry, 91*(1), 1–8.
8. Ron, D., & Walter, P. (2007, July). Signal integration in the endoplasmic reticulum unfolded protein response. *Nature Reviews Molecular Cell Biology, 8*(7), 519.
9. Xin, Q., Ji, B., Cheng, B., Wang, C., Liu, H., Chen, X., Chen, J., & Bai, B. (2014, March 1). Endoplasmic reticulum stress in cerebral ischemia. *Neurochemistry International, 68*, 18–27.
10. Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., & Ron, D. (2000, June). Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nature Cell Biology, 2*(6), 326.
11. Shen, J., Chen, X., Hendershot, L., & Prywes, R. (2002, July 1). ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals. *Developmental Cell, 3*(1), 99–111.
12. Wang, M., Wey, S., Zhang, Y., Ye, R., & Lee, A. S. (2009, September 1). Role of the unfolded protein response regulator GRP78/BiP in development, cancer, and neurological disorders. *Antioxidants & Redox Signaling, 11*(9), 2307–2316.
13. Zhou, H., Zhang, Y., Fu, Y., Chan, L., & Lee, A. S. (2011, July 22). Novel mechanism of anti-apoptotic function of 78-kDa glucose-regulated protein (GRP78): endocrine resistance factor in breast cancer, through release of B-cell lymphoma 2 (BCL-2) from BCL-2-interacting killer (BIK). *The Journal of Biological Chemistry, 286*(29), 25687–25696.
14. Su, Y. C., Wu, J. L., & Hong, J. R. (2011, March 1). Betanodavirus up-regulates chaperone GRP78 via ER stress: Roles of GRP78 in viral replication and host mitochondria-mediated cell death. *Apoptosis, 16*(3), 272–287.
15. Louessard, M., Bardou, I., Lemarchand, E., Thiebaut, A. M., Parcq, J., Leprince, J., Terrisse, A., Carraro, V., Fafournoux, P., Bruhat, A., & Orset, C. (2017, September). Activation of cell

surface GRP78 decreases endoplasmic reticulum stress and neuronal death. *Cell Death and Differentiation, 24*(9), 1518.
16. Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., & Ron, D. (2000, November 1). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Molecular Cell, 6*(5), 1099–1108.
17. Paschen, W. (2003, October 1). Endoplasmic reticulum: A primary target in various acute disorders and degenerative diseases of the brain. *Cell Calcium, 34*(4–5), 365–383.
18. Marciniak, S. J., Garcia-Bonilla, L., Hu, J., Harding, H. P., & Ron, D. (2006, January 16). Activation-dependent substrate recruitment by the eukaryotic translation initiation factor 2 kinase PERK. *The Journal of Cell Biology, 172*(2), 201–209.
19. Mamady, H., & Storey, K. B. (2008, September 1). Coping with the stress: Expression of ATF4, ATF6, and downstream targets in organs of hibernating ground squirrels. *Archives of Biochemistry and Biophysics, 477*(1), 77–85.
20. Feng, D., Wang, B., Wang, L., Abraham, N., Tao, K., Huang, L., Shi, W., Dong, Y., & Qu, Y. (2017, April 1). Pre-ischemia melatonin treatment alleviated acute neuronal injury after ischemic stroke by inhibiting endoplasmic reticulum stress-dependent autophagy via PERK and IRE1 signalings. *Journal of Pineal Research, 62*(3), e12395.
21. Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., & Mori, K. (2001, December 28). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell, 107*(7), 881–891.
22. Shen, J., & Prywes, R. (2004, October 8). Dependence of site-2 protease cleavage of ATF6 on prior site-1 protease digestion is determined by the size of the luminal domain of ATF6. *The Journal of Biological Chemistry, 279*(41), 43046–43051.
23. Okada, T., Yoshida, H., Akazawa, R., Negishi, M., & Mori, K. (2002, September 1). Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. *The Biochemical Journal, 366*(Pt 2), 585.
24. Yoshikawa, A., Kamide, T., Hashida, K., Ta, H. M., Inahata, Y., Takarada-Iemata, M., Hattori, T., Mori, K., Takahashi, R., Matsuyama, T., & Hayashi, Y. (2015, February 1). Deletion of Atf6α impairs astroglial activation and enhances neuronal death following brain ischemia in mice. *Journal of Neurochemistry, 132*(3), 342–353.
25. Yu, Z., Sheng, H., Liu, S., Zhao, S., Glembotski, C. C., Warner, D. S., Paschen, W., & Yang, W. (2017, March). Activation of the ATF6 branch of the unfolded protein response in neurons improves stroke outcome. *Journal of Cerebral Blood Flow and Metabolism, 37*(3), 1069–1079.
26. Tirasophon, W., Welihinda, A. A., & Kaufman, R. J. (1998, June 15). A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes & Development, 12*(12), 1812–1824.
27. Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., & Ron, D. (2002, January). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature, 415*(6867), 92.
28. Yoneda, T., Imaizumi, K., Oono, K., Yui, D., Gomi, F., Katayama, T., & Tohyama, M. (2001, April 27). Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *The Journal of Biological Chemistry, 276*(17), 13935–13940.
29. Oyadomari, S., & Mori, M. (2004, April). Roles of CHOP/GADD153 in endoplasmic reticulum stress. *Cell Death and Differentiation, 11*(4), 381.
30. Kim, I., Xu, W., & Reed, J. C. (2008, December). Cell death and endoplasmic reticulum stress: Disease relevance and therapeutic opportunities. *Nature Reviews Drug Discovery, 7*(12), 1013.
31. Tajiri, S., Oyadomari, S., Yano, S., Morioka, M., Gotoh, T., Hamada, J. I., Ushio, Y., & Mori, M. (2004, April). Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP. *Cell Death and Differentiation, 11*(4), 403.
32. Osada, N., Kosuge, Y., Ishige, K., & Ito, Y. (2010, August 1). Characterization of neuronal and astroglial responses to ER stress in the hippocampal CA1 area in mice following transient forebrain ischemia. *Neurochemistry International, 57*(1), 1–7.

33. Nakagawa, T., & Yuan, J. (2000, August 21). Cross-talk between two cysteine protease families: Activation of caspase-12 by calpain in apoptosis. *The Journal of Cell Biology, 150*(4), 887–894.
34. Badiola, N., Penas, C., Minano-Molina, A., Barneda-Zahonero, B., Fado, R., Sánchez-Opazo, G., Comella, J. X., Sabria, J., Zhu, C., Blomgren, K., & Casas, C. (2011, April). Induction of ER stress in response to oxygen-glucose deprivation of cortical cultures involves the activation of the PERK and IRE-1 pathways and of caspase-12. *Cell Death & Disease, 2*(4), e149.
35. Mouw, G., Zechel, J. L., Gamboa, J., Lust, W. D., Selman, W. R., & Ratcheson, R. A. (2003, February 10). Activation of caspase-12, an endoplasmic reticulum resident caspase, after permanent focal ischemia in rat. *Neuroreport, 14*(2), 183–186.
36. Shibata, M., Hattori, H., Sasaki, T., Gotoh, J., Hamada, J., & Fukuuchi, Y. (2003, May 8). Activation of caspase-12 by endoplasmic reticulum stress induced by transient middle cerebral artery occlusion in mice. *Neuroscience, 118*(2), 491–499.
37. Aoyama, K., Burns, D. M., Suh, S. W., Garnier, P., Matsumori, Y., Shiina, H., & Swanson, R. A. (2005, March). Acidosis causes endoplasmic reticulum stress and caspase-12-mediated astrocyte death. *Journal of Cerebral Blood Flow and Metabolism, 25*(3), 358–370.
38. Martinez, J. A., Zhang, Z., Svetlov, S. I., Hayes, R. L., Wang, K. K., & Larner, S. F. (2010, December 1). Calpain and caspase processing of caspase-12 contribute to the ER stress-induced cell death pathway in differentiated PC12 cells. *Apoptosis, 15*(12), 1480–1493.
39. Wada, T., & Penninger, J. M. (2004 April). Mitogen-activated protein kinases in apoptosis regulation. *Oncogene, 23*(16), 2838.
40. Putcha, G. V., Le, S., Frank, S., Besirli, C. G., Clark, K., Chu, B., Alix, S., Youle, R. J., LaMarche, A., Maroney, A. C., & Johnson, E. M., Jr. (2003, June 19). JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron, 38*(6), 899–914.
41. Nishitoh, H., Matsuzawa, A., Tobiume, K., Saegusa, K., Takeda, K., Inoue, K., Hori, S., Kakizuka, A., & Ichijo, H. (2002, June 1). ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes & Development, 16*(11), 1345–1355.
42. Choi, J. H., Choi, A. Y., Yoon, H., Choe, W., Yoon, K. S., Ha, J., Yeo, E. J., & Kang, I. (2010, December). Baicalein protects HT22 murine hippocampal neuronal cells against endoplasmic reticulum stress-induced apoptosis through inhibition of reactive oxygen species production and CHOP induction. *Experimental & Molecular Medicine, 42*(12), 811.
43. Galluzzi, L., Bravo-San Pedro, J. M., Blomgren, K., & Kroemer, G. (2016, August). Autophagy in acute brain injury. *Nature Reviews Neuroscience, 17*(8), 467.
44. Ogata, M., Hino, S. I., Saito, A., Morikawa, K., Kondo, S., Kanemoto, S., Murakami, T., Taniguchi, M., Tanii, I., Yoshinaga, K., & Shiosaka, S. (2006, December 15). Autophagy is activated for cell survival after endoplasmic reticulum stress. *Molecular and Cellular Biology, 26*(24), 9220–9231.
45. Niu, M., Dai, X., Zou, W., Yu, X., Teng, W., Chen, Q., Sun, X., Yu, W., Ma, H., & Liu, P. (2017, January 1). Autophagy, endoplasmic reticulum stress and the unfolded protein response in intracerebral hemorrhage. *Translational Neuroscience, 8*(1), 37–48.
46. Adhami, F., Schloemer, A., & Kuan, C. Y. (2007, January 8). The roles of autophagy in cerebral ischemia. *Autophagy, 3*(1), 42–44.
47. Malhi, H. (2014, September 1). MicroRNAs in ER stress: Divergent roles in cell fate decisions. *Current Pathobiology Reports, 2*(3), 117–122.
48. Tripathi, A. K., Dwivedi, A., Pal, M. K., Rastogi, N., Gupta, P., Ali, S., BH, M. P., Kushwaha, H. N., Ray, R. S., Singh, S. K., & Duggal, S. (2014, December). Attenuated neuroprotective effect of riboflavin under UV-B irradiation via miR-203/c-Jun signaling pathway in vivo and in vitro. *Journal of Biomedical Science, 21*(1), 39.
49. Ouyang, Y. B., Lu, Y., Yue, S., Xu, L. J., Xiong, X. X., White, R. E., Sun, X., & Giffard, R. G. (2012, January 1). miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. *Neurobiology of Disease, 45*(1), 555–563.

50. Yin, K. J., Deng, Z., Hamblin, M., Xiang, Y., Huang, H., Zhang, J., Jiang, X., Wang, Y., & Chen, Y. E. (2010, May 5). Peroxisome proliferator-activated receptor 未 regulation of miR-15a in ischemia-induced cerebral vascular endothelial injury. *The Journal of Neuroscience, 30*(18), 6398–6408.
51. Yin, K. J., Deng, Z., Huang, H., Hamblin, M., Xie, C., Zhang, J., & Chen, Y. E. (2010, April 1). miR-497 regulates neuronal death in mouse brain after transient focal cerebral ischemia. *Neurobiology of Disease, 38*(1), 17–26.
52. Ouyang, Y. B., Stary CM, Yang, G. Y., & Giffard, R. (2013, January 1). microRNAs: Innovative targets for cerebral ischemia and stroke. *Current Drug Targets, 14*(1), 90–101.
53. Ouyang, Y. B., Lu, Y., Yue, S., & Giffard, R. G. (2012, March 31). miR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. *Mitochondrion, 12*(2), 213–219.
54. Wang, P., Zhang, N., Liang, J., Li, J., Han, S., & Li, J. (2015, November 1). Micro-RNA-30a regulates ischemia-induced cell death by targeting heat shock protein HSPA5 in primary cultured cortical neurons and mouse brain after stroke. *Journal of Neuroscience Research, 93*(11), 1756–1768.
55. Dong, Y. F., Chen, Z. Z., Zhao, Z., Yang, D. D., Yan, H., Ji, J., & Sun, X. L. (2016, December). Potential role of microRNA-7 in the anti-neuroinflammation effects of nicorandil in astrocytes induced by oxygen-glucose deprivation. *Journal of Neuroinflammation, 13*(1), 60.

Chapter 5
The Role of Autophagy in Ischaemic Stroke: Friend or Foe?

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Abstract Autophagy is an evolutionarily conserved process of cellular self-degradation and recycling of redundant cytoplasmic entities by lysosomal enzymes. Moreover, autophagy also plays critical roles in controlling several biochemical and molecular neuronal physiology such as growth, survival and metabolism. The autophagy process constantly occurs at basal level under normal physiological conditions and gets increased during stress conditions such as starvation and hypoxia. In neuronal cells, it is a vital homeostasis mechanism that helps in the maintenance of protein quality control. In various neurological disorders, several crucial pro-survival and anti-apoptotic effects of autophagy have been reported. However, the function of autophagy in ischaemic stroke (IS) is highly controversial and still debated. Some reports show that it protects neurons during IS, while others advocate it to be neurodegenerative. Thus, the present chapter deals with the possible function of autophagy in ischaemic stroke along with the discussion of various factors influencing the action of autophagy in ischaemic stroke.

Keywords Autophagy · Cerebral ischaemia · Ischaemic penumbra · Ischaemic stroke

5.1 Introduction

Stroke is the third leading cause of disability and mortality worldwide [1]. It refers to a condition of sudden obstruction in cerebral blood supply. This can be caused by a number of disorders that can interrupt the blood flow to the brain. Stroke can be subdivided into two classes; the first is ischaemic stroke (decreased blood supply either by thrombosis or by arterial embolism), and the second is haemorrhagic

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stroke (vascular leakage). Ischaemic stroke (ISs) is a general term with reference to a heterogeneous group of aetiologies, e.g. embolism, thrombosis and relative hypoperfusion. However, it is predominantly caused by embolism from the heart and atherothrombosis of intracranial and cranial arteries [2].

Cerebral ischaemia is subcategorised into hypoxic, global and focal ischaemia. Hypoxic ischaemia that is commonly found in neonates occurs during the perinatal period resulting in detrimental long-term neurological morbidity in terms of motor, intellectual, educational and neuropsychological performance deficits (e.g. cerebral palsy, retardation in mental status, disability in learning and epileptic episodes) and even neonatal mortality [3]. Global cerebral ischaemia occurs when blood flow to widespread areas of the central nervous system is severely compromised after transient circulatory arrest with resuscitation, traumatic brain injury or after near-drowning incidents [4]. In contrast, focal ischaemia is marked by a sudden considerable reduction in blood supply to the brain either due to rupture or formation of an occlusion by embolism/thrombosis of the blood vessel. The occluded artery that results in blocked blood flow generates an infarct encompassing all or some part of the territory vasculature. This leads to a condition of oxygen deprivation in tissue, and cells undergo a series of the molecular events leading to excitotoxicity of neurons, dysfunction in mitochondrial activity, acidotoxicity of neurons, disturbance in ionic balance, conditions of oxidative stress and inflammation [5]. Within few minutes, some neurons die immediately leading to an irreversible injury impairing sensory processing, communication, cognition and motor function [6]. The extent of ischaemic injury depends upon the duration or severity of the insult. The motor impairment as the result of IS can lead to short-term or permanent disabilities and also makes the person prone to several neurological disorders such as Alzheimer's or Parkinson's diseases [7]. Neuroimaging techniques have confirmed that around the infarct core, there is an area where the blood supply is marginally sufficient to keep these cells alive. This is called the ischaemic penumbra [8].

Two major strategies for the treatment of IS, namely, reperfusion and neuroprotection, are currently being investigated. In reperfusion, the attempts are made to restore the blood flow in affected areas by thrombotic, antithrombotic and anti-aggregation drugs [9]. Till date, recombinant tissue plasminogen activator (rt-PA) is the only drug that has shown promising results in clinical trials [10, 11]. However, these drugs provide very narrow therapeutic window (only 3 h) and have associated risk of haemorrhage. On the other hand, neuroprotection refers to all the endogenous cytoprotective mechanisms that shield the neuronal death and diminish metabolic pathways that usher cellular injury. Thus, an effective neuroprotective strategy would be to prevent infarct core from expanding by increasing penumbra survival and reducing ischaemic inflammation and reperfusion injury [12, 13].

5.2 Pathophysiology of Ischaemic Stroke

Ischaemic brain injury evolves from a complex sequence of pathophysiological events which progresses during the course of time. The major pathogenic mechanisms of this sequence include excitotoxicity, per-infarct depolarisation, inflammation and apoptosis [14]. For its energetic requirements, the brain primarily depends upon the availability of oxygen and glucose that provide energy by oxidative phosphorylation. When blood flow is interrupted in the brain, this energetic flow fails to maintain the ionic gradients [15]. Consequently, the membrane potential is lost, which results in depolarisation of neurons and glial cells. This causes inactivation of somatodendritic as well as presynaptic voltage-dependent Ca^{2+} channels and release of glutamate like excitatory amino acids in extracellular space which cannot be transmitted back to the cell as it is an energy-requiring process. Thus, the glutamate is accumulated in the extracellular space. The depolarisation of the cell results in activation of NMDA receptors allowing a voltage-dependent inflow of calcium (Ca^{2+}) via phospholipase C and Ins (1,4,5)P3 signalling. Other monovalent ion channels such as AMPA receptor channel also transport the Na^+ and Cl^- into the neurons. Since the influx of Na^+ and Cl^- is much higher than the efflux of K^+, this causes the water to flow inside the cells passively. This results in a condition called oedema which is the earliest marker of stroke and can be studied with MRI or computed tomography [16]. Increased Ca^{2+} concentration in the neurons triggers a series of cytoplasmic as well as nuclear events that further add up to tissue damage. Free radicals are generated through the activation of phospholipase A2 and cyclooxygenase, overwhelming intracellular scavenging processes and leading to lipid peroxidation and membrane damage [17]. These oxygen free radicals, in turn, serve as important signalling mediators that trigger inflammation and apoptosis. These free radicals also disrupt the internal mitochondrial membrane and oxidise the proteins of electron transport chain [18]. Consequently, the mitochondrial outer membrane becomes permeable and promotes mitochondrial swelling, the cessation of ATP production and increased oxygen free radical generation. Mitochondrial outer membrane permeabilisation (MOMP) stimulates the cytochrome C to be released in the cytoplasm and triggers the apoptosis [19]. Penumbra that lies between the fatally damaged core and healthy brain tissues has partially preserved blood flow that maintains energetic balance at some level and keeps the tissues alive for some period of time. If left untreated, the penumbra can progress to infarction owing to ongoing excitotoxicity and lead to other secondary deleterious events, such as spreading depolarisation, postischaemic inflammation and apoptosis [20].

5.3 Various Animal Models to Study IS

In order to understand the mechanisms that underlie cerebral ischaemia and developing treatment therapies, a number of animal stroke models have been designed. There are several reasons that justify manoeuvre of an animal model to study IS. The animal model of stroke is highly reproducible and well controllable and allows unambiguous scrutiny of IS pathophysiology and effects of drug treatment. There are various invasive methods involved in the study of anatomical, physiological and biochemical status of brain tissue that cannot be understood in humans using imaging techniques [21]. Therefore, a number of rodent model of IS have been designed that mimic some aspects of human stroke. IS is a heterogeneous human disorder; therefore, it is impractical to investigate all related pathophysiology in a single model (Fig. 5.1).

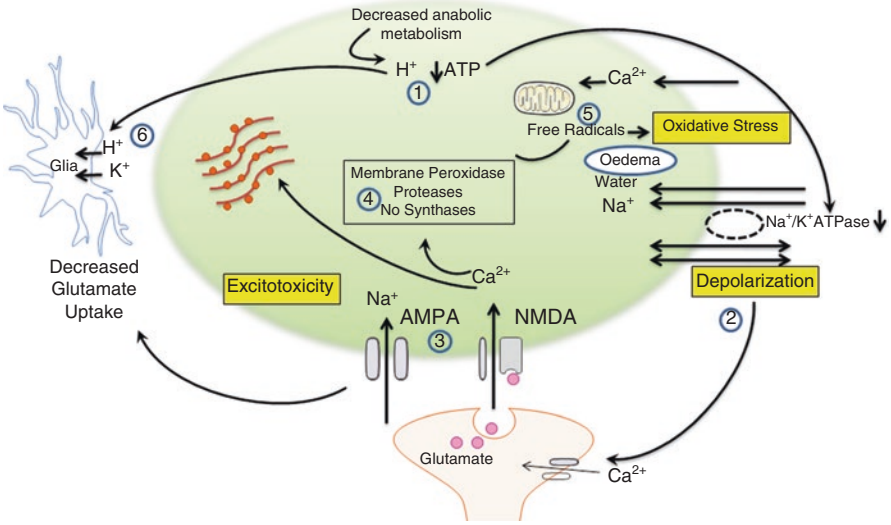

Fig. 5.1 Pathophysiology of ischaemic stroke: oxygen and glucose deprivation leads to decreased production of ATP. Energy flow and ionic gradient of the cells become compromised that results in membrane depolarisation. The depolarisation of cells results in activation of NMDA receptors and somatodendritic as well as presynaptic voltage-dependent Ca^{2+} channels. The excitatory amino acids such as glutamate released in extracellular space cannot be taken up by neurons and glial cells. The monovalent ion channels such as AMPA receptor channel also transports the Na^+ and Cl^- into the neurons. To counterbalance, water flows inside the cells passively and causes oedema. The activation of phospholipase A2 and cyclooxygenase results in free radical generation which can damage cellular macromolecules leading to autophagy, apoptosis and necrosis

5.3.1 Intra-arterial Suture Middle Cerebral Arterial Occlusion Model

The most affected artery that accounts for most of the cases of IS in humans is the occlusion of middle cerebral artery (MCA) and its branches. Thus, the techniques that mimic this type of condition are the closest to human IS [22, 23]. The most common method to generate such condition is an intra-arterial suture in MCA. In this technique, extracranial internal carotid artery (ICA) is clipped by introducing an occluding thread that advances until the origin of the MCA, thus interrupting blood flow into the MCA [24]. This procedure is minimally invasive and does not require craniotomy. The duration of occlusion using thread suture varies from transient blockage of 60–120 min or even permanent infarction. A large body of literature report that the efficacy of infarction is affected by several factors such as quality of thread, insertion length, size and diameter of suture and also different rat and mouse strain. Silicon- and polylysine-coated threads are most often used because these threads reduce the risk of subarachnoid haemorrhage [24] and better adhere to adjoining endothelium [25] and thus reduce inter-animal variability in comparison to uncoated threads. The great advantage of this technique is that analogous to human IS, it also arises from MCA and generates a penumbra likewise. It also offers controlled reperfusion as the thread can be withdrawn any time from the site of suture which is particularly suitable for neuroprotection studies. However, inadequate occlusion of MCA leads to the rupture of vessels and occurrence of subarachnoid haemorrhage. Conversely, the use of silicone-coated thread and laser Doppler-guided placement of the suture can prevent these incidences [26].

5.3.2 Craniotomy Model

This is an invasive procedure where a bone of the skull is temporarily removed to allow direct access to cerebral vasculature [22]. In this procedure, the vessels are either ligated to each other, clipped and hooked to lift the vessels from the surface of the brain that halts blood flow, or sealed by electrocoagulation or photothrombosis [21].

5.3.3 Photothrombosis Model

The animal underwent small craniotomy to expose the right distal middle cerebral artery and injected intravenously a photosensitizing dye (Rose Bengal or erythrosine B) [27]. The thrombotic occlusion is induced by irradiating three separate points with light from an argon laser-activated dye laser. This results in a large consistent temporoparietal cortical infarct. This model has the advantages as it only

requires minimal surgery, allowing the dura to remain intact and circumvent the mechanical trauma to the brain tissues [28].

5.3.4 Endothelin-I Model

Endothelin-I is a peptidergic vasoconstrictor, which may be injected directly as an intracerebral injection or applied directly onto the MCA to cause a condition similar to focal ischaemia [29]. Endothelin-I creates a model of middle cerebral artery occlusion in a dose-dependent manner [30]. However, when injected directly to the cortical surface, it provides a semicircular infarct that intricate all cortical layers. Stereotaxic injections of this compound create infarcts in the white matter of the internal capsule underlying sensorimotor cortex in rats [31]. This is a least-invasive method, which shows less mortality, and the development of a capsular infarct model allows examination of the physiological, anatomical and chemical characteristics of neurons after white matter infarction. It must be noted that endothelin-1 is several fold more potent in conscious rats than numb rats.

5.3.5 Clot Embolic Model of Stroke

In more than 80% of human stroke cases, embolism or thrombosis is the key player [32]. The middle cerebral artery is occluded with autologous blood clot, and utilizing a microcatheter and laser Doppler flowmetry is a precise method to produce an accurate stroke model [33]. Another method is the injection of thrombin-induced clots directly into the intracranial segment of the internal carotid artery [34] or middle carotid artery [35]. Since this model mimics human stroke condition unambiguously, it is very practical in studying the effect of thrombolytic factors [36] and various neuroprotective drugs [37].

5.4 Autophagy and Its Machinery

Autophagy is an evolutionary conserved physiological activity that conserves cellular homeostasis by purging the cell from aberrant protein aggregates and damaged organelles. The term first used in the 1960s is now proved to be an important survival mechanism during acute starvation and contributes to development, growth regulation and longevity. Depending upon the mechanism of transportation of cargo to lysosomal lumen, autophagy has been described into four types: macroautophagy (commonly called as autophagy), microautophagy, chaperone-mediated autophagy and crinophagy [38].

In microautophagy, the cargo to be degraded is directly engulfed by lysosome itself through a sequential process of invagination, protrusion and separation [39]. On the other hand, chaperone-mediated autophagy is a very particular process that executes degradation of the soluble cytosolic proteins containing a target motif marked by the presence of pentapeptide KFERQ. Cargo proteins containing this motif are recognised by cytosolic heat-shock cognate protein-70 (cyt-hsc70) that, along with some other co-chaperone of Hsc70, target the substrate to the lysosomal membrane, where it interacts with the lysosomal membrane protein (lamp) type 2a [40] and undergoes rapid proteolysis by resident hydrolases [41]. Macroautophagy or autophagy as it is commonly called is a sequential process initiated by the sequestering of cytoplasmic constituents to be degraded in a double membrane bound structure (phagophore) forming "autophagosome". These autophagosomes are then transported to the lysosome where they fuse with it to form structures called "autophagolysosomes". Both the inner membrane and material within the autophagolysosomes are degraded by lysosomal hydrolases, which are then transported to the cytoplasm for reuse [42]. In crinophagy, secretory vesicles directly fuse with lysosome, which leads to the degradation of vesicle content [43]. There is a conserved family of Atg genes (AuTophaGy-related), and till now 32 Atg genes are reported in yeast and 14 Atg genes in mammals [44]. The concerted actions of these gene products are responsible for the conjugated mechanism of autophagy. Phagophore formation starts with the interaction of Vps34 with a multifactor complex that also contains Atg6, Atg14 and Vps15. This along with other components interacts with Atg1 and Atg13 to initiate early autophagy [45].

5.5 Autophagy and Its Role in Cerebral Ischaemia

The primary function of autophagic process is to degrade the cellular proteins and organelles that have been sequestered in the cell, which, otherwise, may become the underlying cause of neurodegenerative disorders. Therefore, induction of autophagy may be a promising neuroprotective strategy in neurological disorders [46]. However, molecular mechanisms underlying neuronal autophagy in IS remain poorly understood. In neurons, two types of autophagic processes have been reported: basal autophagy and induced autophagy. In the context of IS, the role of autophagy is little controversial. Some reports suggest that autophagy is responsible for induction of IS, while others indicate its preventive effects. Although the role of autophagy in IS is arguable, on the basis of published data so far, researchers have proposed five possible prospects.

5.5.1 Autophagy Activation in Ischaemic Stroke Protects Neurons

It has demonstrated that rapamycin-induced activation of autophagy protects neurons. They showed that rapamycin induces autophagy by inactivating mTOR complex in neonatal rat under the influence of hypoxia-ischaemia which delays the advancement of neuronal cell mortality, whereas when autophagy was halted, cells rapidly gallop to necrosis [47]. In another study, we have confirmed that the autophagy is a part of a pro-survival network that also includes PI3K/Akt1/mTOR signalling pathway(s) and when any of these pathways are interrupted, the neuroprotective role of rapamycin is zeroed [48]. Nicotinamide phosphoribosyltransferase is the rate-limiting enzyme in NAD^+ biosynthesis in mammals. It is shown to have a protective role in IS through inhibiting neuronal cell death. Wang and colleagues have found out that visfatin increases neuronal survival in middle cerebral artery occlusion (MCAO) model of rats and in oxygen-glucose deprivation (OGD)-cultured cortical neurons through induction of autophagy via regulating TSC2-mTOR-S6K1 signalling pathway in a SIRT1-dependent manner during cerebral ischaemia [49].

5.5.2 Autophagy Is Also Responsible for Neuronal Death After Ischaemic Stroke

In the Levine/Vannucci model, after 24 h of ischaemia/hypoxia, electron microscopy confirmed vacuolisation and extensive lysis of cytoplasmic contents in the damaged cells, suggesting an induction of the autophagic-lysosomal compartment of the programmed cell death [50]. It has also been reported that AMPK-mediated autophagy is detrimental in hypoxia and ischaemic stroke [51]. When Atg7 gene was deleted in mice with occluded common carotid artery, there was a reduction in pyramidal neurons in the hippocampus damage and ischaemic area [52]. Various reports have suggested that the pharmacological inhibitions of autophagy reduce damage in focal cerebral ischaemia. Wan et al have shown that the injection of Vps34 kinase inhibitor, 3-methyladenine (3-MA), immediately after permanent occlusion in middle cerebral artery decreases autophagy activation and relieves postischaemic injury [53]. It has also been found that inhibiting postischaemic hypoxia-inducible factor-1 α (HIF-1α) by 2-methoxyestradiol (2ME) significantly inhibits autophagy and prevents primordial neuron death [54].

5.5.3 Degree of Autophagy Is Critical in Ischaemic Stroke

Researchers have proposed that the degree of autophagy is the deciding factor for the cell fate; the basal level of autophagy is favourable for cell survival, while excessive or diminished autophagy may prove to be deleterious. Kang and Avery in 2008

have shown in *C. elegans* that during starvation, autophagy promotes the ability to survive in *C. elegans*, while inadequate levels of autophagy promote death [55]. This hypothesis is also confirmed by using in vitro as well as in vivo models [56]. Oxygen-glucose deprivation (OGD) induced hypoxic/ischaemic injury followed by reperfusion in primary cortical neurons in vitro [57]. While in vivo neonate rat model, unilateral common carotid artery occlusion and hypoxia create a condition similar to cerebral ischaemia. Increased autophagy was marked by the upregulated ratio of autophagy initiation gene expression. Electron microscopic studies have confirmed the increased autophagic deaths after OGD/reperfusion. However, there was a remarkable decrease in autophagic cell death upon inhibition of autophagy using a specific autophagy inhibitor, 3-MA [57].

5.5.4 Autophagy Contributes to Ischaemic Tolerance After Preconditioning

Preconditioning is often referred to a subthreshold insult that occurs in a cell so that certain signalling pathways can be activated which would provide protection from future fierce ischaemic episodes [58]. Thus, time at which autophagy is induced is crucial for its pro-survival or pro-death functions. In 2009, it was first shown that in PC12 cell model, ischaemic preconditioning (IPC) increases the formation and degradation of autophagosomes [59]. However, when autophagy was inhibited by 3-MA during IPC, it nullifies the neuroprotective effects of IPC. In male Sprague-Dawley rats, tolerance to focal cerebral ischaemia is increased by elevating autophagy through hyperbaric oxygen (HBO) preconditioning [60]. It has also been found that when 3-MA and rapamycin were administered 20 min before the hypoxic/ischaemic injury, 3-MA prevents autophagy by decreasing Beclin-1 expression resulting in neuronal cell death, while rapamycin induces autophagy and provided neuroprotection [47, 61].

5.5.5 Autophagy May Be Disrupted During Ischaemia

Researchers have confirmed that the accumulation of autophagic vacuoles and intracytoplasmic protein aggregates is the root cause of various neurodegenerative diseases [62]. It has been hypothesised that an increase in protein content leads to the impaired lysosomal degradation which results in accumulation of autophagosome. When sham-operated rats were treated with chloroquine, a lysosomal inhibitor, the level of LC3-II was found to be increased; however, postischaemia further changes in LC3-II were not reported. From this study, it was concluded that these accumulated autophagy-associated proteins are due to the failure of autophagic pathway [63]. It has also been thought that failure in the fusion of autophagosome to

lysosome or deficiency of acid phosphatase in lysosome could result in accumulation of autolysosome and autophagosome.

5.6 Concluding Remarks

The molecular mechanism(s) underlying the neuronal autophagy in IS remains poorly understood and highly controversial. The growing body of evidence demonstrated that the physiological and constitutive low level of autophagy acts as an integral part of several pro-survival networks in the neurons that provides protection against stress and injury. On the other hand, at redundant levels, it may also cause tissue injury and necrosis. Thus, the intensity and instance of autophagy are critical to its aftermaths. The mechanism of autophagy in IS and its effect in disease progression must be further investigated. This will help in the development of better therapeutic strategies that will act to modify the cellular survival network in patients with IS.

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References

1. Chu, C. T. (2008, February). Eaten alive. *The American Journal of Pathology, 172*(2), 284–287.
2. Lakhan, S. E., Kirchgessner, A., & Hofer, M. (2009). Inflammatory mechanisms in ischemic stroke: Therapeutic approaches. *Journal of Translational Medicine, 7*(1), 97.
3. Cowan, F., Rutherford, M., Groenendaal, F., Eken, P., Mercuri, E., Bydder, G. M., et al. (2003, March). Origin and timing of brain lesions in term infants with neonatal encephalopathy. *The Lancet, 361*(9359), 736–742.
4. Block, F. (1999, June). Global ischemia and behavioural deficits. *Progress in Neurobiology, 58*(3), 279–295.
5. Ouyang, Y. B., & Giffard, R. G. (2012). ER-mitochondria crosstalk during cerebral ischemia: Molecular chaperones and ER-mitochondrial calcium transfer. *International Journal of Cell Biology, 2012*, 1–8.
6. Carron, S. F., Alwis, D. S., & Rajan, R. (2016, June). Traumatic brain injury and neuronal functionality changes in sensory cortex. *Frontiers in Systems Neuroscience*, *10,* 47. Available from: http://journal.frontiersin.org/Article/10.3389/fnsys.2016.00047/abstract
7. Petty, G. W., Brown, R. D., Whisnant, J. P., Sicks, J. D., O'Fallon, W. M., & Wiebers, D. O. (2000, May 1). Ischemic stroke subtypes: A population-based study of functional outcome, survival, and recurrence. *Stroke, 31*(5), 1062–1068.
8. Sanganalmath, S. K., Gopal, P., Parker, J. R., Downs, R. K., Parker, J. C., & Dawn, B. (2017, February). Global cerebral ischemia due to circulatory arrest: Insights into cellular pathophysiology and diagnostic modalities. *Molecular and Cellular Biochemistry, 426*(1–2), 111–127.
9. Ye, Y., Perez-Polo, J. R., & Birnbaum, Y. (2010, October). Protecting against ischemia-reperfusion injury: Antiplatelet drugs, statins, and their potential interactions: Ye et al. *The Annals of the New York Academy of Sciences, 1207*(1), 76–82.

10. Balucani, C., Levine, S. R., Khoury, J. C., Khatri, P., Saver, J. L., & Broderick, J. P. (2016, April). Acute ischemic stroke with very early clinical improvement: A National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator stroke trials exploratory analysis. *Journal of Stroke and Cerebrovascular Diseases, 25*(4), 894–901.
11. Barreto, A. D., Fanale, C. V., Alexandrov, A. V., Gaffney, K. C., Vahidy, F. S., Nguyen, C. B., et al. (2016, August). Prospective, open-label safety study of intravenous recombinant tissue plasminogen activator in wake-up stroke: Safety study of wake-up stroke thrombolysis. *Annals of Neurology, 80*(2), 211–218.
12. Green, A. R., & Shuaib, A. (2006, August). Therapeutic strategies for the treatment of stroke. *Drug Discovery Today, 11*(15–16), 681–693.
13. Fisher, M. (2011, January 1). New approaches to neuroprotective drug development. *Stroke, 42*, S24–S27.
14. Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999, September). Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences, 22*(9), 391–397.
15. Mongin, A. A. (2007, December). Disruption of ionic and cell volume homeostasis in cerebral ischemia: The perfect storm. *Pathophysiology, 14*(3–4), 183–193.
16. Simard, J. M., Kent, T. A., Chen, M., Tarasov, K. V., & Gerzanich, V. (2007, March). Brain oedema in focal ischaemia: Molecular pathophysiology and theoretical implications. *The Lancet Neurology, 6*(3), 258–268.
17. Chen, H., Yoshioka, H., Kim, G. S., Jung, J. E., Okami, N., Sakata, H., et al. (2011, April 15). Oxidative stress in ischemic brain damage: Mechanisms of cell death and potential molecular targets for neuroprotection. *Antioxidants & Redox Signaling, 14*(8), 1505–1517.
18. Sanderson, T. H., Reynolds, C. A., Kumar, R., Przyklenk, K., & Hüttemann, M. (2013, February). Molecular mechanisms of ischemia–reperfusion injury in brain: Pivotal role of the mitochondrial membrane potential in reactive oxygen species generation. *Molecular Neurobiology, 47*(1), 9–23.
19. Deb, P., Sharma, S., & Hassan, K. M. (2010, June). Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. *Pathophysiology, 17*(3), 197–218.
20. Ford, A. L., An, H., Vo, K. D., Lin, W., & Lee, J.-M. (2012, June). Defining the ischemic penumbra using hyperacute neuroimaging: Deriving quantitative ischemic thresholds. *Translational Stroke Research, 3*(2), 198–204.
21. Kleinschnitz, C., Fluri, F., & Schuhmann, M. (2015). Animal models of ischemic stroke and their application in clinical research. *Drug Design, Development and Therapy, 9*, 3445.
22. Tamura, A., Graham, D. I., McCulloch, J., & Teasdale, G. M. (1981, March). Focal cerebral ischaemia in the rat: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. *Journal of Cerebral Blood Flow & Metabolism, 1*(1), 53–60.
23. Longa, E. Z., Weinstein, P. R., Carlson, S., & Cummins, R. (1989, January). Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke, 20*(1), 84–91.
24. Schmid Elsaesser, R., Zausinger, S., Hungerhuber, E., Baethmann, A., & Reulen, H. J. (1998, October). A critical reevaluation of the intraluminal thread model of focal cerebral ischemia: Evidence of inadvertent premature reperfusion and subarachnoid hemorrhage in rats by laser-Doppler flowmetry. *Stroke, 29*(10), 2162–2170.
25. Belayev, L., Alonso, O. F., Busto, R., Zhao, W., Ginsberg, M. D., & Hsu, C. Y. (1996, September 1). Middle cerebral artery occlusion in the rat by intraluminal suture: Neurological and pathological evaluation of an improved model. *Stroke, 27*(9), 1616–1623.
26. Howells, D. W., Porritt, M. J., Rewell, S. S., O'Collins, V., Sena, E. S., Van der Worp, H. B., et al. (2010, August). Different strokes for different folks: The rich diversity of animal models of focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 30*(8), 1412–1431.

27. Kuroiwa, T., Xi, G., Hua, Y., Nagaraja, T. N., Fenstermacher, J. D., & Keep, R. F. (2009, January 1). Development of a rat model of photothrombotic ischemia and infarction within the caudoputamen. *Stroke, 40*(1), 248–253.
28. Watson, B. D., Dietrich, W. D., Busto, R., Wachtel, M. S., & Ginsberg, M. D. (1985, May). Induction of reproducible brain infarction by photochemically initiated thrombosis. *Annals of Neurology, 17*(5), 497–504.
29. Yanagisawa, M., Kurihara, H., Kimura, S., Goto, K., & Masaki, T. (1988, December). A novel peptide vasoconstrictor, endothelin, is produced by vascular endothelium and modulates smooth muscle Ca^{2+} channels. *Journal of Hypertension, 6*(4), S188–S191.
30. Hughes, P. M., Anthony, D. C., Ruddin, M., Botham, M. S., Rankine, E. L., Sablone, M., et al. (2003, December). Focal lesions in the rat central nervous system induced by endothelin-1. *Journal of Neuropathology and Experimental Neurology, 62*(12), 1276–1286.
31. Frost, S. B., Barbay, S., Mumert, M. L., Stowe, A. M., & Nudo, R. J. (2006, May). An animal model of capsular infarct: Endothelin-1 injections in the rat. *Behavioural Brain Research, 169*(2), 206–211.
32. Albers, G. W. (1995, February). Antithrombotic agents in cerebral ischemia. *The American Journal of Cardiology, 75*(6), 34B–38B.
33. DiNapoli, V. A., Rosen, C. L., Nagamine, T., & Crocco, T. (2006, June). Selective MCA occlusion: A precise embolic stroke model. *Journal of Neuroscience Methods, 154*(1–2), 233–238.
34. Zhang, Z., Zhang, R. L., Jiang, Q., Raman, S. B. K., Cantwell, L., & Chopp, M. (1997, February). A new rat model of thrombotic focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 17*(2), 123–135.
35. Orset, C., Macrez, R., Young, A. R., Panthou, D., Angles-Cano, E., Maubert, E., et al. (2007, October 1). Mouse model of in situ thromboembolic stroke and reperfusion. *Stroke, 38*(10), 2771–2778.
36. Overgaard, K., Sereghy, T., Pedersen, H., & Boysen, G. (1994, May). Effect of delayed thrombolysis with rt-PA in a rat embolic stroke model. *Journal of Cerebral Blood Flow and Metabolism, 14*(3), 472–477.
37. Zhang, L., Zhang, Z. G., Zhang, C., Zhang, R. L., & Chopp, M. (2004, November 11). Intravenous administration of a GPIIb/IIIa receptor antagonist extends the therapeutic window of intra-arterial tenecteplase-tissue plasminogen activator in a rat stroke model. *Stroke, 35*(12), 2890–2895.
38. Eskelinen, E. L. (2005, April). Maturation of autophagic vacuoles in mammalian cells. *Autophagy, 1*(1), 1–10.
39. Mijaljica, D., Prescott, M., & Devenish, R. J. (2011, July). Microautophagy in mammalian cells: Revisiting a 40-year-old conundrum. *Autophagy, 7*(7), 673–682.
40. PeriyasamyThandavan, S., Jiang, M., Schoenlein, P., & Dong, Z. (2009, August). Autophagy: Molecular machinery, regulation, and implications for renal pathophysiology. *The American Journal of Physiology - Renal Physiology, 297*(2), F244–F256.
41. Kiffin, R. (2004, September 1). Activation of chaperone-mediated autophagy during oxidative stress. *Molecular Biology of the Cell, 15*(11), 4829–4840.
42. Glick, D., Barth, S., & Macleod, K. F. (2010, May). Autophagy: Cellular and molecular mechanisms. *The Journal of Pathology., 221*(1), 3–12.
43. Marzella, L., Ahlberg, J., & Glaumann, H. (1981). Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. *Virchows Archives B, Cell Pathology Including Molecular Pathology, 36*(2–3), 219–234.
44. Klionsky, D. J. (2007, November). Autophagy: From phenomenology to molecular understanding in less than a decade. *Nature Reviews Molecular Cell Biology, 8*(11), 931–937.
45. Ravikumar, B., Sarkar, S., Davies, J. E., Futter, M., Garcia-Arencibia, M., Green-Thompson, Z. W., et al. (2010, October 1). Regulation of mammalian autophagy in physiology and pathophysiology. *Physiological Reviews, 90*(4), 1383–1435.
46. Gabryel, B., Kost, A., & Kasprowska, D. (2012). Neuronal autophagy in cerebral ischemia – a potential target for neuroprotective strategies? *Pharmacological Reports, 64*(1), 1–15.

47. Carloni, S., Girelli, S., Scopa, C., Buonocore, G., Longini, M., & Balduini, W. (2010, April). Activation of autophagy and Akt/CREB signaling play an equivalent role in the neuroprotective effect of rapamycin in neonatal hypoxia-ischemia. *Autophagy, 6*(3), 366–377.
48. Singh, A. K., Kashyap, M. P., Tripathi, V. K., Singh, S., Garg, G., & Rizvi, S. I. (2016). Neuroprotection through rapamycin-induced activation of autophagy and PI3K/Akt1/mTOR/CREB signaling against amyloid-β-induced oxidative stress, synaptic/neurotransmission dysfunction, and neurodegeneration in adult rats. *Molecular Neurobiology*. Available from: http://link.springer.com/10.1007/s12035-016-0129-3.
49. Wang, P., Guan, Y. F., Du, H., Zhai, Q. W., Su, D. F., & Miao, C. Y. (2012, January). Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. *Autophagy, 8*(1), 77–87.
50. Adhami, F., Liao, G., Morozov, Y. M., Schloemer, A., Schmithorst, V. J., Lorenz, J. N., et al. (2006, August). Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. *The American Journal of Pathology, 169*(2), 566–583.
51. Li, J., & McCullough, L. D. (2010, March). Effects of AMP-activated protein kinase in cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism, 30*(3), 480–492.
52. Koike, M., Shibata, M., Tadakoshi, M., Gotoh, K., Komatsu, M., Waguri, S., et al. (2008, February). Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. *The American Journal of Pathology, 172*(2), 454–469.
53. Wen, Y. D., Sheng, R., Zhang, L. S., Han, R., Zhang, X., Zhang, X. D., et al. (2008, August 16). Neuronal injury in rat model of permanent focal cerebral ischemia is associated with activation of autophagic and lysosomal pathways. *Autophagy, 4*(6), 762–769.
54. Xin, X. Y., Pan, J., Wang, X. Q., Ma, J. F., Ding, J. Q., Yang, G. Y., et al. (2011, July). 2-methoxyestradiol attenuates autophagy activation after global ischemia. *Canadian Journal of Neurological Sciences/Journal Canadien des Sciences Neurologiques, 38*(04), 631–638.
55. Kang, C., & Avery, L. (2008, January). To be or not to be, the level of autophagy is the question: Dual roles of autophagy in the survival response to starvation. *Autophagy, 4*(1), 82–84.
56. Shi, R., Weng, J., Zhao, L., Li, X. M., Gao, T. M., & Kong, J. (2012, March). Excessive autophagy contributes to neuron death in cerebral ischemia: Autophagy in cerebral ischemia. *CNS Neuroscience & Therapeutics, 18*(3), 250–260.
57. Qin, A. P., Liu, C. F., Qin, Y. Y., Hong, L. Z., Xu, M., Yang, L., et al. (2010, August). Autophagy was activated in injured astrocytes and mildly decreased cell survival following glucose and oxygen deprivation and focal cerebral ischemia. *Autophagy, 6*(6), 738–753.
58. Tu, X., Yang, W., Chen, J., Chen, Y., Chen, Q., Chen, P., et al. (2015, April). Repetitive ischemic preconditioning attenuates inflammatory reaction and brain damage after focal cerebral ischemia in rats: Involvement of PI3K/Akt and ERK1/2 signaling pathway. *Journal of Molecular Neuroscience, 55*(4), 912–922.
59. Park, H. K., Chu, K., Jung, K. H., Lee, S. T., Bahn, J. J., Kim, M., et al. (2009, February). Autophagy is involved in the ischemic preconditioning. *Neuroscience Letters, 451*(1), 16–19.
60. Yan, W., Zhang, H., Bai, X., Lu, Y., Dong, H., & Xiong, L. (2011, July). Autophagy activation is involved in neuroprotection induced by hyperbaric oxygen preconditioning against focal cerebral ischemia in rats. *Brain Research, 1402*, 109–121.
61. Carloni, S., Buonocore, G., & Balduini, W. (2008, December). Protective role of autophagy in neonatal hypoxia–ischemia induced brain injury. *Neurobiology of Disease, 32*(3), 329–339.
62. Komatsu, M., Ueno, T., Waguri, S., Uchiyama, Y., Kominami, E., & Tanaka, K. (2007, March). Constitutive autophagy: Vital role in clearance of unfavorable proteins in neurons. Cell Death and Differentiation. Available from: http://www.nature.com/doifinder/10.1038/sj.cdd.4402120.
63. Liu, C., Gao, Y., Barrett, J., & Hu, B. (2010, October). Autophagy and protein aggregation after brain ischemia. *Journal of Neurochemistry, 115*(1), 68–78.

Chapter 6
Critical Role of Mitochondrial Autophagy in Cerebral Stroke

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Abstract Mitochondria supply energy to cells by generating ATP; thus it can be considered as one of the essential organelles of the cell. For the efficient working of cells, a good quality of mitochondria is essential; thus the elimination of injured or nonfunctional mitochondria by means of mitophagy is a very important process for cell function. Mitophagy showed a neuroprotective property in cerebral ischemia by accurate labeling and entrapment of defective mitochondria into isolation membranes. Then the entrapped mitochondria were digested by lysosomes. Therefore, the regulation of mitophagy in ischemic brain injury may be used as a therapeutic strategy to protect the neuron by the efficient removal of injured mitochondria.

Keyword Mitochondria · Stroke · Cerebral ischemia · Autophagy · Mitophagy

6.1 Introduction

Stroke main outcomes are sudden death or adult disability. Stroke may be ischemic or hemorrhagic. Ischemic stroke alone accounts for 80% of the stroke. Inadequate blood supply to the brain leads to ischemic stroke [1]. Based on the region of the brain affected, ischemic stroke is of two types: global ischemic stroke and focal ischemic stroke. In global ischemic stroke, the blood supply of the entire brain was significantly reduced. However in focal ischemic stroke, the blood supply was reduced in a particular region of the brain blood vessel. Loss of consciousness, impaired voice, blurred vision, and numbness are the principal symptoms of cerebral ischemia. Due to the suppression of the blood flow during the ischemic

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condition in the brain, the level of the ATP was significantly reduced for a short period of time. That leads to enormous cell death in susceptible regions. Therefore cerebral blood flow is one of the crucial factors for regular functioning of the brain. The damaged region of the ischemic brain which cannot be restored is known as the ischemic core region; however we can restore the region surrounding the core known as the penumbra if treatment is available at the right time. If we are able to recover cerebral blood flow during cerebral ischemia, it limits the nerve cell death in the penumbra region. Therapeutic strategy for ischemic brain injury involves reperfusion and neuroprotection. Usually to restore blood flow in cerebral ischemia, antithrombotic drug was administered as soon as possible. The antithrombotic drugs are of three types based on their mechanism of action. Thrombolytic drugs (recombinant tissue plasminogen activator, streptokinase, etc.) break down an already formed clot. Antiplatelet drugs (aspirin, clopidogrel, etc.) prevent the aggregation of the platelet and limit the formation of a secondary clot. Anticoagulant drugs (heparin, warfarin, dabigatran, etc.) prevent the coagulation of the blood and prevent secondary clot formation. The return of blood flow in the ischemic region leads to a secondary injury known as reperfusion injury. To protect against reperfusion injury and secure the penumbral region from further damage, neuroprotection is another therapeutic approach [2]. But still, the use of recognized neuroprotective agents is unclear in clinical studies.

During ischemic brain damage, the death of nerve cells is reported to have three routes, i.e., necrotic, apoptotic, and autophagic cell death [3, 4]. In this chapter, we have discussed the regulation of mitochondrial autophagy in cerebral ischemia. Mitochondria supply energy to cells by generating ATP; thus it can be considered as one of the essential organelles of the cell. For the efficient working of cells, a good quality of mitochondria is essential; thus the elimination of injured or nonfunctional mitochondria by means of mitophagy is a very important process for cell function. A publication report stated that mitophagy protects nerve cell during stroke condition.

6.2 Autophagy

The autophagy word was given by Christian De Duve [5, 6]. Autophagy is a destructive process which allows the degradation of old, damaged, or nonfunctional cytoplasmic material including proteins and organelle through the liposome's catalytic enzymes. Stress condition like starvation or ischemic event induces autophagy. Autophagy regulates both cell survival and cell death [7]. Mitochondrial autophagy is also known as mitophagy; it involves the selective removal of the mitochondria from the cytoplasm of the cell. The mitophagy process in yeast resembles with mammals [8]. The maintenance of good quality mitochondria is very important for the survival of the brain cell as mitochondria have an extremely shorter life span compared with neurons. Mitophagy regulation is found to be disturbed in nervous system disorder including ischemic brain injury [9–14]. Upregulation of mitophagy

enhances elimination of defected mitochondria and has a protective role in cerebral ischemia [11].

6.3 Nonselective Autophagy and Mitophagy

Nonselective autophagy involves the hydrolysis of cytoplasmic contents, while mitophagy involves selective mitochondrial content hydrolysis by liposome through autolysosomes. Nonselective autophagy mainly occurs at the time of nutrient deficiency. Nonselective autophagy involves the degradation of cytosolic material including proteins and organelle to supply ATP. However mitophagy involves selective degradation of damaged or nonfunctional mitochondria from the cytoplasm [15].

6.4 Mitochondria Dynamics: Fission and Fusion of Mitochondria

Mitochondria are the powerhouse of the cell as they are responsible for the generation of energy. Further mitochondria also supervise the programmed death of the cell [16]. Since brain cells cannot store enough energy sources to perform their function, therefore they carry numerous mitochondria in their cytoplasm. The shape of the mitochondria changes according to the fission or fusion. Mitofusins control mitochondrial fusion through the outer mitochondrial membranes. Mitofusins are of two types: mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2). Optic atrophy 1 (OPA1) controls the fusion of the mitochondria through the inner mitochondria membranes [16, 17]. Any impairment in the Mfn1, Mfn2, and OPA1 is responsible for the wide-ranging mitochondrial fragmentation and loss of mitochondrial DNA [18, 19]. To identify the role of fusion mediators in the early development of the embryo, a study has been performed. This study showed that mice lacking these mediators of the fusion die at an initial stage of fetus development, indicating that mitofusins and optic atrophy 1 are very important in the early stage of embryo formation [18, 20]. Similarly another published report stated that a point mutation in mitofusins and OPA1 leads to severe nervous system disorder [21, 22].

Mitochondrial fission results in the splitting of mitochondria into two daughter mitochondria. Regulation of mitochondria splitting is done by dynamin-related protein 1 (Drp1) and fission 1 protein (Fis1) [23]. During the period of the fission, the Drp1 is enrolled to the mitochondria to form the complex with the Fis1. This complex is responsible for the split of the mitochondria [24, 25]. Mitochondrial fission significantly decreases in Drp1-deficient cell; studies have shown lengthened mitochondria in the cell lacking Drp1 [26]. Mitochondrial fusion and fission are greatly affected by disease states. Reports are available showing that in pathological

condition such as neurodegenerative disorders and brain trauma, there are imbalances between fusion and fission events [27]. Furthermore, mitochondrial fission mediates mitochondria-dependent cell death [26]. The discharge of pro-apoptotic factors, such as cytochrome c from depolarized mitochondria into the cytosol, is associated with Drp1-mediated fragmentation of the mitochondria which induced apoptosis [23].

6.5 Molecular Mechanisms of Mitophagy

Mitophagy involves the removal of the selective mitochondria from the cytoplasm of the cell. To remove the selective mitochondria from the cytoplasm, damaged or nonfunctional mitochondria are tagged followed by the entrapment into the isolation membrane and formation of autolysosomes.

The autophagy-related gene (Atg) regulates mitophagy through the autophagy-related protein as per the metabolic demands of the cell. For example, Atg 32 protein helps in the initiation of the mitophagy, and Atg 8 protein is necessary for the sealing of the mitochondria through a membrane in yeast. Another protein known as Atg11 is helpful in the sealing of the mitochondria by isolation membrane. Once the mitochondria are sealed completely by the membrane in autophagosome, the sealed mitochondria are fused with the liposomes which digest the defected mitochondria.

During the differentiation of the red blood cell, mitophagy is responsible for the removal of the red blood cell mitochondria. At the time of mitochondrial removal from the red blood cell, the BNIP3L (NIX) expression increases on the outer mitochondrial membrane and has a WXXL-like motif which forms a linkage to LC3. This complex generates the isolation membranes and forms autophagosomes and fuses liposome to form autolysosome. The catalytic enzymes of the liposome hydrolyzed the mitochondrial content.

When mitochondria are injured and lose membrane potential, the PINK1 accumulates and recruits the E3 ubiquitin ligase parkin from the cytosol particularly to the damaged mitochondrion. Parkin promotes the binding of ubiquitinated substrates such as MARF, Mfn1, Mfn2, and VDAC1 to the outer mitochondrial membrane; p62 is recruited to the mitochondria, binding with the ubiquitinated substrates and linking to LC3 to form an isolated membrane and then fuse with lysosomes [15].

6.6 Mitophagy in Cerebral Ischemia

Adenosine monophosphate-activated protein kinase (AMPK), autophagy proteins 5–12 (Atg5–Atg12), dynamin-related protein 1(DRP1), light chain 3 (LC3), mammalian target of rapamycin (mTOR), mitochondrial assembly regulatory factor

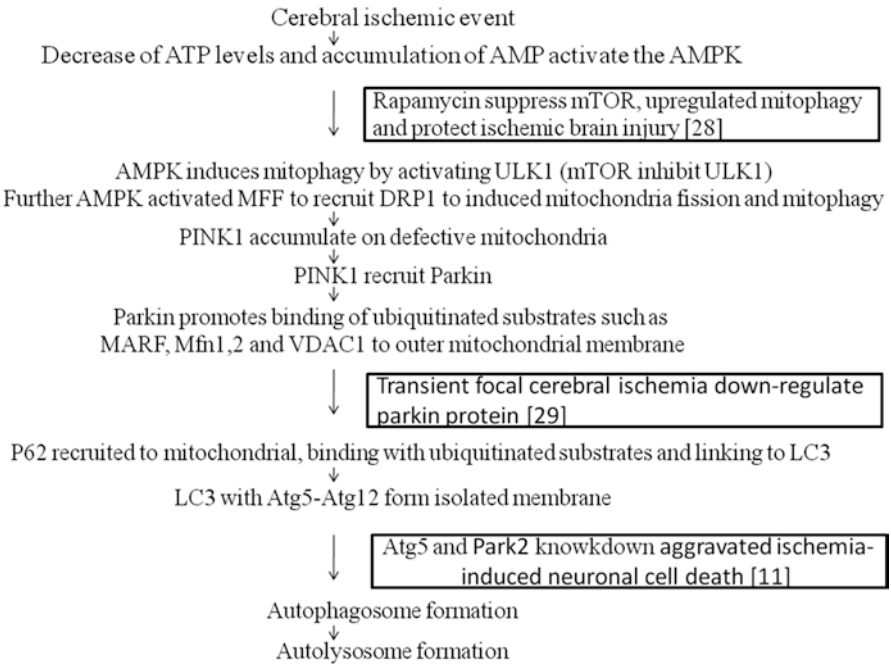

Fig. 6.1 Showing the role of mitophagy mediators in cerebral ischemia

(MARF), mitofusins 1 and 2 (Mfn1, Mfn2), mycophenolate mofetil (MMF), p62/SQSTM1/sequestosome-1 (p62), PTEN-induced putative kinase 1 (PINK1), serine/threonine-protein kinase (ULK1), voltage-dependent anion-channel 1 (VDAC1) (Fig. 6.1).

Mitophagy involves selective removal of mitochondria from the cytoplasm by liposomes. During ischemic event, there is shortage of blood supply to the brain. Blood supplies oxygen and nutrient to the brain, and shortage of blood to the brain event for a short period of 1–2 min causes severe shortage of ATP in the brain. This causes formation of reactive oxygen species and leads to the mitochondrial membrane's potential depolarization. Reduction in ATP levels and accumulation of AMP activate the AMPK. AMPK induces mitophagy by activating ULK1 (mTOR inhibit ULK1). Further AMPK activates MFF to recruit DRP1 to induce mitochondrial fission and mitophagy. PINK1 is accumulated in the defective mitochondria triggering the recruitment of the parkin. Parkin promotes binding of ubiquitinated substrates such as MARF, Mfn1, Mfn2, and VDAC1 to the outer mitochondrial membrane. p62 is recruited to the mitochondria, binding with ubiquitinated substrates and linking to LC3 for the autophagic degradation. LC3 with Atg5–Atg12 forms autophagosomes. Autophagosomes fuse with lysosomes to form the autolysosomes and hydrolyze the mitochondrial contents. Rapamycin suppresses mTOR, defending the rat brain from ischemic injury by upregulating mitophagy. Similarly Parkin and Atg5 knockdown aggravates ischemia-induced neuronal cell death, indicating that

upregulation of mitophagy is responsible for the protection of the brain cell from ischemic brain injury [28, 30, 31].

6.7 Effect of Cerebral Ischemia in Mitochondrial Dynamic Mediators

Ischemic event in the brain shifts the balance of the mitochondrial fission and fusion toward the fission. Dynamin-related GTPases, i.e., Mfn1, Mfn2, and Opa1, regulate the mitochondrial fusion, while Fis1 and Drp1 regulate mitochondrial fission [12, 17, 24, 32]. Two daughter mitochondria are generated after fission. The daughter mitochondria have altered membrane potential. These daughter mitochondria cannot be fused and are more likely to be taken up by autophagosome due to their smaller size and facilitate mitophagy [33]. Both mitochondrial fission and mitophagy are beneficial, working together to eliminate damaged and depolarized mitochondria. A study revealed that the level of the mitochondrial fusion proteins Opa1 and Mfn2 was significantly reduced in the condition of cerebral ischemia [34]. Further another recent study showed that Drp1 protects against ischemic injury by facilitating mitophagy [35] (Fig. 6.2).

Mitofusins Mfn1 and Mfn2 (Mfn1, Mfn2), optic atrophy 1 (Opa1), fission 1 protein (Fis1), dynamin-related protein 1 (Drp1)

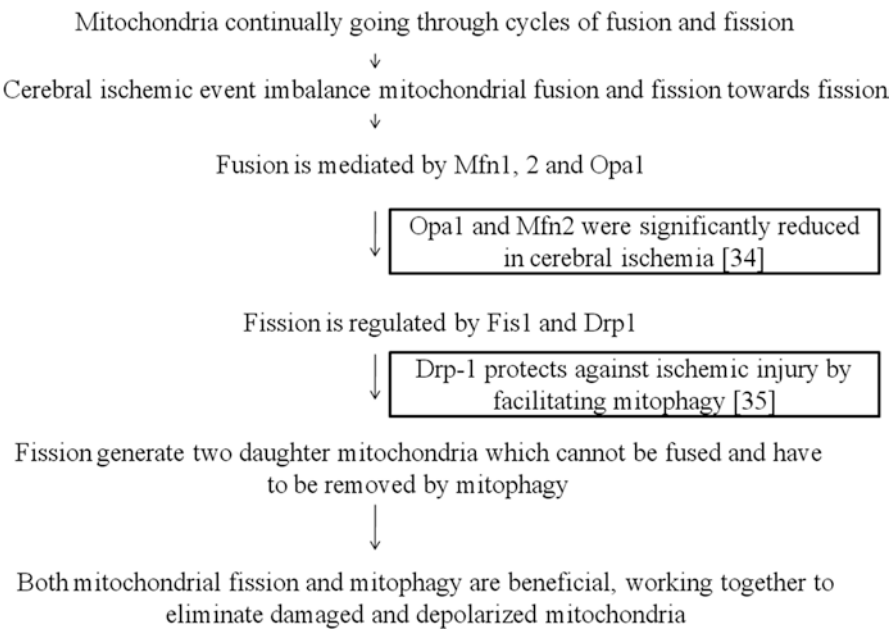

Fig. 6.2 Showing the role of mitochondrial dynamic mediators in cerebral ischemia

6.8 Mitophagy Regulation: An Anti-inflammation Approach in Cerebral Ischemia

Defected mitochondria lead to the release of mitochondrial DNA and reactive oxygen species (ROS) which in turn activate inflammasome [36]. The inflammasome is responsible for the release of the pro-inflammatory marker such as cytokines interleukin 1β and interleukin 18 which leads to programmed cell death due to inflammation. The published report suggests that nuclear factor-κB avoids inflammasome activation by regulating mitophagy to prevent inflammation [37]. Further, studies confirmed that compound that sustains mitochondrial membrane potential intact and inhibited NOD-like receptor (NLRP3) are found to decrease the injury related to the ischemia in the brain [38].

6.9 Mitophagy Regulation: A Neuroprotective Approach in Cerebral Ischemia

Rapamycin shows neuroprotection against cerebral ischemic reperfusion injury through the activation of autophagy [39]. Similarly another recent study reported that rapamycin promotes the L3-II and Beclin-1 that attenuate cerebral ischemia-induced brain injury [14]. The proposed mechanism of action of rapamycin during ischemia is through the increased expression of p62 in mitochondria [14]. Further another compound, methylene blue, shows neuroprotection against cerebral ischemia-reperfusion injury [40]. The exact mechanism of methylene blue is still not known, but it is thought to augment mitophagy [41, 42].

6.10 Conclusion

Mitophagy involves the specific degradation of the damaged or nonfunctional mitochondria. Starvation, oxidative stress, or specific signals including mitochondrial targeting of signaling proteins or modification of mitochondrial proteins induce mitophagy. Since the clearance of the damaged or nonfunctional mitochondria is essential to maintain the homeostasis of cells, the dysfunction of mitophagy is closely related to cerebral diseases. Cerebral ischemic reperfusion injury activates mitophagy. Therefore the regulation of the mitophagy pathway in cerebral ischemic reperfusion injury to protect the nerve cells might be an effective strategy for the treatment of stroke.

References

1. Paliwal, P., Dash, D., & Krishnamurthy, S. (2017). Pharmacokinetic study of piracetam in focal cerebral ischemic rats. *European Journal of Drug Metabolism and Pharmacokinetics*, 1–9.
2. Paliwal, P., Chauhan, G., Gautam, D., Dash, D., Patne, S. C. U., & Krishnamurthy, S. (2018). Indole-3-Carbinol improves neurobehavioral symptoms in a cerebral ischemic stroke model. *Naunyn-Schmiedeberg's Archives of Pharmacology, 391*, 613–625.
3. Lipton, P. (1999). Ischemic cell death in brain neurons. *Physiological Reviews, 79*, 1431–1568.
4. Carloni, S., Girelli, S., Scopa, C., Buonocore, G., Longini, M., & Balduini, W. (2010). Activation of autophagy and Akt/CREB signaling play an equivalent role in the neuroprotective effect of rapamycin in neonatal hypoxia-ischemia. *Autophagy, 6*, 366–377.
5. Deter, R. L., & De Duve, C. (1967). Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *The Journal of Cell Biology, 33*, 437–449.
6. Deter, R. L., Baudhuin, P., & De Duve, C. (1967). Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *The Journal of Cell Biology, 35*, C11–C16.
7. Yu, L., Alva, A., Su, H., Dutt, P., Freundt, E., Welsh, S., Baehrecke, E. H., & Lenardo, M. J. (2004). Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science, 304*(5676), 1500–1502.
8. Liu, L., Sakakibar, a. K., Chen, Q., & Okamoto, K. (2014). Receptor-mediated mitophagy in yeast and mammalian systems. *Cell Research, 24*, 787–795.
9. Santos, R. X., SC, C. a., Wang, X., Perry, G., Smith, M. A., Moreira, P. I., et al. (2010). A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease. *Journal of Alzheimer's Disease, 20*(2), 401–412.
10. Vives-Bauza, C., & Przedborski, S. (2011). Mitophagy: The latest problem for Parkinson's disease. *Trends in Molecular Medicine, 17*(3), 158–165.
11. Zhang, X., Yan, H., Yuan, Y., Gao, J., Shen, Z., Cheng, Y., Shen, Y., Wang, R. R., Wang, X., Hu, W. W., & Wang, G. (2013). Cerebral ischemia-reperfusion-induced autophagy protects against neuronal injury by mitochondrial clearance. *Autophagy, 9*(9), 1321–1333.
12. Zuo, W., Zhang, S., Xia, C. Y., Guo, X. F., He, W. B., & Chen, N. H. (2014). Mitochondria autophagy is induced after hypoxic/ischemic stress in a Drp1 dependent manner: The role of inhibition of Drp1 in ischemic brain damage. *Neuropharmacology, 86*, 103–115.
13. Huang, C., Andres, A. M., Ratliff, E. P., Hernandez, G., Lee, P., & Gottlieb, R. A. (2011). Preconditioning involves selective mitophagy mediated by Parkin and p62/SQSTM1. *PLoS One, 6*(6), e20975.
14. Li, Q., Zhang, T., Wang, J., Zhang, Z., Zhai, Y., Yang, G. Y., & Sun, X. (2014). Rapamycin attenuates mitochondrial dysfunction via activation of mitophagy in experimental ischemic stroke. *Biochemical and Biophysical Research Communications, 444*, 182–188.
15. Youle, R. J., & Narendra, D. P. (2011). Mechanisms of mitophagy. *Nature Reviews Molecular Cell Biology, 12*(1), 9.
16. Kroemer, G., Dallaporta, B., & Resche-Rigon, M. (1998). The mitochondrial death/life regulator in apoptosis and necrosis. *Annual Review of Physiology, 60*(1), 619–642.
17. Chen, H., & Chan, D. C. (2010). Physiological functions of mitochondrial fusion. *Annals of the New York Academy of Sciences, 1201*, 21–25.
18. Chen, H., Chomyn, A., & Chan, D. C. (2005). Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *The Journal of Biological Chemistry, 280*, 26185–26192.
19. Detmer, S. A., & Chan, D. C. (2007). Functions and dysfunctions of mitochondrial dynamics. *Nature Reviews Molecular Cell Biology, 8*, 870–879.
20. Cipolat, S., Rudka, T., Hartmann, D., Costa, V., Serneels, L., Craessaerts, K., Metzger, K., Frezza, C., Annaert, W., D'Adamio, L., & Derks, C. (2006). Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell, 126*(1), 163–175.

21. Züchner, S., Mersiyanova, I. V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J., & Parman, Y. (2004). Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nature Genetics, 36*(5), 449.
22. Alexander, C., Votruba, M., Pesch, U. E., Thiselton, D. L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G., & Bhattacharya, S. S. (2000). OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nature Genetics, 26*(2), 211.
23. Chang, C. R., Manlandro, C. M., Arnoult, D., Stadler, J., Posey, A. E., Hill, R. B., & Blackstone, C. (2010). A lethal de novo mutation in the middle domain of the dynamin-related GTPase Drp1 impairs higher order assembly and mitochondrial division. *Journal of Biological Chemistry, 285*(42), 32494–32503.
24. James, D. I., Parone, P. A., Mattenberger, Y., & Martinou, J. C. (2003). hFis1, a novel component of the mammalian mitochondrial fission machinery. *The Journal of Biological Chemistry, 278*, 36373–36379.
25. Smirnova, E., Shurland, D. L., Ryazantsev, S. N., & van der Bliek, A. M. (1998). A human dynamin-related protein controls the distribution of mitochondria. *The Journal of Cell Biology, 143*, 351–358.
26. Ishihara, N., Nomura, M., & Jofuku, A. (2009). Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nature Cell Biology, 11*, 958–966.
27. Hoppins, S., Lackner, L., & Nunnari, J. (2007). The machines that divide and fuse mitochondria. *Annual Review of Biochemistry, 76*, 751–780.
28. Takagi, H., Matsui, Y., Hirotani, S., Sakoda, H., Asano, T., & Sadoshima, J. (2007). AMPK mediates autophagy during myocardial ischemia in vivo. *Autophagy, 3*, 405–407.
29. Mengesdorf, T., Jensen, P. H., Mies, G., Aufenberg, C., & Paschen, W. (2002). Down-regulation of parkin protein in transient focal cerebral ischemia: A link between stroke and degenerative disease? *Proceedings of the National Academy of Sciences of the United States of America, 99*, 15042–15047.
30. Tang, Y. C., Tian, H. X., Yi, T., & Chen, H. B. (2016). The critical roles of mitophagy in cerebral ischemia. *Protein & Cell, 7*(10), 699–713.
31. Wang, P., Guan, Y. F., Du, H., Zhai, Q. W., Su, D. F., & Miao, C. Y. (2012). Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. *Autophagy, 8*(1), 77–87.
32. Yamamori, T., Ike, S., Bo, T., Sasagawa, T., Sakai, Y., Suzuki, M., Yamamoto, K., Nagane, M., Yasui, H., & Inanami, O. (2015). Inhibition of the mitochondrial fission protein dynamin-related protein 1 (Drp1) impairs mitochondrial fission and mitotic catastrophe after x-irradiation. *Molecular Biology of the Cell, 26*(25), 4607–4617.
33. Gomes, L. C., Di Benedetto, G., & Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nature Cell Biology, 13*, 589–598.
34. Kumari, S., Anderson, L., Farmer, S., Mehta, S. L., & Li, P. A. (2012). Hyperglycemia alters mitochondrial fission and fusion proteins in mice subjected to cerebral ischemia and reperfusion. *Translational Stroke Research, 3*, 296–304.
35. Zuo, W., Yang, P. F., Chen, J., Zhang, Z., & Chen, N. H. (2016). Drp-1, a potential therapeutic target for brain ischaemic stroke. *British Journal of Pharmacology, 173*(10), 1665–1677.
36. Gurung, P., Lukens, J. R., & Kanneganti, T. D. (2015). Mitochondria: Diversity in the regulation of the NLRP3 inflammasome. *Trends in Molecular Medicine, 21*, 193–201.
37. Zhong, Z., Umemura, A., Sanchez-Lopez, E., Liang, S., Shalapour, S., Wong, J., He, F., Boassa, D., Perkins, G., Ali, S. R., & McGeough, M. D. (2016). NF-κB restricts inflammasome activation via elimination of damaged mitochondria. *Cell, 164*(5), 896–910.
38. Zhao, J., Mou, Y., Bernstock, J. D., Klimanis, D., Wang, S., Spatz, M., Maric, D., Johnson, K., Klinman, D. M., Li, X., & Li, X. (2015). Synthetic oligodeoxynucleotides containing multiple telemeric TTAGGG motifs suppress inflammasome activity in macrophages subjected to oxygen and glucose deprivation and reduce ischemic brain injury in stroke-prone spontaneously hypertensive rats. *PLoS One, 10*(10), e0140772.

39. Malagelada, C., Jin, Z. H., Jackson-Lewis, V., Przedborski, S., & Greene, L. A. (2010). Rapamycin protects against neuron death in in vitro and in vivo models of Parkinson's disease. *The Journal of Neuroscience, 30*, 1166–1175.
40. Miclescu, A., Sharma, H. S., Martijn, C., & Wiklund, L. (2010). Methylene blue protects the cortical blood–brain barrier against ischemia/reperfusion-induced disruptions. *Critical Care Medicine, 38*, 2199–2206.
41. Di, Y., He, Y. L., Zhao, T., Huang, X., Wu, K. W., Liu, S. H., Zhao, Y. Q., Fan, M., Wu, L. Y., & Zhu, L. L. (2015). Methylene blue reduces acute cerebral ischemic injury via the induction of mitophagy. *Molecular Medicine, 21*, 420–429.
42. Jin, R., Yang, G., & Li, G. (2010). Inflammatory mechanisms in ischemic stroke: Role of inflammatory cells. *Journal of Leukocyte Biology, 87*, 779–789.

Chapter 7
Application of Neuroimaging Tools in Identification of Pinpoint Location of Blockage

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Abstract Stroke is a significant disease which causes disability and even death to a vast number of persons around the world especially in the modernized and developed countries like the USA. It is caused either by blockage (ischemic stroke = 85%) or by rupture (hemorrhagic stroke) of the blood vessel. Hence we see the ischemic stroke is the most common stroke in which there is a lack of supply of nutrients into and any part of the brain due to blockage of any blood vessel. Due to this, the nerve cells start dying and hence reflected as a hypodense area in the image. There are various imaging modalities to analyze the dysfunctioning of the brain like ultrasound, CT, MRI, PET, SPECT, etc. Over the period CT and MRI had been widely used based on its advantages and limitations depending on the application and availability of these imaging systems. This chapter primarily discusses, in brief, all the conventional imaging modalities and also deals with further improvement in the quality of these imaging systems by the application of image processing which can assist to get a clear picture about the stroke detection by the physician. Image processing involves various steps like removal of noise in the image, image segmentation, image enhancement, etc. After following these image processing steps, it is easy to diagnose an apparent pinpoint location in a precise and accurate manner for the detection of the stroke.

Keywords CT · MRI · PET · SPECT · Ischemia · Hemorrhage

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7.1 Introduction

The brain is a very complex structure of the human body; it performs various tasks like body control and movement, speech or language processing, memory control, conscious control, emotion control, cognition control, etc. [1]. The brain is composed of various neural cells which survive on a continuous supply of one of the essential nutrients like O_2 and glucose provided through the bloodstream. In a situational consequence, if there is a lack of blood supply to the brain, the nerve cells start to die, which may result in the disability of certain parts or the whole body depending upon the location of dead nerve cells which control any particular body part or action. This phenomenon is termed as stroke [2]. It may be a result of two factors: (1) an interruption in blood supply (ischemic stroke = 85%) due to clot formation inside any blood vessel and (2) rupture of any blood vessel (hemorrhagic = 15%). It is important to consider here that brain cells divide and form new cells only for a brief period after the birth and hence in later stages of life, dead brain cells can't be replaced by newer ones [3]. That's why there is a limited chance of rehabilitation after the stroke if it is diagnosed at later stages.

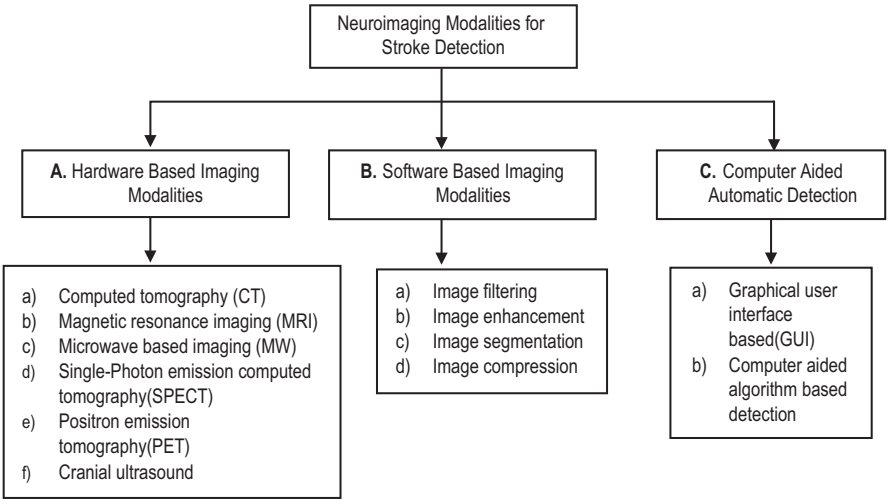
There are various risk factors [4] associated with stroke, which are broadly classified into two: (a) controllable and (b) uncontrollable. The controllable risk factors include unhealthy lifestyles like smoking, drinking alcohol, use of tobacco, etc. and some other factors like high blood pressure, high cholesterol, diabetes mellitus, physical inactivity, etc. Uncontrollable risk factors include hereditary factors like age, gender, race, family history, etc. and transient ischemic stroke, patent foramen ovale (hole in the heart), etc.

According to the World Health Organization (WHO), it costs around 6 million lives each year, and there are around 15 million people who suffer from this disease [5]. It is most common in the age group of above 60 as it ranks as a second leading cause of death to them. Demographic statistics show that women have a longer life span than men and hence have more chances for the occurrence of stroke because it occurs mostly in the older age groups.

Anyone with symptoms of stroke needs an immediate medical help; it doesn't matter if it is a stroke or not after diagnosis. It takes only a fraction of time to disable the functioning of particular organ or the whole body of the person, and also the fact that many deadliest diseases can also be detected while diagnosing stroke, because of the similar nature of the symptoms like in the case of cardiac arrest. The physician needs to consider the various facts [6] to diagnose the stroke like family history of stroke, laboratory tests, cardiac evaluation, imaging studies, angiography, ultrasound, blood flow test, etc. In order to treat the patient, the physician may use anticoagulant and antiplatelet medications and/or surgery if needed.

7.2 Neuroimaging Modalities for Stroke Detection

Stroke is the main cause of a huge number of deaths every year. Over the years it has been found that by proper detection of stroke before death or disability of a person, it can be prevented. And also if after the occurrence of stroke and if the patient gets the symptom of paralysis, it can be rehabilitated. To do so, we need to get prior information about the stroke, which can be achieved by various neuroimaging modalities [6] as shown below.



7.3 Hardware-Based Imaging Modalities

There are various imaging techniques which require a significant hardware like CT machine, MRI machine, ultrasound machine, etc. These imaging techniques are discussed below.

7.3.1 Computed Tomography (CT)

This imaging technique [7] is based upon the radiation effect on human tissue which is plotted in the form of a CT scan where the subject is placed inside a rotating chamber and the radiation pattern is drawn from various angles of incidence of radiation to the subject. Because of its quicker performance, this technique is preferred in emergency situations where the subject is in a critical condition.

7.3.2 Magnetic Resonance Imaging(MRI)

Magnetic resonance imaging [8] is an imaging technique which utilizes non-ionizing radiation to create images of the tissues of the brain. There is no radioactive element involved in this procedure and is completely radiation-free. It is a tool for the detection of early ischemic stroke lesions with a high sensitivity. It can also detect ICH with an accuracy comparable to CT.

7.3.3 Microwave-Based Imaging(MW)

Microwave imaging is a novel technique for the detection of stroke. It is based on the difference between the normal and abnormal brain tissue dielectric properties [9]. It is a noninvasive [10] imaging technique for the monitoring of the brain functioning.

7.3.4 Single-Photon Emission Computed Tomography (SPECT)

It is a functional nuclear imaging [11] technique used to evaluate the regional cerebral perfusion. It operates on gamma rays with the help of a gamma camera. The scanning process monitors the level of biological activities in the 3-D region. Multiple 2-D images are obtained from various angles.

7.3.5 Positron Emission Tomography (PET)

Positron emission tomography is a technique which gives the functional information about the brain in the early phase of disease before the occurrence of any anatomical changes in the brain compared to CT or MRI which only gives anatomical details of the brain [12]. PET scan can show the increased or decreased metabolism rate at the seizure focus point. It can provide information about the pinpoint location of the stroke before surgery.

7.3.6 Cranial Ultrasound

Cranial ultrasound [13] utilizes reflected sound waves to generate the picture of the brain and its inner fluid which flows within its chambers. This test is mostly done on the babies because ultrasound can't penetrate into the hard bones. So it is used to check the problems of the brain just a few days after birth unto the age of 18 months.

7.4 Software-Based Image Processing Modalities

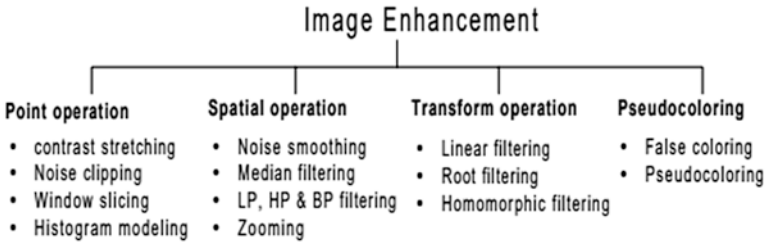
The scanned images from any of the abovesaid imaging modalities contain several types of noises which may provide a wrong information to the visual perception by the physician. An erroneous detection will result in the wrong treatment which can cause death to the patient as there is a very less time for survival during a stroke attack. So there are various image processing techniques which are used in association with these imaging techniques to get a clear view of a picture obtained so that various features can be evaluated correctly to detect the stroke or any other diseases. Various such techniques are discussed below.

7.4.1 Image Filtering

Image obtained by these imaging modalities may contain several types of noises like salt-and-pepper noise (caused by sudden sharp disturbances), Gaussian noise (resulting from random fluctuations), speckle noise (can be modeled by random values multiplied by pixel values), periodic noise, etc. Salt-and-pepper noise can be removed by applying low-pass filtering, median filtering, rank order filtering, an outlier method, etc. Gaussian noise can be cleaned by image averaging, average filtering, Wiener filtering, etc. [14].

7.4.2 Image Enhancement

Image enhancement is a technique which is used to improve the subjective quality of the pictures for human interpretation. To distinguish one object from another, contrast is an important parameter which gives the subjective evaluation of the image quality. A difference in the color brightening pattern can be seen as contrast enhancement. Our eyes are more sensitive toward the contrast than just luminance [15]. Various image enhancement operations are summarized below.



7.4.3 Image Segmentation

Image segmentation is a technique which divides the image into various segments or parts. This is usually done to detect or identify individual objects or any other relevant information from the digitalized images. This task can be performed by various algorithms like thresholding methods (Otsu's method), color-based segmentation (k-means clustering), transform methods (watershed transform), texture methods (texture filters), etc. [16].

7.4.4 Image Compression

With the advancement in technology and the requirement of the better-quality pictures, we now tend to use more and more numbers of megapixels for our cameras, which in turn give a better quality of the image, but at the very same time, it also increases the memory capacity requirement associated with it. So to store a large number of files, we need more space which will not be a good thing as it'll cost you more, both the cost of database and also the weight. Also, it becomes difficult to upload the high-quality images of large size over the Internet if the speed is slow. So there is a need to compress the image size to save the space, but without degrading or compromising the image quality [17].

7.5 Computer-Aided Automatic Detection

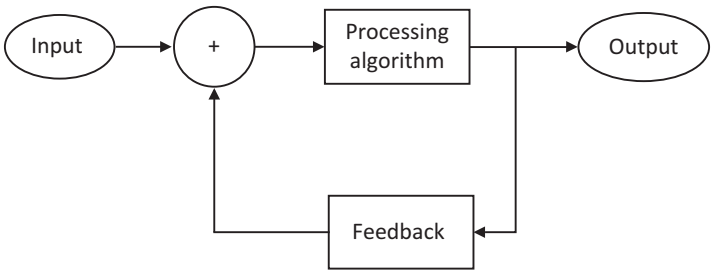
7.5.1 Graphical User Interface Based (GUI)

A graphical user interface is a visual display of some buttons and display screens that perform tasks defined by associated algorithm to that particular button. It is more user-friendly as an ordinary layperson with a sound understanding of the process can use it even if he doesn't know the background programming. It utilizes graphical symbols rather than typing instructions, and hence more emphasis is on the utilization of the mouse rather than keyboard [18].

7.5.2 Computer-Aided Algorithm-Based Detection

A computer-aided algorithm-based detection involves various image processing steps in a single click. All the programming steps are written in the background, and once we click the result button, the input image is transformed into the output image with desired region extraction along with the calculated parameters like area,

eccentricity, energy or intensity values, etc. [19]. The following block diagram can understand the basic understanding of the method.



7.6 Comparison of Neuroimaging Modalities

The following comparison table can understand the comparison of various neuroimaging modalities.

SN	Imaging modality	Application	Sensitivity	Positives	Negatives
1.	Positron emission tomography (PET)	To find the existence of penumbra in the humans	−93.7%	It offers semiquantitative or quantitative hemodynamic data	Radiation
					High cost
		It acts as a gold standard in the evolution of early stroke			Limited availability
2.	Computed tomography (CT)	Non-contrast CT is used for the evaluation of hemorrhagic stroke detection	31% (acute ischemic stroke)	Early signs of stroke	Reduced sensitivity in ischemic stroke detection
			57% (acute stroke under normal parameters)	It is preferred in hemorrhagic stroke detection	
			71%(using soft window and variable)	Wider availability and low cost	
3.	CT perfusion	Used for imaging penumbra	>75% (ischemic stroke)	It is less invasive than angiography and more widely available than MR imaging	Slight chance of cancer from excessive exposure to radiation
		Salvageable tissue indicated by penumbra	>90% (for infarcts in supraterritorial regions)		

(continued)

SN	Imaging modality	Application	Sensitivity	Positives	Negatives
4.	CT angiography	Ischemic stroke detection Intravascular thrombi	It gives equal accuracy as MRA	Increased contrast and sensitivity for early ischemic stroke detection	Chances of an allergic reaction to contrast material
5.	Single-photon emission computed tomography (SPECT)	Another CT with radiotracers such as Xe, HMPAO, or ECD to evaluate CBF and cerebrovascular reserve	90% sensitivity for acute stroke (much higher than CT)	Safe Allows for repeated studies for serial measurements Can provide functional information	It can't measure absolute CBF or metabolism
6.	T1 MRI and T2 MRI	Hemorrhage detection Cacogenic edema T2W1 as a "gold standard"	T2W1 has a greater sensitivity (90%) compared to T1W1 (50%)	Acute infarcts can be clearly visible	There are no known side effects of an MRI scan
7.	Diffusion-weighted imaging (DWI)	Detects the movements of H_2O molecules Ischemic stroke detection	>Non-contrast CT scan	More sensitive for the detection of hyper acute ischemia	Identify patients at sufficiently low risk to warrant ED discharge
8.	Perfusion-weighted imaging	To detect the first event after the ischemic stroke Disturbed CBF detection	97.5% for detection of ischemic stroke	Provides metabolic and hemodynamic data of the brain in the first few hrs poststroke	Frequently overestimates final infarct volume
9.	Diffusion tensor imaging (DTI)	Advanced diffusion MRI Fully utilize the information obtained from diffusion anisotropy causes by biological boundaries to infer the exquisite details of tissue microstructure	–	Used to visualize the restoration of structural integrity and connectivity	–
10.	Functional magnetic resonance imaging (fMRI)	fMRI using blood-O_2 level depends on BOLD, CBF, or CBV contrast To visualize brain activity noninvasively Functional recovery study	High sensitivity	Ease of implementation High sensitivity	–

7.7 Conclusion

In this chapter, we have discussed various imaging techniques which have been compared with one another, and in addition to that, there are also few advanced image processing techniques that were discussed which are nowadays utilized for a better quality of image at a reasonably required memory space to store that picture. Imaging modalities can be broadly classified under the terms radioactive and nonradioactive imaging systems, since it is a safe side to allow the nonradioactive imaging to the patient as it will not cause any harm to the patient as in the case of MRI. So it is always preferable to use MRI for stroke detection, but it's not the case everywhere due to its limited availability and its huge cost. Also in emergency situations, CT is preferable as it takes less time to operate and it also has a better availability in most of the areas. So there should be a trade-off between the radiation dose and health hazard to the patient, and suitable imaging system should be utilized. Once after imaging is done, there is a need to enhance the quality of the image, so proper image processing steps should be followed to get a clearer view of the picture in order to detect the diseased area. For example, in the present case of detecting the pinpoint location of the stroke area firstly, we have to remove the noise associated with the scanned image, and after that the image should be enhanced to give a bright and clear view, and finally the stroke area has to be segmented and extracted as to detect the stroke location.

References

1. [Online]. Available: https://medicine.yale.edu/intmed/genmed/ourresearch/iris/stroke247601_174718_30867.pdf
2. [Online]. Available: http://www.webmd.com/heart-disease/stroke
3. [Online]. Available: https://www.ahajournals.org/doi/pdf/10.1161/01.STR.0000255757.12198.0f
4. [Online]. Available: https://www.nhlbi.nih.gov/health/health-topics/topics/stroke/atrisk
5. [Online]. Available: http://www.strokeassociation.org/STROKEORG/AboutStroke/Impact-of-Stroke-Stroke-statistics_UCM_310728_Article.jsp
6. [Online]. Available: https://www.strokeassociation.org/idc/groups/stroke-public/@wcm/@hcm/documents/downloadable/ucm_309713.pdf
7. [Online]. Available: http://cdn.iiit.ac.in/cdn/cvit.iiit.ac.in/images/ConferencePapers/2009/Mayank09Amethod.pdf
8. [Online]. Available: https://radiopaedia.org/articles/mri-introduction
9. [Online]. Available: https://en.wikipedia.org/wiki/Microwave_imaging
10. Scapaticci, L. R., Di Donato, L., et al. (2012). A feasibility study on microwave imaging for brain stroke monitoring. *Progress In Electromagnetics Research B, 40*, 305–324.
11. Mountz, J. M., Modell, J. G., et al. (1990, January). Prognostication of recovery following stroke using the comparison of CT and technetium 99m HM-PAO SPECT. *The Journal of Nuclear Medicine, 31*, 61–66.
12. [Online]. Available: http://www.petscaninfo.com/zportal/portals/pat/brain.
13. [Online]. Available: http://www.webmd.com/brain/cranial-ultrasound#1

14. [Online]. Available: https://eclass.teicrete.gr/modules/document/file.php/TM152/Books/Matlab-Image_Processing_Tutorial.pdf
15. [Online]. Available: https://sisu.ut.ee/imageprocessing/book/5
16. [Online]. Available: https://in.mathworks.com/discovery/image-segmentation.html
17. [Online]. Available: http://electronicsforu.com/electronics-projects/lossless-image-compression-using-matlab
18. [Online]. Available: http://study.com/academy/lesson/what-is-a-graphical-user-interface-gui-definition-components-examples.html
19. Tyan, Y.-S., & Wu, M.-C. (2014). Ischemic stroke detection system with a computer-aided diagnostic ability using an unsupervised feature perception enhancement method. *International Journal of Biomedical Imaging, 2014*, 947539.

Chapter 8
Emerging Role of Electromagnetic Field Therapy in Stroke

Chandra Kant Singh Tekam, Amit Kumar Tripathi, Gaurav Kumar, and Ranjana Patnaik

Abstract Interest in the clinical utilization of the magnetic field is increasing globally. Various articles have suggested the use of magnetic fields to initiate neuroprotective and neuro-regenerative effects on different biological systems that are of critical importance for the treatment of various injuries. Electromagnetic therapy provides a secure method for the direct treatment of injuries. The magnetic field may cause cell proliferation, genotoxic effects, changes in cell membrane permeability, and osteoblast formation. Some studies focus on the application of magnetic fields with different frequency bands and its corresponding effects at a cellular level. It has recently found that the static magnetic field (SMF) and the pulsating magnetic field (PEMF) can enhance the therapeutic outcome owing to anti-inflammatory and neuro-regenerative effects in animals and humans. In this chapter, we have included various mechanisms for neuroprotection and many experimental pieces of evidence to support the hypothesis that magnetic fields might constitute a non-invasive mode of therapy. Meanwhile, some of the experimental studies demonstrate the occurrence of protection, and various other articles propose that magnetic fields influence biochemical systems.

Keywords Stroke · Static magnetic field · Pulsed magnetic field · Ischemic neuronal damage · DNA damage

8.1 Introduction

In the present scenario, medicine is based primarily on the development of drug delivery methods that deal with the interaction of a particular chemical substance and its effects on cells, tissue, and metabolic activities, and is further utilized by the

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pharmaceutical industries in drug development methodologies. Most of the drugs affect diseased and healthy tissues simultaneously, which could lead to adverse side effects. In contrast, electromagnetic therapy provides a secure method for curing the site of disease or injury [1].

After World War II, the clinical application of electromagnetic therapy adopted in countries such as Russia, Japan, and Romania, and development in the following years paved the way for the increased use of static magnetic field (SMF) and pulsing electromagnetic field (PEMF) in the treatment of musculoskeletal and neurological disorders [2].

Various research articles have proposed that magnets can affect the large number of biological processes [3, 4]. Many have put forth experimental evidence that can prove that selective magnetic fields are capable of in vivo and in vitro effects on the organism [5].

8.2 Importance of Electromagnetic Therapy

For decades, we have witnessed treatment methodologies that originate in natural remedies that were used for centuries before the advancement of pharmaceutical technologies. Decades ago, natural drugs developed with plant extracts and knowledge about the medicinal and therapeutic practices were communicated through oral teachings. These natural drugs were not only the mixture of various ingredients, but the methods of preparations are a far more important part of the healing, without the occurrence of adverse side effects. As time passed, medical schools became involved in the development of chemical compound-based pharmaceutical products instead of natural medicine, and the substitution of natural medicine. The excessive use of chemically evolved drugs led to drug overdose, causing deadly side effects. When physicians prescribed a medication based on the age and body weight on the assumption that the target tissue will receive some of the medicine, but what about the rest of the dose? This excess amount of medicine led to adverse effects. These negative effects of pharmaceutics forced the researchers and people toward the application of alternative medicine [6, 7]. Now the application of magnetic fields as a therapeutic procedure has emerged as an option. Bio-electromagnetism is a discipline that evaluates the electromagnetic phenomena that occur in a biological system when exposed to the magnetic field [8] in the low-frequency band (<300 Hz) of the electromagnetic spectrum [7, 9].

8.3 Physical Basis of the Generation of a Magnetic Field

In this chapter, we describe the fundamentals of the generation of artificial magnetic fields and their possible therapeutic application. “A magnetic field is a result of moving charge in space, and it affects the behavior of paramagnetic and

diamagnetic materials." It consists of magnetic flux lines that travel from one end of the magnet to another [4].

According to Ampere's law, the movement of charges results in a physical parameter called the magnetic field expressed in a quantity called magnetic field intensity (H). The effect of magnetic field intensity depends upon the distance from charges [10].

The magnetic fields are classified into following categories:

(a) Stationary magnetic field (SMF)
(b) Pulsed electromagnetic field (PEMF)

8.3.1 Stationary Magnetic Field

A SMF has the same direction and magnitude with time because it incorporates an electric current of a continuous nature. Hence, the generated magnetic field is also continuous [11].

The continuous magnetic field is not preferred for two essential reasons:

1. The current flows through solenoid wire, which causes the Joule effect.

$$\text{Heat} = k\,I\left(i\right)^2$$

The generated heat in solenoid wire is directly proportional to the square of current flowing in the wire.

$$K = \text{constant}$$

2. The creation of uniform magnetic fields is a difficult task.

Various studies on human populations primarily focus on the effects of SMF, but the available experimental evidence is not sufficient [11].

Some articles on cell culture study have proposed the use of magnetic fields based upon promising biological changes. Experimental evidence has established the fact that SMF leads to variations in the direction of forces on cellular components, which are prone to magnetic flux lines, such as hemoglobin and free radicals. [10, 11].

8.3.2 Pulsed Electromagnetic Field

A PEMF is characterized by the current waveform changing the intensity and direction of the field concerning time, which finally generates the electromagnetic field. This magnetic field can be further classified as pulsed or alternate. The waveform shows frequencies from 6 up to 500 Hz [4]. The high frequency of the waveform can induce a significant amount of biological current in the cells, and hence have significantly greater biological impact [9].

The extremely low-frequency band-generated magnetic fields are non-ionizing [7]. The waveform required for the generation of PEMF could be asymmetric and quasi-rectangular [4]. However, various low-frequency sources generate a sinusoidal waveform [9]. Certain low-level EMFs can produce a specific response, depending on the magnitude and frequency [7].

Stimulation of PEMF can be achieved using the following methods:

(a) *Inductive coupling*

This technique does not involve the electrode being in direct contact with the subject. According to Faraday's law of induction, the variation in the flux density of the PEMF induces an electric field, which generates current in the biological system [12].

(b) *Capacitive coupling*

In this technique, the therapeutic protocol consists of the placement of opposing polarity electrodes in direct contact with the skin surface in the vicinity of the target tissue [12].

8.4 Primary Biological Effects of Magnetic Fields

Various methodologies exist for SMF and PEMF. Numerous articles explain the different mechanisms for this physical phenomenon. In this chapter, we try to explain nearly all knowledge on the mechanism of magnetic fields.

8.4.1 Cell Proliferation and Cell Cycle Regulation

Wiskirchen et al. conducted a study using SMF (1.5T) with treatment duration (3 weeks). The result indicated negligible improvement in population doublings or cumulative population doublings in exposed groups. It was also observed that constant exposure to an SMF (1.5T) has no significant effect on the proliferation of human fetal lung fibroblast (HFLF) cells [13].

Raylman et al. propounded another theory using the magnetic field of intensity (7T) for treatment duration (64 h). The results show a significant reduction in viable cell count in melanoma, HTB-63, HTB-77 IP3, and CCL-86 cell lines. In addition, further exposure of the same field inhibits the growth of cell lines in vitro [14].

Buemi et al. conducted a study using a SMF (0.5 mT) with periodic examination (2, 4, 6 days) of the cellular generation/death in renal cells and cortical astrocyte cultures from rats. The reports show a gradual decrease in apoptosis and cellular proliferation, but a successive increase in cells with a necrotic morphology in the exposed group [15].

The experimental evidence regarding the clinical efficiency of PEMF and SMF suggests that PEMF might be more effective than SEMF. The application of PEMF

does not have a significant effect on bone marrow fibroblasts [16] and sub-confluent chondrocytes [17].

There is much experimental evidence that concludes that PEMF exposure affects the proliferation of subconfluent chondroblasts [18], subconfluent osteoblasts [19], and subconfluent human osteoblast cells [20].

Diniz et al. proposed a hypothesis based upon the experimental evidence that PEMF stimulation causes an increase in bone cell proliferation until the cells become confluent and begin to form the multilayers, but the difference in the DNA content is not present in the treatment group [21].

8.4.2 Genotoxic Effects

(a) *Mutation*

Ikehata et al. conducted a study using SMF (5T) for a possible mutation using the bacterial mutagenicity test. The experimental results showed that no mutagenic effect was observed on the strains of *Salmonella* typhimurium and *E. coli*. The mutation effects in the treatment group were higher after treatment with N-ethyl-N0-nitro-N-nitrosoguanidine, N-methyl-N0-nitro-N-nitrosoguanidine, ethyl methanesulfonate, 4-nitroquinoline-N-oxide, 2-amino-3-methyl-3H-midazo[4,5-f]quinoline or 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide [22].

(b) *DNA damage*

Zmyślony et al. conducted a study using SMF (7 mT) irradiation on a lymphocyte. The results did not demonstrate any significant effect on DNA, not even with the incubation of lymphocytes with $FeCl_2$ (10 mg/ml). However, when the $FeCl_2$-incubated lymphocytes were simultaneously exposed to an SMF (7 mT) static magnetic field, the damaged cell count increased to 20% [23].

(c) *Metabolic activity*

Onodera et al. conducted a study using the magnetic field (10T), and the results propounded a distinct phenomenon. It was observed that the viability of phytohemagglutinin (PHA)-activated T cells is reduced, with an increase in the death of PHA-stimulated lymphocytes by apoptosis. It also shows no effects on immune cells in the cell division phase and a minimal effect in nondivision phases [24].

Sabo et al. conducted a study using SMF (1T) for treatment duration (72 h). It was reported that a significant reduction in metabolic activities in a leukemic cell line in addition to this inhibitory effect is present owing to antineoplastic drugs [25].

Yamaguchi et al. conducted the study using SMF (0.2T) for the treatment duration (6–8 months), with careful observation under light and electron microscopy techniques. The results were based upon the proliferation rate of human gingival fibroblasts, lactate production, glucose consumption, adenosine

triphosphate content, and cell morphology, but no significant differences were observed [26].

(d) *Gene expression*

Hirai et al. conducted a study using SMF (100 mT) with an exposure duration of 15 min. The magnetic field exposure resulted in a potential increment in the binding of activator protein-1 through the expression of Fos-related antigen 2 (Fra-2), c-Jun, and transcription factor (Jun-D) proteins present in hippocampal neurons [27].

Hiraoka et al. conducted a study using the magnetic field range (0.18–0.2T) with an exposure duration of 2–24 h. The results showed a change in the expression of mRNA following 6 h irradiation time. The magnetic field exposure ($t = 4$ h) led to a potential increase in the expression of tumor suppressing factor (c-Fos) with simultaneous heat treatment (451 °C) and duration (10–15 min) [28].

8.4.3 Effects on Cellular Membrane Permeability

Liboff et al. conducted a study using SMF exposure (20 mT) with a duration of 30 min on cellular membrane. It was discovered that the effect of the magnetic field on CaM activation was markedly dependent on the calcium concentration [29].

Aldinucci et al. conducted a study using SMF exposure (4.75T) and PEMF (0.7 mT) for the pulsed component on human lymphocytes at a frequency of 500 MHz using an NMR apparatus for the duration of 1-h. It was observed that simultaneous SMF and PEMF increase the Ca^{2+} influx on either unstimulated or (PHA)-stimulated lymphocytes [30].

Miyamoto et al. conducted another study on the cell membrane effect of the magnetic field and proposed that exposure to magnetic fields ($H < 1.6T$) shows no effect on Rb+ influxes inside HeLa cells [31].

Sonnier et al. conducted a study using a magnetic field of 2T with a wide temperature range (10–451 °C).The experimental results suggested that it might not cause any significant changes in Rb+ influx and there was no evidence for a phase transition point of the cell membrane within the temperature range (10−371 °C). The SMF of intensity (0.1, 0.5, 7.5 mT) was used with the patch clamp method to quantify the sodium (Na+) and potassium (K+) charge movement through the cell membrane [32].

8.5 Role of Electromagnetic Therapy in Ischemic Stroke

Grant et al. proposed a model for the treatment of transient focal ischemia. In this model, MRI and histological slides were employed to evaluate the extent of injury following occlusion and reperfusion. The treatment groups both consisted of six

animals, and the application of magnetic field exposure was started 10 min after and continued throughout reperfusion. The experimental results show that the magnetic field reduced the cortical edema by 65% in the experimental group compared with the sham-exposed group. The brain slices (post histological evaluation) revealed damage of the neural cells inside the middle cerebral artery, lateral neocortex, and neostriatum. These data suggest that use of a magnetic field against the development of neuronal damage following occlusion and reperfusion might be effective [33].

Albertini et al. in 1999 proposed another method comprising PEMF treatment with an intensity of 3 mT and a frequency of 75 Hz. The results showed a reduction in the permanent damage of the myocardium and delayed cell death followed by the permanent ligation of the left anterior descending coronary artery. The limitations of the method proposed above are that the effects are sustain for a very short time (18 h) and after 6 days no effect was observed in the sham-exposed group or the experimental group [34].

Di Carlo et al. conducted series of experiments involving treatment with a magnetic field and found a cytoprotective effect on chick embryos [35]. It was observed that chick embryos survived using a magnetic field with a potentially lethal hypoxic situation until the survival rate of sham-exposed embryos dropped below 50% [35]. In another experiment, a field intensity of 8 mT with a frequency of 60 Hz was applied 1 h before the introduction of hypoxia.

After the investigation of PEMF exposure and other effects of the potential mechanism, it was proposed that the preconditioning of the exposure group could protect against UV rays [35].

Ronchi et al. conducted a study involving a magnetic field of 11.4 mT and 36.1 mT, broadband RF frequency of (0.2–200 MHz), treatment duration of 3 weeks with short exposure. The experimental evidence indicated separate heart function after ischemia–reperfusion [36]. The changes observed in the functional properties indicated developing ischemic tolerance with an increase in HSP-70 levels. It found that chronic magnetic exposure causes a decrease in protection, although the duration of exposure is longer [35].

8.6 Conclusion

We have seen much experimental evidence to show the effects of SMF and PEMF at the cellular and molecular level; however, the effect of SMF alone is not sufficient in comparison with PEMF. Hence we cannot make any assumptions. The experimental studies concerning the therapeutic effects of SMF and PEMF in assessing membrane permeability, metabolic activity, and treatment protocol for ischemia–reperfusion injuries are limited, hence the development of treatment. The mechanism is necessary for evaluation of the complete influence of magnetic fields for further study. The experimental evidence in various research articles concerning the flux density of SMF and duration of exposure makes it hard to compare them directly, but the conclusion can be drawn that SMF and PEMF alone have minimal

effects on cell growth and genetic toxicity. However, various articles have strongly proposed that a magnetic field with ionizing radiation and certain chemical compounds can modify its effects in an overall context.

Basic Terminology

1. Magnetic induction –the capacity of a magnetic field to induce a magnetic phenomenon at a certain point. It is measured in gauss.
2. Magnetic flow –the current of any magnitude through a surface. Magnetic flow is measured by the number of lines that cross a surface. This number changes depending on the distance and position that has the surface respective to the magnetic field.
3. Power lines –show the field direction at each point.
4. Magnetic intensity –the strength that a field exerts on an electromagnetic charge unit placed in a point in that field in a time unit.
5. Frequency –the number of times per second that change between alternate polarities. It is measured in Hertz.
6. Frequency spectra –a frequency range.
7. Apoptosis – programmed cell death.
8. BCL-2 family of protein –consists of members that either promote or inhibit the apoptosis and control apoptosis by governing mitochondrial outer membrane permeabilization.
9. SMF – stationary magnetic field.
10. PEMF – pulsed electromagnetic field.
11. DC– direct current.
12. ELF – extremely low frequency.
13. MF – medium frequency.
14. DNA – deoxyribose nucleic acid.
15. HFLF – human fetal lung fibroblast.

References

1. Markov, M. S. (2007). Therapeutic application of static magnetic fields. *The Environmentalist, 27*(4), 457–463.
2. Adey, W. R., (1993). Electromagnetic technology and the future of bioelectromagnetics. In *Electricity and magnetism in biology and medicine* (pp. 101–108). Plenary Lecture, Proceedings of the First World Congress of Electricity and Magnetism in Biology and Medicine, Buena Vista, Florida.
3. Adey, W. R. (2004). Potential therapeutic applications of nonthermal electromagnetic fields: Ensemble organization of cells in tissue as a factor in biological field sensing. In P. J. Rosch & M. S. Markov (Eds.), *Bioelectromagnetic medicine* (p. 1). Boca Raton: CRC.

4. Bassett, C. A. (1989). Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields (PEMFs). *Critical Reviews in Biomedical Engineering, 17*(5), 451–529.
5. Okano, H., Masuda, H., & Ohkubo, C. (2005). Effects of 25 mT static magnetic field on blood pressure in reserpine-induced hypotensive Wistar-Kyoto rats. *Bioelectromagnetics, 26*(1), 36–48.
6. Vallbona, C., & Richards, T. (1999). Evolution of magnetic therapy from alternative to traditional medicine. *Physical Medicine and Rehabilitation Clinics of North America, 10*(3), 729–754.
7. Rubik, B. (1997). Bioelectromagnetics & the future of medicine. *Administrative Radiology Journal: AR, 16*(8), 38–46.
8. Shupak, N. M., Prato, F. S., & Thomas, A. W. (2003). Therapeutic uses of pulsed magnetic-field exposure: A review. *Radio Science Bulletin, 307*(12), 9À30.
9. Juutilainen, J., & Lang, S. (1997). Genotoxic, carcinogenic and teratogenic effects of electromagnetic fields. Introduction and overview. *Mutation Research, Reviews in Mutation Research, 387*(3), 165–171.
10. Khoromi, S., Blackman, M. R., Kingman, A., Patsalides, A., Matheny, L. A., Adams, S., Pilla, A. A., & Max, M. B. (2007). Low-intensity permanent magnets in the treatment of chronic lumbar radicular pain. *Journal of Pain and Symptom Management, 34*(4), 434–445.
11. Colbert, A. P., Wahbeh, H., Harling, N., Connelly, E., Schiffke, H. C., Forsten, C., Gregory, W. L., Markov, M. S., Souder, J. J., Elmer, P., & King, V. (2009). Static magnetic field therapy: A critical review of treatment parameters. *Evidence-Based Complementary and Alternative Medicine, 6*(2), 133–139.
12. Trock, D. H. (2000). Electromagnetic fields and magnets: Investigational treatment for musculoskeletal disorders. *Rheumatic Disease Clinics of North America, 26*(1), 51–62.
13. Wiskirchen, J., Groenewaeller, E. F., Kehlbach, R., Heinzelmann, F., Wittau, M. H. P. R., Rodemann, H. P., Claussen, C. D., & Duda, S. H. (1999). Long-term effects of repetitive exposure to a static magnetic field (1.5 T) on the proliferation of human fetal lung fibroblasts. *Magnetic Resonance in Medicine, 41*(3), 464–468.
14. Raylman, R. R., Clavo, A. C., & Wahl, R. L. (1996). Exposure to strong static magnetic field slows the growth of human cancer cells in vitro. *Bioelectromagnetics, 17*(5), 358–363.
15. Buemi, M., Marino, D., Di Pasquale, G., Floccari, F., Senatore, M., Aloisi, C., Grasso, F., Mondio, G., Perillo, P., Frisina, N., & Corica, F. (2001). Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. *Nephron, 87*(3), 269–273.
16. Farndale, R. W., & Murray, J. C. (1985). Pulsed electromagnetic fields promote collagen production in bone marrow fibroblasts via athermal mechanisms. *Calcified Tissue International, 37*(2), 178–182.
17. Norton, L. A. (1982). Effects of a pulsed electromagnetic field on a mixed chondroblastic tissue culture. *Clinical Orthopaedics and Related Research, 167*, 280–290.
18. Sakai, A., Suzuki, K., Nakamura, T., Norimura, T., & Tsuchiya, T. (1991). Effects of pulsing electromagnetic fields on cultured cartilage cells. *International Orthopaedics, 15*(4), 341–346.
19. Shomura, K. (1997). Effects of pulsing electromagnetic field on the proliferation and calcification of osteoblast-like cell line, MC3T3-E1. *The Journal of Japan Orthodontic Society, 56*(4), 211–223.
20. De Mattei, M., Caruso, A., Traina, G. C., Pezzetti, F., Baroni, T., & Sollazzo, V. (1999). Correlation between pulsed electromagnetic fields exposure time and cell proliferation increase in human osteosarcoma cell lines and human normal osteoblast cells in vitro. *Bioelectromagnetics, 20*(3), 177–182.
21. Diniz, P., Shomura, K., Soejima, K., & Ito, G. (2002). Effects of pulsed electromagnetic field (PEMF) stimulation on bone tissue like formation are dependent on the maturation stages of the osteoblasts. *Bioelectromagnetics, 23*(5), 398–405.
22. Ikehata, M., Koana, T., Suzuki, Y., Shimizu, H., & Nakagawa, M. (1999). Mutagenicity and co-mutagenicity of static magnetic fields detected by bacterial mutation assay. *Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis, 427*(2), 147–156.

23. Zmyślony, M., Palus, J., Jajte, J., Dziubaltowska, E., & Rajkowska, E. (2000). DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz). *Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis, 453*(1), 89–96.
24. Onodera, H., Jin, Z., Chida, S., Suzuki, Y., Tago, H., & Itoyama, Y. (2003). Effects of 10-T static magnetic field on human peripheral blood immune cells. *Radiation Research, 159*(6), 775–779.
25. Sabo, J., Mirossay, L., Horovcak, L., Sarissky, M., Mirossay, A., & Mojzis, J. (2002). Effects of static magnetic field on human leukemic cell line HL-60. *Bioelectrochemistry, 56*(1), 227–231.
26. Yamaguchi, H., Hosokawa, K., Soda, A., Miyamoto, H., & Kinouchi, Y. (1993). Effects of seven months' exposure to a static 0.2 T magnetic field on growth and glycolytic activity of human gingival fibroblasts. *Biochimica et Biophysica Acta (BBA) – General Subjects, 1156*(3), 302–306.
27. Hirai, T., Nakamichi, N., & Yoneda, Y. (2002). Activator protein-1 complex expressed by magnetism in cultured rat hippocampal neurons. *Biochemical and Biophysical Research Communications, 292*(1), 200–207.
28. Hiraoka, M., Miyakoshi, J., Li, Y. P., Shung, B., Takebe, H., & Abe, M. (1992). Induction of c-fos gene expression by exposure to a static magnetic field in HeLaS3 cells. *Cancer Research, 52*(23), 6522–6524.
29. Liboff, A. R., Cherng, S., Jenrow, K. A., & Bull, A. (2003). Calmodulin-dependent cyclic nucleotide phosphodiesterase activity is altered by 20 μT magnetostatic fields. *Bioelectromagnetics, 24*(1), 32–38.
30. Aldinucci, C., Garcia, J. B., Palmi, M., Sgaragli, G., Benocci, A., Meini, A., Pessina, F., Rossi, C., Bonechi, C., & Pessina, G. P. (2003). The effect of exposure to high flux density static and pulsed magnetic fields on lymphocyte function. *Bioelectromagnetics, 24*(6), 373–379.
31. Miyamoto, H., Yamaguchi, H., Ikehara, T., & Kinouchi, Y., (1996). Effects of electromagnetic fields on K+ (Rb+) uptake by HeLa cells. In *Biological effects of magnetic and electromagnetic fields* (pp. 101–119). Springer US.
32. Sonnier, H., Kolomytkin, O., & Marino, A. (2003). Action potentials from human neuroblastoma cells in magnetic fields. *Neuroscience Letters, 337*(3), 163–166.
33. Grant, G., Cadossi, R., & Steinberg, G. (1994). Protection against focal cerebral ischemia following exposure to a pulsed electromagnetic field. *Bioelectromagnetics, 15*(3), 205–216.
34. Albertini, A., Zucchini, P., Noera, G., Cadossi, R., Napoleone, C. P., & Pierangeli, A. (1999). Protective effect of low-frequency low energy pulsing electromagnetic fields on acute experimental myocardial infarcts in rats. *Bioelectromagnetics, 20*(6), 372–377.
35. Di Carlo, A., White, N., Guo, F., Garrett, P., & Litovitz, T. (2002). Chronic electromagnetic field exposure decreases HSP70 levels and lowers cytoprotection. *Journal of Cellular Biochemistry, 84*(3), 447–454.
36. Ronchi, R., Marano, L., Braidotti, P., Bianciardi, P., Calamia, M., Fiorentini, C., & Samaja, M. (2004). Effects of broad band electromagnetic fields on HSP70 expression and ischemia-reperfusion in rat hearts. *Life Sciences, 75*(16), 1925–1936.

Chapter 9
Stem Cell-Based Therapy for Ischemic Stroke

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Abstract Stroke is still a leading cause of death and physical disability among adults. For stroke patients, there is a need for improved and effective therapy. Tissue plasminogen activator is a single FDA-approved drug available for treatment with short window of opportunity (it must be applied within 4.5 h of symptom onset). Over the years, several clinical trials of potential drugs have failed to show positive results. Stem cell therapy currently holds great promise as a neuroregenerative medical strategy for stroke by replenishing the lost brain functions. In the clinical arena of stroke therapy, animal studies revealed that the therapeutic efficacy of stem cells, including mesenchymal stem cells, neural stem cells, embryonic stem cells, and inducible pluripotent stem cells, may be due to angiogenesis, endogenous neurogenesis, neurorestoration, neuroprotection, and modulation of inflammation and immune responses. In this chapter, the current status, therapeutic potential, and the detailed factors of stem cell-based therapy for ischemic stroke are presented and discussed.

Keywords Stem cell therapy · Stroke · Neuroregenerative · Neuroprotection · Neurorestoration

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9.1 Introduction

In ischemic stroke, the blood flow is suddenly reduced owing to embolic or thrombotic occlusion in the cerebral artery resulting in deprivation of oxygen and other nutrients in the nerve cells. This leads to disruption of the synaptic architecture in addition to serious loss and dysfunction of neurons and glial cells, including oligodendroglia and astroglia. After removal of occlusion, the highly oxygenated blood returns to the ischemic tissue (reperfusion). Because of reperfusion, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative damage and nitrosative stress in the ischemic tissue respectively. The combination of oxidative damage and ischemia result in neuronal cell death, by apoptosis, necrosis, and autophagy, in addition to loss of neural function. The "clot-busting" tissue plasminogen activator (tPA) is the only FDA-approved drug available for the treatment of acute ischemic stroke. Nevertheless, therapy with tPA has significant limitations, notably the narrow therapeutic window of 4.5 h [1], which limits its use to a small population (2–4%) of patients. Administering the tPA outside the recommended window increases the risk of intracerebral hemorrhages, worsening the injury. Currently, endovascular clot retrieval therapy for acute ischemic stroke is gaining wide interest in the scientific community. Some randomized controlled trials provide significant evidence that clot retrieval by mechanical thrombectomy using a stent retriever improves outcomes after ischemic stroke. The limitations of this therapy are that it is a complex procedure, and needs to be executed by experienced, trained, and competent hands with great rapidity.

Additionally, owing to the slow turnover of neural cells and limited renewal ability, the endogenous cell replacement mechanism is not sufficient to restore the loss of adult neuronal cells. However, the intracerebral transplantation studies carried out in the 1980s established a close relationship between cell therapy and brain plasticity. Thereafter, several experiments were performed to show the capability of damaged brain structures to adopt exogenous immature neurons and to incorporate these neurons into the renewal process after a brain lesion. In vivo studies in humans demonstrate that neurogenesis takes place throughout life in the subventricular zone of the olfactory bulb and the subgranular zone/dentate gyrus of the hippocampus [2, 3]. These studies have formed the basis for experimental work exploring the potential role of stem cell transplantation therapy in the treatment of ischemic stroke.

Stem cell therapy currently holds great promise as a neuroregenerative medical strategy for stroke by replenishing the lost brain functions via replacing the damaged neurons with stem cells and stimulating the secretion of various endogenous neurotrophic factors. Stem cells (SCs) are undifferentiated long-lived cells with self-renewal ability and multipotency. In the clinical arena of stroke therapy, animal studies revealed that the neural stem cells (NSCs), mesenchymal stem cell (MSCs), embryonic stem cells (ESCs), and inducible pluripotent stem cells (iPSCs) have therapeutic efficacy. This chapter briefly summarizes the important findings covering the suitable sources of stem cells, the functional benefits of stem cell transplant therapy, potential mechanisms, preconditioning approaches, and the most efficient delivery routes in neurorestoration therapies.

9.2 Stem Cell Transplantation for Stroke

Pre-clinical studies have successfully demonstrated the therapeutic benefits of stem cell therapy in animal models of ischemic stroke. For the treatment of stroke with stem cells, endogenous and exogenous approaches are widely used. With an endogenous approach, stem cells already present within the individual are stimulated by administering drugs or growth factors that mobilize the stem cell to replace damaged tissue. For example, administration of granulocyte-colony stimulating factor (G-CSF), erythropoietin (EPO), and endothelial growth factor (EGF) can stimulate endogenous stem cells to replace damaged tissue after middle cerebral artery occlusion (MCAO) in the rat [4–7]. With an exogenous approach, the transplantation of stem cells is carried out in the patient suffering with stroke. The stem cells may be delivered locally (e.g., direct intracerebral implantation) or systemically (e.g., intravenously) or they can be administered after in vitro culturing [8]. For the treatment of ischemic stroke, stem cell therapy focuses on a regenerative strategy that requires restoration of neural elements and support of structures such as blood vessels. Several types of exogenous stem cells have been tested in ischemic stroke, both in clinical and in experimental studies, and are briefly discussed below.

9.3 Neural Stem Cells

Neural stem cells (NSCs) are multipotent, self-renewing, and mitotically active cells present in the developing and the adult central nervous system (CNS). They proliferate for an almost indefinite period and form neurospheres (multicellular free-floating spheres), which impulsively differentiate into CNS daughter cells such as neurons, oligodendrocytes, and astrocytes after withdrawal of growth factors [9]. Endogenous NSCs can be isolated from the entire embryonic and the adult CNS. The two brain regions, i.e., the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) at the dentate gyrus of the hippocampus have been recognized as highly specialized CNS germinal niches. These regions contain slowly proliferating putative CNS stem cells that give rise to functional neurons and glia. Palmer et al. have reported that neurogenesis can also occur in the cortex, striatum, and septum brain regions [10, 11], although, some researchers suggest that the NSCs and progenitor cells present in the spinal cord and brain might be astroglial cells [12, 13]. Therefore, these findings are still highly controversial.

There are obstacles to the use of endogenous NSC from these two sources, for instance, the need for multiple fetal donors to treat a stroke patient could raise ethical concerns and not be feasible in large-scale clinical trials; the isolation of adult NSCs for autologous transplantation requires brain biopsies; and a long period in culture preparation is needed for their expansion. A variety of molecules have been shown to stimulate the proliferation of endogenous NSCs in the adult brain, such as brain-derived neurotrophic factor (BDNF), Wnt proteins, glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), epidermal

growth factor (EGF), basic fibroblast growth factor (bFGF), and erythropoietin [14]. These factors hold great promise for future therapeutic applications because they can regulate and enhance endogenous neurogenesis.

Bone marrow and adipose-derived MSCs, embryonic stem cells (ESCs), embryonic NSCs, iPSCs, and fetal and adult nervous systems are prominent sources of exogenous NSCs [15]. In vitro proliferation of these cells is stimulated by various growth factors including but not limited to EGF, leukemia-inhibiting factor (LIF), and FGF. Different environmental factors such as exposure to differentiation-inducing factor (retinoic acid) or withdrawal of mitogens induce the differentiation of these stem cells into specific glial and neuronal phenotypes [16]. Owing to the aforementioned features, these cells act as potential candidates for replacing the lost neuronal cells in stroke [17, 18].

Studies revealed that in comparison to embryonic stem cells, the tumorigenicity of human fetal NSCs is quite low, and these cells express a low level of major histocompatibility complex (MHC) molecules that obviate the necessity for immunosuppression [19]. There is also evidence that adult NSCs possess great stability in maintaining a differentiated state and have a lower oncogenic potential [20]. In vitro culture of NSCs possesses the disadvantage of limiting differentiating capability and expansion whereas use of embryonic stem (ES)-derived neural stem/progenitor cells (NSPCs) may form neural overgrowth or teratoma, if undifferentiated ES continues in the transplant pool [21]. Acute and chronic CNS inflammation may disturb the functional and anatomical relationships between the cell components of SVZ and SGZ, thereby impairing the restoration capacity of the endogenous stem cell compartment. Therefore, practices aimed at mobilizing endogenous NSCs from germinal niche(s) in vivo may be therapeutically inefficacious in inflammatory CNS disorders including stroke [9]. Thus, transplantation of NSCs may represent an alternative, and possibly more effective, therapeutic approach. Experimental evidence shows that transplantation of NSCs, which are obtained from various cell sources and administered through different routes, reduces the brain infarction and promotes recovery of neurological functions in a rodent model of ischemic stroke. In addition to these outcomes, individual contradictory results also exist.

During stem cell transplantation, the type, dose, and timing of the transplanted cells play a significant role and are responsible for diverse results [17]. The study also suggests that neuronal plasticity can be introduced by providing exogenous stimuli that play an important role in cell-based therapy [22]. In stroke animal models, it has been observed that the therapeutic effects of transplanted NSCs are enhanced by over-expression of BDNF, GDNF, neurotrophin-3, FGF-2, and VEGF. Also, the migration abilities, secretion of neurotrophic factors, along with the proliferation and survival of the NSCs, can be amplified by genetic modifications.

The intraparenchymal transplantation of NSCs via a focal stereotactic method faces limitations as wide areas of the brain are affected by both ischemic and hemorrhagic stroke. Studies have revealed that in response to chemoattractant stimuli, the stem cells present in the systemic circulation are capable of homing to injury

regions; this mechanism is similar to immune cell trafficking including rolling" on and adhesion to the endothelial transmigration and endothelium. For selective homing into injured areas of the CNS, adult NSCs recapitulate lymphocyte-like pathways after intravenous injection. For cell adhesion and migration, NSCs expressing integrin proteins (such as α4) and cell adhesion molecules (CAMs such as CD44) that interact with specific ligands (expressed by inflamed endothelial cells) vascular cell adhesion molecule 1 (VCAM1) and hyaluronic acid respectively. Several chemokines activate chemokine receptors (such as CXCR3, CXCR4, CCR1, CCR2, and CCR5) expressed on the plasma membrane of adult NSCs, leading to activation of α4 integrins that enable adhesion and migration of transplanted cells across the inflamed endothelium [23]. The chemokines and proinflammatory cytokines produced by blood-borne inflammatory cells, CNS-resident cells, and transplanted adult NSCs organize these sequential events, resulting in activation of G-protein coupled receptors (GPCRs) and cell migration across the endothelium [9].

A study performed by Toda et al. to investigate the effects of adult NSC transplantation in rat hippocampus revealed that after implantation, NSCs integrated and differentiated into neurons and astrocytes 2 weeks after transient global ischemia, and that significant recovery was observed in learning and memory function of the rat model [24]. NSCs isolated from the SVZ of rat brain, when transplanted into the cisterna magna of the rat 2 days after MCAO, showed significant behavioral recovery after 3–4 weeks [25]. Those NSCs that were obtained from the external germinal zone of the cerebellum and immortalized with the v-Myc oncogene, have been shown to differentiate into neuronal phenotypes and integrate into the ischemic brain [26]. Intravascular cell transplantation could offer the benefit of avoiding the necessity for invasive brain surgery and ensure better distribution of the NSCs into the injured brain areas [27].

Transplanting more numbers of adult NSCs did not result in increased neuronal differentiation and a greater number of surviving cells. Optimal dose and time of cell transplantation variation depends on different animal models, infusion routes, and cell sources, but for the cell survival, maximal activation of microglia before transplantation has proved to be more beneficial. Furthermore, recent research has demonstrated that transplantation of human embryonic NSCs promotes post-stroke angiogenesis, i.e., is associated with improvement of neurological deficits in the rodent stroke model at 7 and 14 days [25]. Immunomodulation by downregulation of proinflammatory cytokines (tumor necrosis factor-alpha: TNF-alpha and interleukin-6: IL-6) may contribute to the beneficial effects of cell-based therapies in stroke [22].

The available preclinical and clinical experimental results suggest that NSC therapy might be capable of brain restoration after stroke and can be further developed as an effective therapeutic approach. The major obstacles to this approach include insufficient transplant efficacy, limited expansion and survival, as along with important safety issues, which should be overcome by future advanced research, leading to translation of these results of basic research into clinical therapy.

9.4 Embryonic Stem Cells

Human ESCs are pluripotent, demonstrate a strong potential to differentiate into any cell type of the body, and have the capability for unlimited self-renewal. ESCs are isolated from the inner cell mass of 5-day-old human blastocysts [8]. Under specific environmental conditions, ESCs can differentiate into neural lineage. For example, with the presence of fibroblast growth factor 2 and the absence of epidermal growth factor and leukemia inhibitory factor, they can differentiate into neuronal cell types that express glutamatergic, dopaminergic or GABAergic markers [28]. Therefore, ESCs are considered to be a promising unlimited renewable source of stem cells for transplantation into the degenerating brain.

Nonetheless, the clinical implementation of ESCs remains controversial owing to ethical issues and concerns regarding the use of human embryos. Autologous transplantation of these cells would not be possible and allogeneic cell grafting id associated with a risk for immune rejection [29]. Additionally, there is a risk for tumorigenesis or teratoma formation with pluripotent cells when the tissue pool contains undifferentiated cells. For this reason, pluripotent ESCs are commonly directed into a less potent primed state (predifferentiation) before the transplantation for stroke that promotes differentiation into the neural progenitor cells (NPCs) type and minimizes tumorigenesis [30]. After transplantation, these NPCs may differentiate into mature neurons in the brain parenchyma. However, in the face of their favorable characteristics for therapeutics, ESCs have been approved by the FDA for a phase I clinical trial in thoracic spinal cord injury [31]. Wei et al. successfully demonstrated that the intrastriatal grafting of mouse ESCs resulted in differentiation into neuronal-like cells, reduction of the ischemic lesion, and partial restoration of the motor function in an MCAO rat model of ischemic stroke [28].

A study conducted by Erdö et al. in 2003 revealed that xenotransplanted murine ESCs were able to migrate along the corpus callosum and differentiate into neuronal cell types in the border zone of the ischemic lesion, whereas when the same murine ESCs were transplanted as an allograft, they did not migrate to the lesion site and they produced highly malignant teratocarcinomas near the site of implantation, no matter whether or not the ESCs were differentiated or predifferentiated in vitro to the NPCs. This study raised safety concerns about the use of ESCs for clinical purposes [32]. Transplantation studies on ESCs in animal models have shown promising results, although further extensive knowledge is required before translating these studies to human therapy [33].

9.5 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) or mesenchymal stromal cells are a subset of nonhematopoietic adult multipotent cells that originate from the mesoderm. Friedenstein and his colleagues in 1974 were the first to isolate and characterize

these cells. They have the ability for self-renewal and multilineage differentiation into mesoderm lineages (such as osteocytes, chondrocytes, and adipocytes), in addition to endodermic cells and ectodermic cells [34]. In vitro studies demonstrated that MSCs express NeuN-neuronal markers, capable of differentiating into neural cells, and are able to migrate toward the lesion in the brain, which constitutes a promising therapeutic approach to stroke treatment. MSCs exist in almost all tissues and can be isolated from the bone marrow, umbilical cord, adipose tissue, fetal liver, lung, and muscle, and are successfully expanded in vitro [35]. MSCs fulfill the following criteria established by the International Society of Cellular Therapy [36]: (1) the ability to differentiate into osteoblasts, chondroblasts, and adipocytes in vitro; (2) adherence to plastic; and (3) expression of CD73, CD90, and CD105 as along with a lack of expression of CD14, CD19, CD34, CD45, CD11b, CD79a, and HLA class II. Because of their extensive self-renewal capacity, their presence during young and fetal life, their ease of isolation from various sources and being free of both ethical concerns and teratoma formation, great interest has developed in MSCs for stem cell therapy. Additionally, MSCs employ their regenerative potential via the production of several paracrine factors [37]. They are able to promote production of IL-6, VEGF, hepatocyte growth factor (HGF), glial-derived neurotrophic factor (GDNF), BDNF, neurotrophin-3 (NT3), thrombospondins, and fibroblast growth factor (FGF). Preclinical experimental studies have demonstrated that MSC transplantation significantly improves the neurological function in animal stroke models. Genetic modification with neurotrophic factors such as GDNF, angiopoietin-1, FGF-2, BDNF, and placental growth factor (PlGF) have fortified the effective roles of MSCs. It appears that MSCs possess reduced expression of MHC antigens and T-cell stimulatory molecules and are hence suitable for therapeutic use as allografts. Although MSCs are able to cross the blood–brain barrier (BBB) [38], following transplantation by intracerebral or intravenous routes, very few cells are actually found in the ischemic boundary sites and even fewer of these cells express neural markers [39]. However, after transplantation, MSCs transdifferentiate into the neural lineage and induce neurogenesis, synapse formation, and angiogenesis in rodents [40]. The presentation of C-X-C chemokine receptor type 4 (CXCR-4) on MSCs and the increased level of some chemokines such as stromal cell-derived factor 1 (SDF-1) in the surrounding environment mediates the migration capability of these cells [41].

Migration of MSCs into the injured brain area exhibits involvement of various potential factors such as monocyte chemoattractant protein-1 (MCP-1), VEGF, macrophage inflammatory protein-1a (MIP-1a), and IL-8 [42]. Enhanced endogenous neuronal repair, i.e., plasticity, angiogenesis, and neurogenesis, is observed when MSC transplantation is carried out 2–3 weeks after ischemic insult. Functional recovery in the ischemic rat was also reported after MSC administration, even at 1 year [43]. It has also been observed that intravenous or intra-arterial delivery of MSCs is more effective than intracerebral delivery because it is less invasive, easily utilized in clinical practice, and more neuroprotective [44].

In the course of the acute phase of cerebral ischemia, the expression of microglial and neuronal IL-6 is elevated in the injured penumbra. Microglia have also been

associated with the pathogenesis of a number of neurological disorders including stroke. An excessive or sustained activation of microglia contributes to apoptotic cell death. It is demonstrated that transplantation of bone marrow-derived MSCs results in the suppression of activated microglia and in delayed neuronal death [45]. Human MSCs have also been shown to stimulate angiogenesis in focal cerebral injury by increasing the expression of angiopoietin 1 and 2 and α- tubulin [46]. However, there are still some hurdles to be overcome before the widespread utility of MSCs. Further extensive research is needed on interactions between the inflammatory milieu and MSCs and the therapeutic mechanisms of MSCs.

9.5.1 Bone Marrow-Derived Mesenchymal Stem Cells

Bone marrow-derived mesenchymal stem cells (BMSCs) express markers for mesenchymal or endothelial cells (CD73, CD90, and CD105) and adhesion molecules (CD29, CD106, and CD166), but do not express hematopoietic stem cell markers (CD11, CD14, CD34, CD45, CD79, CD19, and HLA-DR) [36]. Experimental evidence has shown that BMSCs improve the pathological processes of primary ischemic stroke through multiple mechanisms of action, including modulating the immune system, inducing angiogenesis, inhibiting apoptosis, and secreting neurotrophic factors [39, 43, 44, 47]. When BMSCs are transplanted by intravenous infusion, the numbers of axons and myelin sheaths increase in the rat hippocampus, corpus striatum, and corpus callosum. Also, axons in the ischemic zone grow along the extending direction of reactive astrocytes [48]. However, it is unclear how BMSCs induce synaptic plasticity. BMSCs may be used in gene therapy by introducing target genes as genetic carriers and in combining cell therapy. Thus, transfected BMSCs with an exogenous gene can be implemented in the treatment of cerebral infarction. By contrast, combining BMSCs with a drug is a simple, highly efficient, and feasible stroke treatment method and produces not only the collective effect of dual therapy, but also synergistic effects that can effectively promote the recovery of neurological function. For example, BMSCs combined with sodium ferulate accelerate migration of cells toward the ischemic region in an MCAO rat model by upregulating CXCR-4 and SDF-1α, increasing glucose transporter 1 expression, leading to stimulating glucose metabolism in the peri-infarct zone, in addition to markedly reducing infarct size [47]. Hypoxia preconditioning significantly improves BMSC migration, targeted migration, and survival. When preconditioned and nonpreconditioned BMSCs are exposed to 6 h of lethal anoxia, it is observed that the number of preconditioned cells is higher than the number of nonpreconditioned cells [49]. Overall, transplantation of BMSCs is an important method in the future treatment of ischemic stroke.

9.5.2 Adipose Mesenchymal Stem Cells

Adipose mesenchymal stem cells (AdMSCs) or adipose-derived stem cells (ASCs) can be easily obtained from adipose tissue and differentiated into both the ectodermal and endodermal lineages. Although, studies have showed that under specific conditions the ASCs can differentiate into neuronal cells that express neuronal markers (NeuN and nestin) and glial markers (S100, p75) [50]. Expression of CD105/SH2, CD90, and CD73 proteins and the lack of expression of HLA-DR proteins, CD45, CD14 or CD11b, CD79a or CD19 and CD34 are the major characteristics of ASCs [51]. A characteristic bone marrow progenitor cell marker, stromal-derived factor-1 (STRO-1), is also expressed in ASCs. The expression of a marker usually present in bone marrow progenitor cells has also been present in ASCs. Transplantation of human ASCs as spheroids after hypoxic preconditioning was found to improve recovery from ischemia in murine models [52]. Another study has determined that ASCs exposed to hypoxia or ischemia secrete cytokines that can improve vasculogenesis and cell proliferation directly [53]. When ADMSCs were transplanted via the internal carotid artery they migrated to the brain infarct region and mainly localized in the boundary zone and ischemic core of the lesion [54]. These findings revealed that the autologous transplantation ADMSCs can inhibit cellular apoptosis, attenuate astroglial reactivity, improve neurological function, and promote cellular proliferation after ischemic stroke. In addition, ASCs are not associated with tumor formation and can be genetically modified to produce regulatory molecules that suppress tumor formation.

9.5.3 Human Umbilical Cord Mesenchymal Stem Cells

The umbilical cord consists of a mixture of both the mononuclear fraction of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). Human umbilical cord mesenchymal stem cells (hUC-MSCs) can differentiate into cardiomyocytes, adipocytes, osteocytes, and dopaminergic neurons [55]. An advantage of umbilical cord-derived MSCs is that administration of regulatory T-cells (1–5% from HUC cells) can improve neurogenesis in the aging brain and prevent graft versus host rejection [56]. Additionally, hUC-derived MSCs have many advantages: they exhibit greater proliferative activity than BM-MSCs [57], they have low immunogenicity, a lower risk of viral contamination, a limited number of ethical issues, and are easily harvested and manipulated without harming the baby or the mother [58]. hUC-MSCs derived from Wharton's jelly (a gelatinous substance within the umbilical cord) have a relatively high harvest rate compared with MSCs derived from cord blood or bone marrow. Thus, the hUC appears to be a promising resource for stem

cell-based regenerative medicine. These cells have CD29, CD73, CD105, CD44, CD146, and CD200 [59]. Although hUC-MSCs possess a wide range of properties and great interest with regard to cell therapy, they still face certain hurdles, such as low survival after injection, reduced differentiation potential in vivo, and a lack of homing to the lesion site. To improve the therapeutic efficacy of MSCs, genetic modification with therapeutic genes can improve their migratory properties and locally deliver biological agents [60]. To evaluate their neuroprotective effect, several preclinical studies have been performed in adult animals. In an adult rat model of stroke, undifferentiated hUC-MSCs were implanted intracranially (2 weeks after MCAO) into the damaged hemisphere of immunosuppressed stroke rats. Three weeks after implantation it was observed that most of the implanted MSCs were present in the damaged hemisphere and some of these cells expressed neuron-specific markers (nestin). After transplantation, improved neurobehavioral function and reduced infarct volume relative to control rats were seen [61]. Another study demonstrated that hUC-MSCs also induced functional improvements when more than 3×10^6 cells were injected intravenously in a rat model of stroke. A third study demonstrated that after transplantation, proliferation of progenitor cells was enhanced in the subventricular zone and in the ischemic boundary zone, synaptogenesis, vascular density, and reduced apoptosis were observed [62]. Umbilical cord-MSCs also have immunoregulatory properties. Interestingly, the decrease pro-inflammatory cytokine levels such as interferon-ɣ, TNF-α, and TNF-β, and suppress lymphocyte proliferation [63]. As hUC-MSCs are non-immunogenic and have immunomodulatory effects, there is no need for immunosuppression when these cells are xenotransplanted.

9.5.4 Menstrual Blood-Derived Mesenchymal Stem Cells

The human endometrium undergoes dynamic remodeling in addition to more than 400 cycles of regeneration during the reproductive period of the woman. It is composed of two layers, the basal layer of loose conjunctive tissue and a functional layer that is always undergoing restructuration. In 2007, Meng et al. extracted a stem cell-like population from menstrual blood termed “endometrial regenerative cells” (ERCs), which are capable of differentiating into multiple lineages [63]. The morphology of MBSCs is typical of MSCs; they possess in vitro proliferative potential and self-renewal characteristics, and they are able to differentiate into various cell lineages including cardiomyocytes, myocytes, neuronal cells, respiratory epithelium, osteocytes, endothelial cells, adipose cells, and pancreatic cells when supplemented with induction media [64]. MBSCs express characteristic MSC markers, such as CD105, CD 59, CD44, CD29, CD73, CD90, and CD1 (42), but do not express CD34 (epithelial and hematopoietic stem cell marker), CD45 (leukocyte marker), stromal-derived factor-1 (STRO-1), and CD31 (epithelial cell marker)

[65]. The stromal-like menstrual blood stem cells that possess CD117 marker are associated with high survival proliferation and migration [66]. Recently, Borlongan et al. performed an in vitro study with an oxygen glucose deprivation (OGD) stroke model and found that co-culturing menstrual blood-derived stem cells with OGD-exposed primary rat neurons significantly reduced cell death and upregulated VEGF, BDNF, and NT-3 trophic factors [67]. Additionally, either intracerebral or intravenous transplantation of MBSCs without immunosuppression significantly reduced histological and behavioral impairments. As mentioned above, MBSCs can rapidly differentiate and expand under laboratory conditions and in clinical application, autologous stem cells would be ideal to avoid graft rejection issues. Therefore, they may serve as an easily accessible, ideal source of stem cells for tissue engineering and cell therapy for stroke.

9.6 Hematopoietic Stem Cells

A major source of hematopoietic stem cells (HSCs) is bone marrow. HSCs can also be isolated from peripheral blood and umbilical cord blood. They have self-renewable capacity and multipotency and are responsible for the generation and maintenance of blood cells and lymphoid cells [68]. The characterization of hematopoietic lineage phenotypes is done on the basis of LKS selection (Lin^- $c\text{-}Kit^+$ $Sca1^+$). They are present in the bone marrow, circulating blood, and in the fetal liver. They possess a high rate of regeneration potential and produce billions of new blood cells every day to maintain the level [69, 70]. Studies revealed that HSCs reside near the blood vessels of the bone marrow [71] and in the endosteal region [72]. These regions are formed by nestin-positive MSCs, osteoblasts, Schwann cells, Cxcl12-expressing abundant-reticular cells, and perivascular cells [63]. HSCs are suitable for both allogeneic and autologous use and are not associated with ethical issues. Preclinical studies of $CD34^+$ cells (BMSCs include populations of endothelial stem cells, HSCs, and progenitor cells) have shown significant functional recovery in rodent models of stroke. The transplantation of these cells intravenously resulted in neurogenesis and increased perilesional angiogenesis in mice at 48 h poststroke [73]. In another study, $CD34^+$ cells were directly transplanted intracerebrally 1 week after the induction of stroke, neurogenesis and angiogenesis were observed, and transplanted cells were differentiated and expressed markers for neurons and glial cells [74]. When hematopoietic stem cell-rich $Sca1^+$ bone marrow cells (enriched in hematopoietic stem cells) were injected intravenously in an MCAO mouse stroke model, it was observed that HSCs protected a sizeable number of mice subjected to stroke and reduced the neurological morbidity in surviving mice [75]. These results suggest that HSCs might be potentially beneficial for improving ischemic stroke-induced degeneration.

9.7 Inducible Pluripotent Stem Cells

Inducible pluripotent stem cells (iPSCs) are stem cell populations generated from various somatic cells through reprogramming by transcription factors. Yamanaka received the Nobel Prize for creating the iPSCs. His group initially derived iPSCs from mouse fibroblasts in 2006 [76] and from human fibroblasts in 2007 [77]. The reprogramming of somatic cells back to iPSCs could be achieved by transduction of four pluripotency-associated transcription factors, namely SOX2 and OCT4 (also known as POUF51 and OCT3), along with either NANOG and LIN28 or Krüppel-like factor 4 (KLF4) and MYC [78]. The derived human iPSCs all have properties of human ESCs, such as the ability to form many or all somatic cell types of the body and grow indefinitely. Therefore, iPSCs are the most promising seed cells for stem cell-replacement therapy. However, both ESCs and iPSCs have an inherent predisposition to tumor formation and there is a higher probability of teratoma formation from iPSCs than from ESCs. As it has been demonstrated that tumorigenic activity is lost after differentiation, the use of differentiated cells derived from iPSCs is significantly safer [79]. The development of iPSCs makes cell transplantation a promising therapy for stroke. A study conducted by Jiang et al. demonstrated that iPSCs injected into the contralateral and ipsilateral peri-infarct regions of rat after MCAO migrated to damaged areas of the brain and showed some differentiation into neuron-like cells, in addition to significantly reducing the lesion volumes and recovering the sensorimotor function [80]. In another study, Chen et al. explored the neurotherapeutic properties of iPSCs in combination with fibrin glue in MCAO rats. They revealed that the subdural transplantation of iPSCs significantly improved the behavior of rats and reduced the infarct volume of the brain [81]. In conclusion, the iPSC transplantation techniques open a new door in stem cell research and offer favorable opportunities for patient-specific, pluripotent cell-based regenerative medicine.

9.7.1 Reprogramming Methods for Human Somatic Cells into iPSCs

Human somatic cells can be reprogrammed into iPSCs by the delivery of reprogramming factors using integrating or non-integrating approaches. The integrating reprogramming is carried out by using retroviruses (generated by retroviral vectors pMSCV, pMXs, and pLib), lentiviruses (derived from HIV) or transposons (piggyBac), resulting in human iPSC lines that have randomly distributed insertion of the transgenes into the cell genome [82]. Although integrating reprogramming has become a popular strategy for generating iPSCs, this approach has a major drawback that at the site of integration, retrovirus insertion can cause gene disruption [83]. Additionally, insertion of a virus genome into the vicinity of an endogenous gene may result in gene silencing or transcription activation and can have major

consequences. For instance, retrovirus-mediated insertions can cause cancer in vivo or enhance self-renewal properties, and mutations caused by insertion may be passed through the germline to the next generation. For these reasons, efforts have been made toward safer strategies, i.e., non-integrating approaches for generating human iPSCs without any resultant permanent genetic modification. Identifying small molecules that are able to reactivate the expression of endogenous pluripotency factors and delivery of recombinant peptides and proteins can be an effective alternative approach. Therefore, these methods could benefit both future cell transplantation therapies and disease modelling.

9.8 Preconditioning Strategy in Stem Cell Transplantation Therapy

Stem cell transplantation therapy is a rapidly developing promising regenerative medicine for stroke. However, several problems and complications still need to be fixed before positive clinical application. On the other hand, many recent preclinical studies have demonstrated the benefits of the recognized mechanisms of ischemic or hypoxic preconditioning. Preconditioning with ischemia or hypoxia stimulates endogenous defense mechanisms and shows neuroprotective effects against stroke. Studies demonstrate that the regenerative and tolerative potential of stem cells and progenitor cells is considerably enhanced after sub-lethal hypoxic exposure.

So far, various preconditioning trigger mechanisms have been evaluated on different stem cells [84–87]. After preconditioning, progenitors and stem cells generally show increased neuronal differentiation, much better cell survival, improved homing to the lesion site, and enhanced paracrine effects important for increased trophic support. Transplantation of preconditioned cells helps to promote neurological functional recovery and suppress inflammatory factors and immune responses [84]. For example, preconditioning with sublethal hypoxia and EPO considerably increased the tolerance of treated cells in the harsh environment of the peri-infarct regions and ischemic core [85]. Preconditioning of MSCs by treating with pharmacological agents and growth factors (such as FGF-2, TGF-α, and IGF-1) show increased paracrine potentials. Currently, the preconditioning of stem cells and its preclinical/clinical application are attracting significant attention in the field of stem cell translational research and in the field of preconditioning research.

9.9 Conclusion

Stem cell therapy is an emerging area of research in the preclinical/clinical area for the treatment of ischemic stroke. Owing to the beneficial characteristics of stem cell replacement therapy and the need to transplant a pure and appropriate stem cell

population, researchers are continually looking for better and more suitable transplantation strategies with minimal patient risk. A kind of experimental evidence suggests that endogenous and exogenous stem cell transplantation might have the potential to treat ischemic stroke in animal models. After transplantation, stem cells have demonstrated positive therapeutic effects by suppressing apoptosis and neuroinflammation and promoting neurogenesis, angiogenesis, and synaptogenesis, in addition to protecting the BBB with a low rate of adverse effects, although preclinical to clinical to bedside translation of stem cell therapy is still in its infancy owing to a number of important issues that have yet to be resolved. Experimental study should be conducted to address several questions related to stem cell therapy:

1. Which cell type or growth factor is more appropriate for stroke patients who may vary with regard to the extent of their lesion, neurovascular risk factors, age or location?
2. What are the optimal timing of treatment, optimal delivery routes, and optimal dose?
3. Will the in vitro expansion of cell therapy use be associated with increased long-term immunogenicity and tumorgenicity?

Further, both preclinical and clinical studies are needed to control cell differentiation and survival, and to examine the clinical safety of stem cell delivery to the injured brain.

References

1. Stemer, A., & Lyden, P. (2010, January 1). Evolution of the thrombolytic treatment window for acute ischemic stroke. *Current Neurology and Neuroscience Reports, 10*(1), 29–33.
2. Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998, November 1). Neurogenesis in the adult human hippocampus. *Nature Medicine, 4*(11).
3. Curtis, M. A., Kam, M., Nannmark, U., Anderson, M. F., Axell, M. Z., Wikkelso, C., Holtås, S., van Roon-Mom, W. M., Björk-Eriksson, T., Nordborg, C., & Frisén, J. (2007, March 2). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science, 315*(5816), 1243–1249.
4. Shyu, W. C., Lin, S. Z., Yang, H. I., Tzeng, Y. S., Pang, C. Y., Yen, P. S., & Li, H. (2004, September 28). Functional recovery of stroke rats induced by granulocyte colony-stimulating factor–stimulated stem cells. *Circulation, 110*(13), 1847–1854.
5. Jin, K., Wang, X., Xie, L., Mao, X. O., Zhu, W., Wang, Y., Shen, J., Mao, Y., Banwait, S., & Greenberg, D. A. (2006, August 29). Evidence for stroke-induced neurogenesis in the human brain. *Proceedings of the National Academy of Sciences, 103*(35), 13198–13202.
6. Talwar, T., & Srivastava, M. V. (2014, January). Role of vascular endothelial growth factor and other growth factors in post-stroke recovery. *Annals of Indian Academy of Neurology, 17*(1), 1.
7. Shin, Y. K., & Cho, S. R. (2016, March 30). Exploring erythropoietin and G-CSF combination therapy in chronic stroke patients. *International Journal of Molecular Sciences, 17*(4), 463.
8. Banerjee, S., Williamson, D., Habib, N., Gordon, M., & Chataway, J. (2010, November 10). Human stem cell therapy in ischaemic stroke: A review. *Age and Ageing, 40*(1), 7–13.

9. Martino, G., & Pluchino, S. (2006, May 1). The therapeutic potential of neural stem cells. *Nature Reviews Neuroscience, 7*(5), 395.
10. Palmer, T. D., Markakis, E. A., Willhoite, A. R., Safar, F., & Gage, F. H. (1999, October 1). Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS. *The Journal of Neuroscience, 19*(19), 8487–8497.
11. Palmer, T. D., Ray, J., & Gage, F. H. (1995, October 31). FGF-2-responsive neuronal progenitors reside in proliferative and quiescent regions of the adult rodent brain. *Molecular and Cellular Neurosciences, 6*(5), 474–486.
12. Doetsch, F., Caille, I., Lim, D. A., García-Verdugo, J. M., & Alvarez-Buylla, A. (1999, June 11). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell, 97*(6), 703–716.
13. Alvarez-Buylla, A., Seri, B., & Doetsch, F. (2002, April 30). Identification of neural stem cells in the adult vertebrate brain. *Brain Research Bulletin, 57*(6), 751–758.
14. Andres, R. H., Choi, R., Steinberg, G. K., & Guzman, R. (2008, November). Potential of adult neural stem cells in stroke therapy. *Regenerative Medicine, 3*(6), 893–905.
15. Garzón-Muvdi, T., & Quiñones-Hinojosa, A. (2010, January 1). Neural stem cell niches and homing: Recruitment and integration into functional tissues. *ILAR Journal, 51*(1), 3–23.
16. Keyoung, H. M., Roy, N. S., Benraiss, A., Louissaint, A., Suzuki, A., Hashimoto, M., Rashbaum, W. K., Okano, H., & Goldman, S. A. (2001, September 1). High-yield selection and extraction of two promoter-defined phenotypes of neural stem cells from the fetal human brain. *Nature Biotechnology, 19*(9), 843–850.
17. Hao, L., Zou, Z., Tian, H., Zhang, Y., Zhou, H., & Liu, L. (2014, February). Stem cell-based therapies for ischemic stroke. *BioMed Research International, 26*, 2014.
18. Lindvall, O., & Kokaia, Z. (2011). Stem cell research in stroke. *Stroke, 142*(8), 2369–2375.
19. Hori, J., Ng, T. F., Shatos, M., Klassen, H., Streilein, J. W., & Young, M. J. (2003, July 1). Neural progenitor cells lack immunogenicity and resist destruction as allografts. *Stem Cells, 21*(4), 405–416.
20. Grompe, M. (2002, September 1). Adult versus embryonic stem cells: It's still a tie. *Molecular Therapy, 6*(3), 303–305.
21. Seminatore, C., Polentes, J., Ellman, D., Kozubenko, N., Itier, V., Tine, S., Tritschler, L., Brenot, M., Guidou, E., Blondeau, J., & Lhuillier, M. (2010, January 1). The postischemic environment differentially impacts teratoma or tumor formation after transplantation of human embryonic stem cell-derived neural progenitors. *Stroke, 41*(1), 153–159.
22. Kim, J. Y., Kawabori, M., & Yenari, M. A. (2014, June 1). Innate inflammatory responses in stroke: Mechanisms and potential therapeutic targets. *Current Medicinal Chemistry, 21*(18), 2076–2097.
23. Pluchino, S., & Cossetti, C. (2013, September 1). How stem cells speak with host immune cells in inflammatory brain diseases. *Glia, 61*(9), 1379–1401.
24. Toda, H., Takahashi, J., Iwakami, N., Kimura, T., Hoki, S., Mozumi-Kitamura, K., Ono, S., & Hashimoto, N. (2001, December 4). Grafting neural stem cells improved the impaired spatial recognition in ischemic rats. *Neuroscience Letters, 316*(1), 9–12.
25. Jiang, Q., Zhang, Z. G., Ding, G. L., Zhang, L., Ewing, J. R., Wang, L., Zhang, R., Li, L., Lu, M., Meng, H., & Arbab, A. S. (2005, November 15). Investigation of neural progenitor cell induced angiogenesis after embolic stroke in rat using MRI. *NeuroImage, 28*(3), 698–707.
26. Magnitsky, S., Walton, R. M., Wolfe, J. H., & Poptani, H. (2008, October 31). Magnetic resonance imaging detects differences in migration between primary and immortalized neural stem cells. *Academic Radiology, 15*(10), 1269–1281.
27. Guzman, R., Choi, R., Gera, A., De Los Angeles, A., Andres, R. H., & Steinberg, G. K. (2008, March 14). Intravascular cell replacement therapy for stroke. *Neurosurgical Focus, 24*(3–4), E15.
28. Wei, L., Cui, L., Snider, B. J., Rivkin, M., Steven, S. Y., Lee, C. S., Adams, L. D., Gottlieb, D. I., Johnson, E. M., Yu, S. P., & Choi, D. W. (2005, July 31). Transplantation of embryonic stem cells overexpressing Bcl-2 promotes functional recovery after transient cerebral ischemia. *Neurobiology of Disease, 19*(1), 183–193.

29. Chau, M., Zhang, J., Wei, L., & Yu, S. P. (2016, April 1). Regeneration after stroke: Stem cell transplantation and trophic factors. *Brain Circulation, 2*(2), 86.
30. Bühnemann, C., Scholz, A., Bernreuther, C., Malik, C. Y., Braun, H., Schachner, M., Reymann, K. G., & Dihné, M. (2006, October 3). Neuronal differentiation of transplanted embryonic stem cell-derived precursors in stroke lesions of adult rats. *Brain, 129*(12), 3238–3248.
31. Keirstead, H. S., Nistor, G., Bernal, G., Totoiu, M., Cloutier, F., Sharp, K., & Steward, O. (2005, May 11). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *The Journal of Neuroscience, 25*(19), 4694–4705.
32. Erdö, F., Bührle, C., Blunk, J., Hoehn, M., Xia, Y., Fleischmann, B., Föcking, M., Küstermann, E., Kolossov, E., Hescheler, J., & Hossmann, K. A. (2003, July). Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. *Journal of Cerebral Blood Flow and Metabolism, 23*(7), 780–785.
33. Barkho BZ, & Zhao, X. (2011, December 1). Adult neural stem cells: Response to stroke injury and potential for therapeutic applications. *Current Stem Cell Research & Therapy, 6*(4), 327–338.
34. Wei, X., Yang, X., Han, Z. P., Qu, F. F., Shao, L., & Shi, Y. F. (2013, June). Mesenchymal stem cells: A new trend for cell therapy. *Acta Pharmacologica Sinica, 34*(6), 747.
35. Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P., Lorenz, H. P., & Hedrick, M. H. (2001, April 1). Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering, 7*(2), 211–228.
36. Dominici, M. L., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Krause, D. S., Deans, R. J., Keating, A., Prockop, D. J., & Horwitz, E. M. (2006, December 31). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy, 8*(4), 315–317.
37. Linero, I., & Chaparro, O. (2014, September 8). Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. *PLoS One, 9*(9), e107001.
38. Kopen, G. C., Prockop, D. J., & Phinney, D. G. (1999, September 14). Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proceedings of the National Academy of Sciences, 96*(19), 10711–10716.
39. Zhao, L. R., Duan, W. M., Reyes, M., Keene, C. D., Verfaillie, C. M., & Low, W. C. (2002, March 31). Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rats. *Experimental Neurology, 174*(1), 11–20.
40. Shen, L. H., Li, Y., Chen, J., Zhang, J., Vanguri, P., Borneman, J., & Chopp, M. (2006, December 31). Intracarotid transplantation of bone marrow stromal cells increases axon-myelin remodeling after stroke. *Neuroscience, 137*(2), 393–399.
41. Delcroix, G. J., Schiller, P. C., Benoit, J. P., & Montero-Menei, C. N. (2010, March 31). Adult cell therapy for brain neuronal damages and the role of tissue engineering. *Biomaterials, 31*(8), 2105–2120.
42. Fiedler, J., Leucht, F., Waltenberger, J., Dehio, C., & Brenner, R. E. (2005, August 26). VEGF-A and PlGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. *Biochemical and Biophysical Research Communications, 334*(2), 561–568.
43. Shen, L. H., Li, Y., Chen, J., Cui, Y., Zhang, C., Kapke, A., Lu, M., Savant-Bhonsale, S., & Chopp, M. (2007, July 1). One-year follow-up after bone marrow stromal cell treatment in middle-aged female rats with stroke. *Stroke, 38*(7), 2150–2156.
44. Du, S., Guan, J., Mao, G., Liu, Y., Ma, S., Bao, X., Gao, J., Feng, M., Li, G., Ma, W., & Yang, Y. (2014, December 31). Intra-arterial delivery of human bone marrow mesenchymal stem cells is a safe and effective way to treat cerebral ischemia in rats. *Cell Transplantation, 23*(1), S73–S82.

45. Ohmi, K., Greenberg, D. S., Rajavel, K. S., Ryazantsev, S., Li, H. H., & Neufeld, E. F. (2003, February 18). Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB. *Proceedings of the National Academy of Sciences, 100*(4), 1902–1907.
46. Ma, X. L., Liu, K. D., Li, F. C., Jiang, X. M., Jiang, L., & Li, H. L. (2013, May 1). Human mesenchymal stem cells increases expression of α-tubulin and angiopoietin 1 and 2 in focal cerebral ischemia and reperfusion. *Current Neurovascular Research, 10*(2), 103–111.
47. Zhao, Y., Lai, W., Xu, Y., Li, L., Chen, Z., & Wu, W. (2013, December 1). Exogenous and endogenous therapeutic effects of combination sodium ferulate and bone marrow stromal cells (BMSCs) treatment enhance neurogenesis after rat focal cerebral ischemia. *Metabolic Brain Disease, 28*(4), 655–666.
48. Li, G., Yu, F., Lei, T., Gao, H., Li, P., Sun, Y., Huang, H., & Mu, Q. (2016, June). Bone marrow mesenchymal stem cell therapy in ischemic stroke: Mechanisms of action and treatment optimization strategies. *Neural Regeneration Research, 11*(6), 1015.
49. Kim, H. W., Mallick, F., Durrani, S., Ashraf, M., Jiang, S., & Haider, K. H. (2012, October 15). Concomitant activation of miR-107/PDCD10 and hypoxamir-210/Casp8ap2 and their role in cytoprotection during ischemic preconditioning of stem cells. *Antioxidants & Redox Signaling, 17*(8), 1053–1065.
50. Tsuji, W., Rubin, J. P., & Marra, K. G. (2014, July 26). Adipose-derived stem cells: Implications in tissue regeneration. *World Journal of Stem Cells, 6*(3), 312.
51. Lindroos, B., Suuronen, R., & Miettinen, S. (2011). The potential of adipose stem cells in regenerative medicine. *Stem Cell Reviews, 7*, 269–290.
52. Bhang, S. H., Cho, S. W., La, W. G., Lee, T. J., Yang, H. S., Sun, A. Y., Baek, S. H., Rhie, J. W., & Kim, B. S. (2011). Angiogenesis in ischemic tissue produced by spheroid grafting of human adipose-derived stromal cells. *Biomaterials, 32*, 2734–2747.
53. Eto, H., Suga, H., Inoue, K., Aoi, N., Kato, H., Araki, J., Doi, K., Higashino, T., & Yoshimura, K. (2011). Adipose injury-associated factors mitigate hypoxia in ischemic tissues through activation of adipose-derived stem/progenitor/stromal cells and induction of angiogenesis. *The American Journal of Pathology, 178*(5), 2322–2332.
54. Jiang, W., Liang, G., Li, X., Li, Z., Gao, X., Feng, S., Wang, X., Liu, M., & Liu, Y. (2014, May 1). Intracarotid transplantation of autologous adipose-derived mesenchymal stem cells significantly improves neurological deficits in rats after MCAo. *Journal of Materials Science Materials in Medicine, 25*(5), 1357–1366.
55. Covas, D. T., Siufi, J. L., Silva, A. R., & Orellana, M. D. (2003, September). Isolation and culture of umbilical vein mesenchymal stem cells. *Brazilian Journal of Medical and Biological Research, 36*(9), 1179–1183.
56. Shahaduzzaman, M., Golden, J. E., Green, S., Gronda, A. E., Adrien, E., Ahmed, A., Sanberg, P. R., Bickford, P. C., Gemma, C., & Willing, A. E. (2013, December 1). A single administration of human umbilical cord blood T cells produces long-lasting effects in the aging hippocampus. *Age, 35*(6), 2071–2087.
57. Dalous, J., Larghero, J., & Baud, O. (2012, February 8). Transplantation of umbilical cord-derived mesenchymal stem cells as a novel strategy to protect the central nervous system: Technical aspects, preclinical studies, and clinical perspectives. *Pediatric Research, 71*(4–2), 482–490.
58. Malgieri, A., Kantzari, E., Patrizi, M. P., & Gambardella, S. (2010). Bone marrow and umbilical cord blood human mesenchymal stem cells: State of the art. *International Journal of Clinical and Experimental Medicine, 3*(4), 248.
59. Delorme, B., Ringe, J., Gallay, N., Le Vern, Y., Kerboeuf, D., Jorgensen, C., Rosset, P., Sensebe, L., Layrolle, P., Häupl, T., & Charbord, P. (2008, March 1). Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. *Blood, 111*(5), 2631–2635.

60. Hodgkinson, C. P., Gomez, J. A., Mirotsou, M., & Dzau, V. J. (2010). Genetic engineering of mesenchymal stem cells and its application in human disease therapy. *Human Gene Therapy, 21*, 1513–1526.
61. Koh, S. H., Kim, K. S., Choi, M. R., Jung, K. H., Park, K. S., Chai, Y. G., Roh, W., Hwang, S. J., Ko, H. J., Huh, Y. M., & Kim, H. T. (2008, September 10). Implantation of human umbilical cord-derived mesenchymal stem cells as a neuroprotective therapy for ischemic stroke in rats. *Brain Research, 1229*, 233–248.
62. Zhang, L., Li, Y., Zhang, C., Chopp, M., Gosiewska, A., & Hong, K. (2011). Delayed administration of human umbilical tissue-derived cells improved neurological functional recovery in a rodent model of focal ischemia. *Stroke, 42*, 1437–1444.
63. Meng, X., Ichim, T. E., Zhong, J., Rogers, A., Yin, Z., Jackson, J., Wang, H., Ge, W., Bogin, V., Chan, K. W., & Thébaud, B. (2007, November 15). Endometrial regenerative cells: A novel stem cell population. *Journal of Translational Medicine, 5*(1), 57.
64. Sousa, B. R., Parreira, R. C., Fonseca, E. A., Amaya, M. J., Tonelli, F. M., Lacerda, S., Lalwani, P., Santos, A. K., Gomes, K. N., Ulrich, H., & Kihara, A. H. (2014, January 1). Human adult stem cells from diverse origins: An overview from multiparametric immunophenotyping to clinical applications. *Cytometry Part A, 85*(1), 43–77.
65. Gargett, C. E., & Masuda, H. (2010). Adult stem cells in the endometrium. *Molecular Human Reproduction, 16*, 818–834.
66. Cho, N. H., Park, Y. K., Kim, Y. T., Yang, H., & Kim, S. K. (2004, February 29). Lifetime expression of stem cell markers in the uterine endometrium. *Fertility and Sterility, 81*(2), 403–407.
67. Borlongan, C. V., Kaneko, Y., Maki, M., Yu, S. J., Ali, M., Allickson, J. G., Sanberg, C. D., Kuzmin-Nichols, N., & Sanberg, P. R. (2010, April 1). Menstrual blood cells display stem cell-like phenotypic markers and exert neuroprotection following transplantation in experimental stroke. *Stem Cells and Development, 19*(4), 439–452.
68. Becker, A. J., Mc, C. E., & Till, J. E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature, 197*, 452–454.
69. Kirschstein, R., & Skirboll, L. R. (2001). Hematopoietic stem cells. In Nih (Ed.), *Stem cells: Scientific progress and future research direction* (pp. 43–58). Bethesda: National Institutes of Health—Department of Health and Human Services.
70. Mayle, A., Luo, M., Jeong, M., & Goodell, M. A. (2013). Flow cytometry analysis of murine hematopoietic stem cells. *Cytometry Part A Journal of the International Society for Analytical Cytology, 83*, 27–37.
71. Kiel, M. J., Yilmaz, O. H., Iwashita, T., Yilmaz, O. H., Terhorst, C., & Morrison, S. J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell, 121*, 1109–1121.
72. Calvi, L. M., Adams, G. B., Weibrecht, K. W., & Weber, J. M. (2003, October 23). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature, 425*(6960), 841.
73. Taguchi, A., Soma, T., Tanaka, H., Kanda, T., Nishimura, H., Yoshikawa, H., Tsukamoto, Y., Iso, H., Fujimori, Y., Stern, D. M., & Naritomi, H. (2004, August 1). Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *The Journal of Clinical Investigation, 114*(3), 330.
74. Shyu, W. C., Lin, S. Z., Chiang, M. F., Su, C. Y., & Li, H. (2006). Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. *The Journal of Neuroscience, 26*, 3444–3453.
75. Felfly, H., Muotri, A., Yao, H., & Haddad, G. G. (2010, October 31). Hematopoietic stem cell transplantation protects mice from lethal stroke. *Experimental Neurology, 225*(2), 284–293.
76. Takahashi, K., & Yamanaka, S. (2006, August 25). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell, 126*(4), 663–676.
77. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., & Yamanaka, S. (2007, November 30). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell, 131*(5), 861–872.

78. Schmidt, R., & Plath, K. (2012, October 22). The roles of the reprogramming factors Oct4, Sox2 and Klf4 in resetting the somatic cell epigenome during induced pluripotent stem cell generation. *Genome Biology, 13*(10), 251.
79. Han, W., Zhao, Y., & Fu, X. (2010, April 1). Induced pluripotent stem cells: The dragon awakens. *Bioscience, 60*(4), 278–285.
80. Jiang, M., Lv, L., Ji, H., Yang, X., Zhu, W., Cai, L., Gu, X., Chai, C., Huang, S., Sun, J., & Dong, Q. (2011, August 1). Induction of pluripotent stem cells transplantation therapy for ischemic stroke. *Molecular and Cellular Biochemistry, 354*(1–2), 67–75.
81. Chen, S. J., Chang, C. M., Tsai, S. K., Chang, Y. L., Chou, S. J., Huang, S. S., Tai, L. K., Chen, Y. C., Ku, H. H., Li, H. Y., & Chiou, S. H. (2010, March 1). Functional improvement of focal cerebral ischemia injury by subdural transplantation of induced pluripotent stem cells with fibrin glue. *Stem Cells and Development, 19*(11), 1757–1767.
82. Cooray, S., Howe, S. J., & Thrasher, A. J. (2012 January 1). Retrovirus and lentivirus vector design and methods of cell conditioning. *Methods in Enzymology, 507*, 29.
83. Lowry, W. E., Richter, L., Yachechko, R., Pyle, A. D., Tchieu, J., Sridharan, R., Clark, A. T., & Plath, K. (2008). Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 2883–2888.
84. Yu, S. P., Wei, Z., & Wei, L. (2013, February 1). Preconditioning strategy in stem cell transplantation therapy. *Translational Stroke Research, 4*(1), 76–88.
85. Wei, L., Fraser, J. L., Lu, Z. Y., Hu, X. Y., & Yu, S. P. (2012). Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiology of Disease, 46*, 635–645.
86. Liu, X. B., Wang, J. A., Ji, X. Y., Yu, S. P., & Wei, L. (2014). Preconditioning of bone marrow mesenchymal stem cells by prolyl hydroxylase inhibition enhances cell survival and angiogenesis in vitro and after transplantation into the ischemic heart of rats. *Stem Cell Research & Therapy, 5*(5), 111.
87. Taban, Z. F., Khatibi, S., Halabian, R., & Roushandeh, A. M. (2016). The effects of preconditioning on survival of mesenchymal stem cells in vitro. *Gene, Cell and Tissue, 3*(4), e40229.

Chapter 10
Emerging Role of microRNAs in Cerebral Stroke Pathophysiology

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Abstract Cerebral stroke is a major cause of death and physical disability throughout the world, yet therapeutic options remain limited. The outcomes of stroke injury are critical, causing an extensive burden to both the individual patient and society. Current interventions for stroke injury have been demonstrated to be inadequate, mostly attributable to a lack of understanding of the cellular and molecular changes that occur following an ischemic cerebral stroke. MicroRNAs (miRNAs) are small, endogenous, noncoding RNA molecules that have capacity as post-transcriptional negative regulators of a target mRNA by base-pairing with the 3′- untranslated region (3′-UTR). Novel methodologies are being produced to get miRNA-related therapeutics into the brain over an intact BBB, including chemical modification, use of targeting molecules and methods of disrupting the BBB. However, circulating miRNAs are novel, stable, and potential biomarkers for the early diagnosis of acute stroke in humans. These miRNA profiles also indicate the severity of stroke results related to age and sex in rodents. In this chapter, we focus on the pathophysiological role of miRNAs as novel diagnostic and prognostic biomarkers, in addition to promising therapeutic interventions in cerebral stroke patients.

Keywords MicroRNAs · Blood–brain barrier · Biomarkers · Stroke · Antagomir

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Abbreviations

BDNF	Brain-derived neurotrophic factor
COX2	Cyclooxygenase 2
e-NOS	Endothelial-NOS
FAP-1	Fas-associated protein-tyrosine phosphatase 1
FasL	Fas ligand
FGF2	Fibroblast growth factor 2
GAX	Growth arrest-specific homeobox
GLT-1	Glutamate transporter-1
GluR2	Glutamate receptor 2
HOXA5	Homeobox A5
HSPA12B	Heat shock protein A12B
iASPP	Inhibitory member of the apoptosis-stimulating proteins of p53 family
IGF-1	Insulin-like growth factor 1
IL	Interleukin
KIT	Kit ligand
MDA	Malondialdehyde
MMP-9	Metalloproteinases 9
MnSOD	Manganese SOD
MyD88	Myeloid differentiation primary response gene 88
NCX1	Sodium–calcium exchanger-1
NMDA	N-Methyl-D-aspartate
NPC	Neuronal progenitor cell
Nrf2	Nuclear factor erythroid-2 related factor 2
PUMA	p53 upregulated modulator of apoptosis
ROS	Reactive oxygen species
SOCS1	Suppressor of cytokine signaling 1
SOD	Superoxide dismutase
Sox9	Sry-box 9
TGF-β	Transforming growth factor-β
TLR	Toll-like receptor
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

10.1 Introduction

Stroke is one of the most devastating of all neurological disabilities, accounting for 5.5 million deaths annually, with 44 million physical disabilities worldwide. The outcomes of stroke injuries are intense and persistent, causing a high burden to both the individual patient and society because of their increasing incidence and

mortality. It has an economic impact, mainly in low- and middle-income countries [1]. Stroke is categorized into two different types: ischemic and hemorrhagic stroke. Ischemic stroke is responsible for 80% of all strokes, whereas hemorrhagic stroke accounts for 15%, and the other 5% are of an unknown etiology [2]. In the present chapter, we discuss the emerging pathophysiological role of miRNAs in the diagnosis, prognosis, and therapeutic interventions of cerebral stroke, and how miRNAs are involved in the post-stroke recovery and repair pathways.

10.2 MicroRNA Biogenesis

Micro-RNAs are small nonprotein coding RNA molecules ~24 nucleotides (nt) in length. They are transcribed into long primary transcripts in the nucleus and then transported to the cytoplasm, where they are processed to form mature miRNAs. Sequential endonucleolytic cleavage steps are required to develop mature miRNA from miRNA genes, starting with long primary miRNA transcripts (pri-miRNA), which are processed to ~70-nt precursor miRNA (pre-miRNA), and then to mature miRNA. A short (5–7 nt long) sequence, known as the seed sequence, in the miRNA determines the specificity of binding to the mRNA, so that miRNAs can bind multiple mRNAs and mRNAs can be bound by multiple miRNAs, creating a novel regulatory complex layer to post-transcriptional control of the target genes (Fig. 10.1). Several authors reported both the repressive and the inducing nature of miRNA for different target genes in the pathogenesis of stroke (Tables 10.1 and 10.2). Furthermore, several recent lines suggested that pri-mRNA and pre-miRNA might potentially interact with the target gene and downregulate expression. miRNAs can target specific genes by inhibiting mRNA translation or degrading the mRNA molecules by binding to their 3′-untranslated (UTR) region of mRNA [39].

Alteration in the expression of miRNAs has been shown in many diseases, such as cancer, cardiovascular disease, diabetes, and neurological disorder. Now, miRNAs act as an essential gene expression regulator to modulate cardiovascular and cerebrovascular development. As previously reported, 30% of protein expression is regulated by miRNAs. They are emerging as potential biomarkers and therapeutic targets in stroke and other related diseases [2, 40–47].

Non-invasive clinical methods such as CT and MRI have diagnostic and prognostic powers, but have lower accessibility and involve higher costs. However, other diagnostic tools include interleukin-6 (IL-6), matrix metallopeptidase-9 (MMP-9), and C-reactive protein (CRP), whose specificity and capacity to recognize intense stroke and its related hazard factors are unclear [48]. Thus, because of a limited therapeutic window for thrombolysis, there is a need for the development of a new biomarker for the prediction of stroke. For this reason, several studies have been conducted to explore the promising role of miRNAs as diagnostic circulating biomarkers (Table 10.1) that could differentiate stroke from nonstroke patients [49].

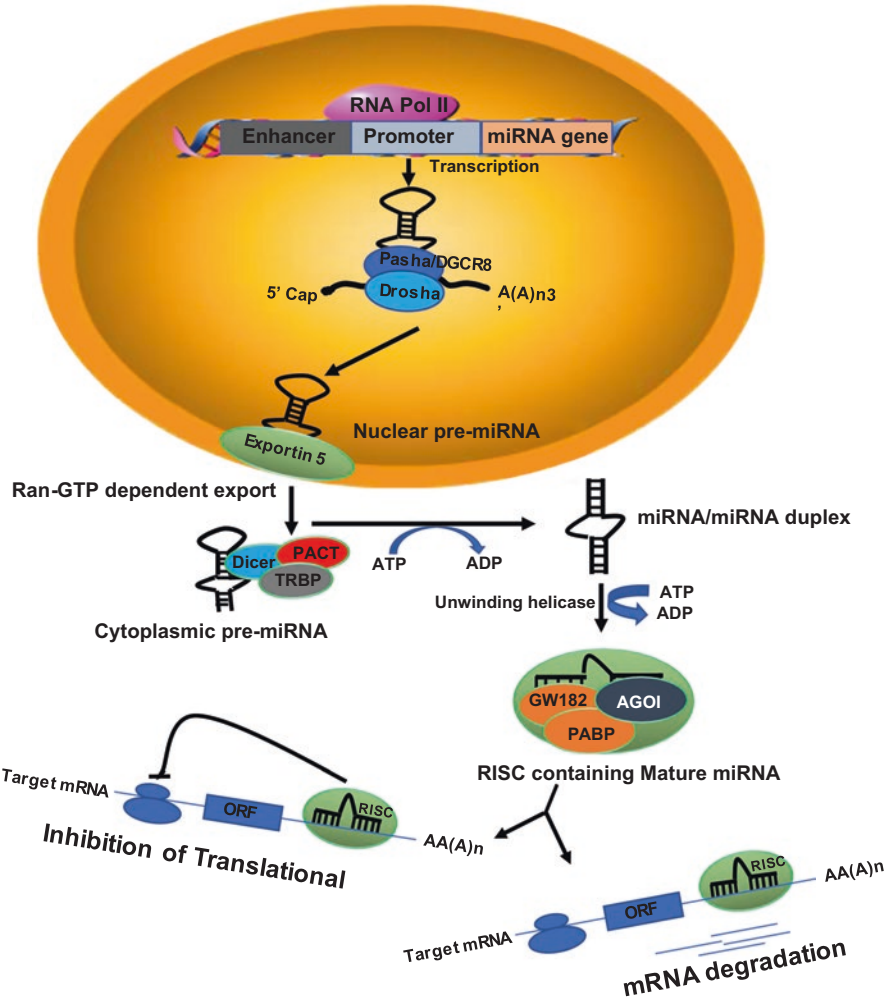

Fig. 10.1 miRNA biogenesis and function

10.3 Current Therapy and the Possible Role of miRNA in Neuroprotection

The fundamental pathophysiology of stroke is highly complex, consisting of impairments of many signaling cascade and pathological processes such as excitotoxicity, oxidative stress, inflammation, and apoptosis. Potential restorative regions to repay these pathogenic procedures incorporate advancing angiogenesis, neurogenesis, and neuroprotection (Fig. 10.2). As of now, viable treatment for ischemic stroke is restricted to recombinant tissue plasminogen activator (rtPA=alteplase). rtPA is the only approved intravenously administered pharmaceutical thrombolytic agent

Table. 10.1 miRNAs and their target genes related to stroke pathogenesis, angiogenesis, and neurogenesis

miRNA	Function of miRNA	Main target genes	References
miR-21	NPC regulation	Wnt and TGF-β	[3, 4]
miR-29a	Protects astrocyte GLT-1	PUMA	[5]
miR-107	Glutamate accumulation	GLT-1	[6]
miR-223	Attenuates NMDA-induced calcium influx	GluR2	[7]
miR-181c	NF-κB activation	TLR4	[8]
miR-let-7c-5p	Neuroprotection against inflammation	Caspase-3	[9]
Anti-miR-103-1	Cellular calcium and sodium homeostasis	NCX1	[10]
miR-134	Increases neuronal apoptosis	HSPA12B	[11]
miR-93	Upregulation of SOD enzymes	Nrf2	[12]
miRNA-384-5p and miRNA-494	Increases neuronal cell death	Bcl-2	[13]
miR-145	Increases oxidative damage	SOD	[14]
miR-29	Induction of Fas receptors	FAP-1	[15]
miR-155	Upregulation of TLR4	SOCS1, MyD88	[16]
miR-21	Apoptosis inhibition	FasL	[17]
miR-99a	Prevents neural apoptosis	Caspase-3	[18]
miR-101	ROS production	COX2	
miR-146a	ROS production	COX-2	[19]
miR-497	Increasing neuronal cell death	Bcl-2 and Bcl-w	[20]
miR-146a	Anti-inflammatory effect	IL-1β and IL-6	[19]
miR-223	Attenuates NMDA-induced calcium influx	NR2B	[7]
miR-221 and miR-222	Decreases tube formation	KIT and e-NOS	[21]
miR-424	Inhibits oxidative damage	MDA	[22]
miR-181c	Decreases neuronal apoptosis	TNF-α	[14]
miR-let-7c	Decreases inflammation	iNOS, TNF-α, and IL-6	[23]
miR-16, -20a, and -20b	Anti-angiogenic agent	VEGF	[24]
miR-181a	Anti-inflammatory effect	IL1-α	[25]
miR-181c	Proinflammatory gene expressions	NF-κB	[8]
miR-491-5p	Inhibits cellular invasion	MMP-9	[26]
miR-25	Apoptosis inhibition	FasL	[27]
miR-9	Decreases neuronal apoptosis	Bcl2L11	[28]
miR-134 antagomir	Neurogenesis	BDNF	[29]
miR-106b-5p	Decreases neuronal apoptosis	Mcl-1	[30]
miR-124	Promotes neuronal apoptosis	iASPP	[3, 4]
miR-181a	Astrocyte dysfunction	Bcl-2	[31]
miR-Let7f antagomir	Neuroprotection	IGF-1	[32]

(continued)

Table. 10.1 (continued)

miRNA	Function of miRNA	Main target genes	References
miR-134	Alleviates ischemic injury	Bcl-2	[29]
miR-124a	Neurogenesis inhibition	JAG1/Notch	[33]
miR-34a	NPC regulation	Notch, Wnt, and TGF-β	[3, 4]
miR-145 antagomir	Inhibition of oxidative stress	SOD2	[14]
miR-181a antagomir	Decreases brain ischemia injury	NF-κB	[34]
miR-125b	Excitotoxic neuronal damage	NR2A	[35]
miR-210	Promotes angiogenesis	VEGF	[36]
miR-124	Promotes neural differentiation	Sox9	[33]
miR-15a	Suppresses post-stroke angiogenesis	FGF2	[37]
miR-130a	Promotes angiogenesis	GAX and HOXA5	[38]

Table 10.2 miRNA expression in stroke cases

Upregulated miRNA	Downregulated miRNA
hsa-let-7e, hsa-miR-125b-2*, hsa-miR-1261, hsa-miR-129-5p, hsa-miR-1321, hsa-miR-135b, hsa-miR-145, hsa-miR-184, hsa-miR-187*, hsa-miR-196a*, hsa-miR-198, hsa-miR-200b*, hsa-miR-549, hsa-miR-214, hsa-miR-220c, hsa-miR-25*, hsa-miR-585, hsa-miR-553, hsa-miR-99a, hsa-miR-26b*, hsa-miR-602, hsa-miR-27a*, hsa-miR-611, hsa-miR-34b, hsa-miR-623, hsa-miR-210, hsa-miR-943, hsa-miR-525-5p, hsa-miR-488, hsa-miR-498, hsa-miR-675, hsa-miR-920, hsa-miR-933, hsa-miR-552, hsa-miR-494, hsa-miR-671-5p, hsa-miR-490-3p, hsa-miR-617, hsa-miR-668, hsa-miR-659, hsa-miR-381, hsa-miR-637, hsa-miR-627, hsa-miR-422a, hsa-miR-483-5p, hsa-miR-638, hsa-miR-370	hsa-let-7a, hsa-let-7b*, hsa-let-7c hsa-let-7d*, hsa-let-7f, hsa-let-7g hsa-let-7i, hsa-miR-106b*, hsa-miR-126 hsa-miR-1299, hsa-miR-130a hsa-miR-151-5p, hsa-miR-18a* hsa-miR-182, hsa-miR-183, hsa-miR-186 hsa-miR-192, hsa-miR-20a, hsa-miR-208a hsa-miR-22*, hsa-miR-222 hsa-miR-362-5p, hsa-miR-363, hsa-miR-23b, hsa-miR-501-5p hsa-miR-30b, hsa-miR-324-5p, hsa-miR-335, hsa-miR-342-3p, hsa-miR-342-5p, hsa-miR-30e*, hsa-miR-320b, hsa-miR-423-3p, hsa-miR-532-5p, hsa-miR-574-5p, hsa-miR-7, hsa-miR-886-5p, hsa-miR-576-5p, hsa-miR-629 hsa-miR-23a, hsa-miR-361-5p, hsa-miR-340, hsa-miR-652 hsa-miR-500*, hsa-miR-92a, hsa-miR-502-3p, hsa-miR-26b, hsa-miR-502-5p, hsa-miR-320d, hsa-miR-574-3p, hsa-miR-331-3p, hsa-miR-625, hsa-miR-30c, hsa-miR-505*, hsa-miR-493*, hsa-miR-500, hsa-miR-93*, hsa-miR-96

* miRNA dysregulation (both down and upreguation) in ischemic stroke can be associated with its inhibition of apoptosis and oxidative stress, suggesting a potential therapeutic target and potential biomarker for stroke dignosis and prognosis

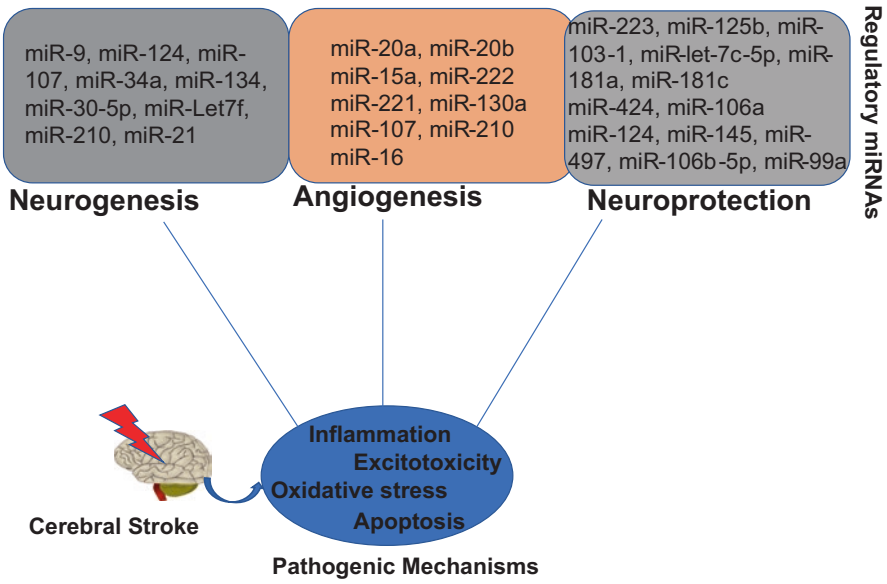

Fig. 10.2 Ischemic stroke and the role of potential therapeutic miRNAs

available for embolus-induced ischemic stroke treatment [50, 52]. In any case, rtPA is constrained by its narrow therapeutic window, which must be offered within 6 h of the beginning of the stroke, hence making it appropriate to only a minority of stroke patients [52]. Additionally, alongside its advantageous thrombolytic function, rtPA has detrimental effects including intracranial hemorrhage and neurotoxicity [52]. Other elective medications incorporate the utilization of other thrombotic agents, mechanical thrombectomy, and antiplatelet agents, for example, aspirin [53]. But there is an unmet clinical need for the development of a novel therapeutic formulation that can regulate the expression of potentially deleterious genes. It can regulate genes that contribute to the neuroprotection, neurogenesis, and angiogenesis, and enhance recovery with repair mechanisms in ischemic stroke patients.

The use of miRNAs as key regulators of gene expression has opened up another field of biomedical research: emerging diagnostic biomarkers and therapeutic interventions for ischemic stroke patients. Despite being only about 22 nt long, miRNAs are thought not to readily cross the blood–brain barrier (BBB) because of their strongly hydrophilic nature. Other factors, such as stability, affect the potential therapeutic use of miRNAs in the brain. Locked nucleic acids (LNAs) are a 2′-modification where 4′-carbon has been attached to the 2′-OH group. These LNAs mediated chemical modification of miRNA can alter their stability against nuclease and cross BBB. Alterations in miRNA expression levels may be needed, either as prevention or treatment of cerebral stroke. Following a stroke, the BBB is disrupted so there is simple access to the infarcted region, yet novel methodologies are being created to get miRNA-related therapeutics into the cerebrum over an unblemished BBB. Chemical modification of rabies infection glycoprotein [54], and opening of

BBB by utilizing mannitol have been made. Cerebral stroke targeted therapy can be made by encapsulation with pegylated immunoliposomes and use of plasmids encoding pri-miRNA [55, 56]. Antagomirs were shown to efficiently target miRNAs when injected via intracerebroventricular (ICV) administration [57]. Subsequent studies have suggested that the therapeutic efficacy of the ICV administration of the miR-497 antagomir might subsequently decrease in the infarct area [20]. Besides, Selvamani et al. used antagomir-based treatment to show complex sex-specific miRNA connections whereby the ICV implantation of the Let7f antagomir displayed neuroprotection only in ovariectomized female rats [32]. Other studies performed by Buller and coworkers demonstrated in a rat model of middle cerebral artery occlusion that miR-121 selectively downregulates Faslg, a TNF-α family member, and that targeted inhibition of miR-121 was cytoprotective in vivo [17].

10.4 Development of Therapeutic Approaches Using miRNA Mimics and Inhibitors

There are two major methods for developing miRNA-based therapeutics: mimics to overexpress the miRNA level, and oligonucleotide inhibitors of miRNA (antagomirs) to decrease miRNA expression. Micro-RNA mimics are small, chemically modified, duplex RNA molecules that faithfully mimic the function of miRNA and load the active strand into ribonucleoprotein complex (RNA-induced silencing complex; RISC), which then binds the target mRNA to induce translational inhibition. miRNA mimics are increasingly being used to explore the biological effect of specific miRNA in cell function and restore a functional loss of beneficial miRNAs. Antagomirs (anti-miRs or blockmiR) are chemically engineered, single-stranded antisense oligonucleotides bearing a complementary sequence to the mature miRNA. Antagomirs are used to silence endogenous levels of the miRNA and increase expression of its mRNA targets. Antagomirs can be utilized as pharmacological inhibition of a few respiratory illnesses and have been utilized broadly in malignancy [58]. Another method is the encapsulation of miR mimics in the liposome-based delivery system to assist target cell uptake and examine its biological effect [59].

10.5 Techniques for miRNA Expression Analysis

miRNA sequences are identified based on the cataloged miRBase Sequence Database. But the most common step is the analysis of miRNA expression levels among different tissues, developmental stages, and disease conditions. miRNA expression levels can be studied using several methods: microarray analysis, nanoString miRNA expression analysis, quantitative real-time PCR (qRT-PCR),

Northern blots, in situ hybridization, and solution hybridization. Of these techniques, qRT-PCR is the most precise and sensitive method. The approaches commonly used for identifying the candidate genes targeted by miRNAs are computational target prediction and experimental target identification strategies.

10.6 Computational Target Gene Prediction

A wide range of computational miRNA target programs is available online for the prediction of miRNA target sites in the 3′ UTR of target mRNAs. These online programs include TargetScan (http://www.targetscan.org), miRbase (http://www.mirbase.org/), ElMMo (http://www.mirz.unibas.ch), MicroCosm Targets (http://www.ebi.ac.uk/enright-srv/microcosm), Pictar (http://pictar.org), and DIANA-microT (http://microrna.gr/microT). All these programs utilize seed sequence complementarity for the prediction of the target gene. These programs are based on sequence alignment of the miRNA seed to the 3′ UTR of candidate target genes [60]. The major disadvantage of this approach is that not all binding sites are in the 3′ UTR. Different current algorithmic programmed uses conservation of miRNA and mRNA target sites over species as a factor to help rank the probability that the objective is genuine. The predicted target mRNAs may be either false-positive or false-negative predictions. Therefore, experimental validation of the target gene is an important step in defining the functions of individual miRNAs.

10.7 Experimental Approaches to miRNA Target Validation

The following experimental approaches are utilized for miRNA target validation:

1. *First approach*: In this approach mRNA is purified after treatment with miRNA mimic or inhibitor/antagomir and analyzed by microarray hybridization or sequencing.
2. *Second approach*: The main regulatory action of miRNAs is to inhibit translation and decrease the amount of protein made from the target mRNA. Changes in protein expression levels can be signaled by using stable isotope labeling of amino acids or by Western blot.

   The major limitation with both these strategies is that they identify candidate genes that could be either direct or indirect targets of the tested miRNA.
3. *Third approach*: This is based on the immunoprecipitation strategy and experimentally validates candidate targets; the RNA-induced silencing complex (RISC) loaded with a specific miRNA is purified by immunoprecipitation along with its bound mRNA targets. This technique has been used in the target identification of hypoxia-induced mmiR-210.

4. *Fourth approach*: This is the method currently used to validate a sequence as a direct target of an miRNA. It depends on the differential activity of a reporter gene, typically luciferase attached to a candidate 3′UTR sequence in the presence or absence of miRNA. Numerous miRNA targets have been approved utilizing the Luciferase Reporter System after cerebral ischemia [20, 61–63].

10.8 miRNA as a Prognostic Biomarker in Ischemic Stroke

Several miRNAs have been widely investigated as biomarkers to distinguish disease from nondisease cases. miRNA expression profiles reflect the transient movement of stroke and additionally particular etiologies. A panel of 32 miRNAs that could differentiate stroke pathophysiologies during the acute phase was identified using a customized TaqMan Low-Density Array. Furthermore, it was found that five miRNAs, miR-125b-2*, -27a*, -422a, -488, and -627 are consistently altered in stroke. Elevated levels of circulating miR-16 after stroke likely express induction of apoptosis in response to stroke [64, 65]. The proliferation of white blood cells or the inflammatory response to stroke may also contribute to the induction of miRNAs and result in increases in its concentration in plasma [66]. In addition, circulating miRNA profiles can predict age- and sex-dependent stroke severity. Thus, although both age and sex eventually influence miRNA expression, each of these variables prevails at different times in the evolution of the serum miRNA response to the infarct. In late improvements, miRNA expression profiling has been examined under stroke conditions, and these examinations show that miRNAs have developed as key regulators in ischemic stroke pathophysiologies. Both expanded and diminished miRNA levels may be required, either as counteractive action or treatment of stroke. Novel methodologies are being developed to get miRNA-related therapeutics into the brain across an intact BBB, including chemical modification, use of targeting molecules, and methods of disrupting the BBB.

10.9 Circular RNA

Circular RNAs (circRNAs) are noncoding RNAs (ncRNAs), created in eukaryotic cells during post-transcriptional processes. They are more stable than linear RNAs, and their 3′ and 5′ end combined forms a covalently closed continuous (CCC) loop and possesses resistance to exonuclease-intervened degradation [67]. Some circRNAs have recently shown potential as gene regulators. CircRNAs are not distributed equally in the neuronal cells in the brain, but are greatly enriched in the synapses. These ncRNA species can be used as potential clinical biomarkers in complex neurological disorders of the central nervous system. For example, ciRS-7 was found to be a natural microRNA sponge for miRNA-7 and a potential regulator of Parkinson's disease- and Alzheimer's disease-related genes [68]. The circRNAs

carry important information, either when they are present inside the cells, or in circulation, or in exosomes released from the synapses. The potential role of circRNAs in stroke remains largely unknown. CircRNA Hectd1 levels were generally increased in ischemic brain tissues of mouse stroke models and this finding was also confirmed in plasma of ischemic stroke patients [69].

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References

1. Mukherjee, D., & Patil, C. G. (2011). Epidemiology and the global burden of stroke. *World Neurosurgery, 76*(6), S85–S90.
2. Beal, C. C. (2010). Gender and stroke symptoms: A review of the current literature. *Journal of Neuroscience Nursing, 42*(2), 80–87.
3. Liu, X., Li, F., Zhao, S., Luo, Y., Kang, J., Zhao, H., Yan, F., Li, S., & Ji, X. (2013). MicroRNA-124–mediated regulation of inhibitory member of apoptosis-stimulating protein of p53 family in experimental stroke. *Stroke, 44*(7), 1973–1980.
4. Liu, F. J., Lim, K. Y., Kaur, P., Sepramaniam, S., Armugam, A., Wong, P. T. H., & Jeyaseelan, K. (2013). microRNAs involved in regulating spontaneous recovery in embolic stroke model. *PLoS One, 8*(6), e66393.
5. Ouyang, Y. B., Xu, L., Lu, Y., Sun, X., Yue, S., Xiong, X. X., & Giffard, R. G. (2013). Astrocyte-enriched miR-29a targets PUMA and reduces neuronal vulnerability to forebrain ischemia. *Glia, 61*(11), 1784–1794.
6. Yang, Z. B., Zhang, Z., Li, T. B., Lou, Z., Li, S. Y., Yang, H., Yang, J., Luo, X. J., & Peng, J. (2014). Up-regulation of brain-enriched miR-107 promotes excitatory neurotoxicity through down-regulation of glutamate transporter-1 expression following ischaemic stroke. *Clinical Science, 127*(12), 679–689.
7. Harraz, M. M., Eacker, S. M., Wang, X., Dawson, T. M., & Dawson, V. L. (2012). MicroRNA-223 is neuroprotective by targeting glutamate receptors. *Proceedings of the National Academy of Sciences, 109*(46), 18962–18967.
8. Zhang, L., Li, Y. J., Wu, X. Y., Hong, Z., & Wei, W. S. (2015). MicroRNA-181c negatively regulates the inflammatory response in oxygen-glucose-deprived microglia by targeting Toll-like receptor 4. *Journal of Neurochemistry, 132*(6), 713–723.
9. Ni, J., Wang, X., Chen, S., Liu, H., Wang, Y., Xu, X., Cheng, J., Jia, J., & Zhen, X. (2015). MicroRNA let-7c-5p protects against cerebral ischemia injury via mechanisms involving the inhibition of microglia activation. *Brain, Behavior, and Immunity, 49*, 75–85.
10. Vinciguerra, A., Formisano, L., Cerullo, P., Guida, N., Cuomo, O., Esposito, A., Di Renzo, G., Annunziato, L., & Pignataro, G. (2014). MicroRNA-103-1 selectively downregulates brain NCX1 and its inhibition by anti-miRNA ameliorates stroke damage and neurological deficits. *Molecular Therapy, 22*(10), 1829–1838.
11. Chi, W., Meng, F., Li, Y., Li, P., Wang, G., Cheng, H., Han, S., & Li, J. (2014). Impact of microRNA-134 on neural cell survival against ischemic injury in primary cultured neuronal cells and mouse brain with ischemic stroke by targeting HSPA12B. *Brain Research, 1592*, 22–33.
12. Wang, P., Liang, X., Lu, Y., Zhao, X., & Liang, J. (2016). MicroRNA-93 downregulation ameliorates cerebral ischemic injury through the Nrf2/HO-1 defense pathway. *Neurochemical Research, 41*(10), 2627–2635.

13. Zhai, F., Zhang, X., Guan, Y., Yang, X., Li, Y., Song, G., & Guan, L. (2012). Expression profiles of microRNAs after focal cerebral ischemia/reperfusion injury in rats. *Neural Regeneration Research, 7*(12), 917.
14. Dharap, A., Bowen, K., Place, R., Li, L. C., & Vemuganti, R. (2009). Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. *Journal of Cerebral Blood Flow & Metabolism, 29*(4), 675–687.
15. Schickel, R., Park, S. M., Murmann, A. E., & Peter, M. E. (2010). miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. *Molecular Cell, 38*(6), 908–915.
16. Wen, Y., Zhang, X., Dong, L., Zhao, J., Zhang, C., & Zhu, C. (2015). Acetylbritannilactone modulates microRNA-155-mediated inflammatory response in ischemic cerebral tissues. *Molecular Medicine, 21*(1), 197.
17. Buller, B., Liu, X., Wang, X., Zhang, R. L., Zhang, L., Hozeska-Solgot, A., Chopp, M., & Zhang, Z. G. (2010). MicroRNA-21 protects neurons from ischemic death. *The FEBS Journal, 277*(20), 4299–4307.
18. Tao, Z., Zhao, H., Wang, R., Liu, P., Yan, F., Zhang, C., Ji, X., & Luo, Y. (2015). Neuroprotective effect of microRNA-99a against focal cerebral ischemia-reperfusion injury in mice. *Journal of the Neurological Sciences, 355*(1), 113–119.
19. Iyer, A., Zurolo, E., Prabowo, A., Fluiter, K., Spliet, W. G., van Rijen, P. C., Gorter, J. A., & Aronica, E. (2012). MicroRNA-146a: A key regulator of astrocyte-mediated inflammatory response. *PLoS One, 7*(9), e44789.
20. Yin, K. J., Deng, Z., Huang, H., Hamblin, M., Xie, C., Zhang, J., & Chen, Y. E. (2010). miR-497 regulates neuronal death in mouse brain after transient focal cerebral ischemia. *Neurobiology of Disease, 38*(1), 17–26.
21. Suárez, Y., Fernández-Hernando, C., Pober, J. S., & Sessa, W. C. (2007). Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circulation Research, 100*(8), 1164–1173.
22. Zhao, H., Wang, J., Gao, L., Wang, R., Liu, X., Gao, Z., Tao, Z., Xu, C., Song, J., Ji, X., & Luo, Y. (2013). MiRNA-424 protects against permanent focal cerebral ischemia injury in mice involving suppressing microglia activation. *Stroke, 44*(6), 1706–1713.
23. Banerjee, S., Xie, N., Cui, H., Tan, Z., Yang, S., Icyuz, M., Abraham, E., & Liu, G. (2013). MicroRNA let-7c regulates macrophage polarization. *The Journal of Immunology, 190*(12), 6542–6549.
24. Hua, Z., Lv, Q., Ye, W., Wong, C. K. A., Cai, G., Gu, D., Ji, Y., Zhao, C., Wang, J., Yang, B. B., & Zhang, Y. (2006). MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. *PLoS One, 1*(1), e116.
25. Xie, W., Li, M., Xu, N., Lv, Q., Huang, N., He, J., & Zhang, Y. (2013). MiR-181a regulates inflammation responses in monocytes and macrophages. *PLoS One, 8*(3), e58639.
26. Yan, W., Zhang, W., Sun, L., Liu, Y., You, G., Wang, Y., Kang, C., You, Y., & Jiang, T. (2011). Identification of MMP-9 specific microRNA expression profile as potential targets of anti-invasion therapy in glioblastoma multiforme. *Brain Research, 1411*, 108–115.
27. Zhang, J. F., Shi, L. L., Zhang, L., Zhao, Z. H., Liang, F., Xu, X., Zhao, L. Y., Yang, P. B., Zhang, J. S., & Tian, Y. F. (2016). MicroRNA-25 negatively regulates cerebral ischemia/reperfusion injury-induced cell apoptosis through Fas/FasL pathway. *Journal of Molecular Neuroscience, 58*(4), 507–516.
28. Wei, N., Xiao, L., Xue, R., Zhang, D., Zhou, J., Ren, H., Guo, S., & Xu, J. (2016). MicroRNA-9 mediates the cell apoptosis by targeting Bcl2l11 in ischemic stroke. *Molecular Neurobiology, 53*(10), 6809–6817.
29. Huang, W., Liu, X., Cao, J., Meng, F., Li, M., Chen, B., & Zhang, J. (2015). miR-134 regulates ischemia/reperfusion injury-induced neuronal cell death by regulating CREB signaling. *Journal of Molecular Neuroscience, 55*(4), 821–829.
30. Liu, P., Zhao, H., Wang, R., Wang, P., Tao, Z., Gao, L., Yan, F., Liu, X., Yu, S., Ji, X., & Luo, Y. (2014). MicroRNA-424 protects against focal cerebral ischemia and reperfusion injury in mice by suppressing oxidative stress. *Stroke, 9*, e91661.

31. Moon, J. M., Xu, L., & Giffard, R. G. (2013). Inhibition of microRNA-181 reduces forebrain ischemia-induced neuronal loss. *Journal of Cerebral Blood Flow & Metabolism, 33*(12), 1976–1982.
32. Selvamani, A., Sathyan, P., Miranda, R. C., & Sohrabji, F. (2012). An antagomir to microRNA Let7f promotes neuroprotection in an ischemic stroke model. *PLoS One, 7*(2), e32662.
33. Cheng, L. C., Pastrana, E., Tavazoie, M., & Doetsch, F. (2009). miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nature Neuroscience, 12*(4), 399–408.
34. Xu, L. J., Ouyang, Y. B., Xiong, X., Stary, C. M., & Giffard, R. G. (2015). Post-stroke treatment with miR-181 antagomir reduces injury and improves long-term behavioral recovery in mice after focal cerebral ischemia. *Experimental Neurology, 264*, 1–7.
35. Edbauer, D., Neilson, J. R., Foster, K. A., Wang, C. F., Seeburg, D. P., Batterton, M. N., Tada, T., Dolan, B. M., Sharp, P. A., & Sheng, M. (2010). Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. *Neuron, 65*(3), 373–384.
36. Mellios, N., Huang, H. S., Grigorenko, A., Rogaev, E., & Akbarian, S. (2008). A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. *Human Molecular Genetics, 17*(19), 3030–3042.
37. Yin, K. J., Olsen, K., Hamblin, M., Zhang, J., Schwendeman, S. P., & Chen, Y. E. (2012). Vascular endothelial cell-specific microRNA-15a inhibits angiogenesis in hindlimb ischemia. *Journal of Biological Chemistry, 287*(32), 27055–27064.
38. Chen, Y., & Gorski, D. H. (2008). Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. *Blood, 111*(3), 1217–1226.
39. Sepramaniam, S., Armugam, A., Lim, K. Y., Karolina, D. S., Swaminathan, P., Tan, J. R., & Jeyaseelan, K. (2010). MicroRNA 320a functions as a novel endogenous modulator of aquaporins 1 and 4 as well as a potential therapeutic target in cerebral ischemia. *Journal of Biological Chemistry, 285*(38), 29223–29230.
40. Jickling, G. C., & Sharp, F. R. (2015). Biomarker panels in ischemic stroke. *Stroke, 46*(3), 915–920.
41. Sepramaniam, S., Tan, J. R., Tan, K. S., DeSilva, D. A., Tavintharan, S., Woon, F. P., Wang, C. W., Yong, F. L., Karolina, D. S., Kaur, P., & Liu, F. J. (2014). Circulating microRNAs as biomarkers of acute stroke. *International Journal of Molecular Sciences, 15*(1), 1418–1432.
42. Onwuekwe, I. O., & Ezeala-Adikaibe, B. (2012). Ischemic stroke and neuroprotection. *Annals of Medical and Health Sciences Research, 2*(2), 186–190.
43. Guyot, L. L., Diaz, F. G., O'Regan, M. H., McLeod, S., Park, H., & Phillis, J. W. (2001). Real-time measurement of glutamate release from the ischemic penumbra of the rat cerebral cortex using a focal middle cerebral artery occlusion model. *Neuroscience Letters, 299*(1), 37–40.
44. Ohta, K., Graf, R., Rosner, G., & Heiss, W. D. (2001). Calcium ion transients in peri-infarct depolarizations may deteriorate ion homeostasis and expand infarction in focal cerebral ischemia in cats. *Stroke, 32*(2), 535–543.
45. Annunziato, L., Pignataro, G., & Di Renzo, G. F. (2004). Pharmacology of brain Na+/Ca2+ exchanger: From molecular biology to therapeutic perspectives. *Pharmacological Reviews, 56*(4), 633–654.
46. Boscia, F., Gala, R., Pignataro, G., De Bartolomeis, A., Cicale, M., Ambesi-Impiombato, A., Di Renzo, G., & Annunziato, L. (2006). Permanent focal brain ischemia induces isoform-dependent changes in the pattern of Na+/Ca2+ exchanger gene expression in the ischemic core, periinfarct area, and intact brain regions. *Journal of Cerebral Blood Flow & Metabolism, 26*(4), 502–517.
47. Tortiglione, A., Pignataro, G., Minale, M., Secondo, A., Scorziello, A., Di Renzo, G. F., Amoroso, S., Caliendo, G., Santagada, V., & Annunziato, L. (2002). Na+/Ca2+ exchanger in Na+ efflux-Ca2+ influx mode of operation exerts a neuroprotective role in cellular models of in vitro anoxia and in vivo cerebral ischemia. *Annals of the New York Academy of Sciences, 976*(1), 408–412.

48. Jickling, G. C., & Sharp, F. R. (2011). Blood biomarkers of ischemic stroke. *Neurotherapeutics, 8*(3), 349.
49. Zhan, X., Jickling, G. C., Tian, Y., Stamova, B., Xu, H., Ander, B. P., Turner, R. J., Mesias, M., Verro, P., Bushnell, C., & Johnston, S. C. (2011). Transient ischemic attacks characterized by RNA profiles in blood. *Neurology, 77*(19), 1718–1724.
50. Hacke, W., Kaste, M., Fieschi, C., von Kummer, R., Davalos, A., Meier, D., Larrue, V., Bluhmki, E., Davis, S., Donnan, G., & Schneider, D. (1998). Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). *The Lancet, 352*(9136), 1245–1251.
51. Albers, G. W., Bates, V. E., Clark, W. M., Bell, R., Verro, P., & Hamilton, S. A. (2000). Intravenous tissue-type plasminogen activator for treatment of acute stroke: The Standard Treatment with Alteplase to Reverse Stroke (STARS) study. *JAMA, 283*(9), 1145–1150.
52. Wahlgren, N., Ahmed, N., Dávalos, A., Ford, G. A., Grond, M., Hacke, W., Hennerici, M. G., Kaste, M., Kuelkens, S., Larrue, V., & Lees, K. R. (2007). Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): An observational study. *The Lancet, 369*(9558), 275–282.
53. Kuebler, P., & Genentech, Inc., 2007. *Method of treating stroke with thrombolytic agent*. U.S. Patent Application 11/832,291.
54. Son, S., Jang, J., Youn, H., Lee, S., Lee, D., Lee, Y. S., Jeong, J. M., Kim, W. J., & Lee, D. S. (2011). A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. *Biomaterials, 32*(21), 4968–4975.
55. Pardridge, W. M. (2004). Intravenous, non-viral RNAi gene therapy of brain cancer. *Expert Opinion on Biological Therapy, 4*(7), 1103–1113.
56. Pardridge, W. M. (2007). shRNA and siRNA delivery to the brain. *Advanced Drug Delivery Reviews, 59*(2), 141–152.
57. Ruberti, F., Barbato, C., & Cogoni, C. (2012). Targeting microRNAs in neurons: Tools and perspectives. *Experimental Neurology, 235*(2), 419–426.
58. Liu, X. S., Chopp, M., Zhang, R. L., Tao, T., Wang, X. L., Kassis, H., Hozeska-Solgot, A., Zhang, L., Chen, C., & Zhang, Z. G. (2011). MicroRNA profiling in subventricular zone after stroke: MiR-124a regulates proliferation of neural progenitor cells through Notch signaling pathway. *PLoS One, 6*(8), e23461.
59. Bouchie, A. (2013). First microRNA mimic enters clinic. *Nature Biotechnology, 31*, 577.
60. Leclercq, M., Diallo, A. B., & Blanchette, M. (2017). Prediction of human miRNA target genes using computationally reconstructed ancestral mammalian sequences. *Nucleic Acids Research, 45*(2), 556–566.
61. Ouyang, Y. B., Lu, Y., Yue, S., Xu, L. J., Xiong, X. X., White, R. E., Sun, X., & Giffard, R. G. (2012). miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. *Neurobiology of Disease, 45*(1), 555–563.
62. Zhu, F., Liu, J. L., Li, J. P., Xiao, F., Zhang, Z. X., & Zhang, L. (2014). MicroRNA-124 (miR-124) regulates Ku70 expression and is correlated with neuronal death induced by ischemia/reperfusion. *Journal of Molecular Neuroscience, 52*(1), 148–155.
63. Ouyang, Y. B., Lu, Y., Yue, S., & Giffard, R. G. (2012). miR-181 targets multiple Bcl-2 family members and influences apoptosis and mitochondrial function in astrocytes. *Mitochondrion, 12*(2), 213–219.
64. Yin, K. J., Hamblin, M., & Eugene Chen, Y. (2015). Angiogenesis-regulating microRNAs and ischemic stroke. *Current Vascular Pharmacology, 13*(3), 352–365.
65. Bhalala, O. G., Srikanth, M., & Kessler, J. A. (2013). The emerging roles of microRNAs in CNS injuries. *Nature Reviews Neurology, 9*(6), 328–339.
66. Allen, C. L., & Bayraktutan, U. (2009). Oxidative stress and its role in the pathogenesis of ischaemic stroke. *International Journal of Stroke, 4*(6), 461–470.
67. Jeck, W. R., Sorrentino, J. A., Wang, K., Slevin, M. K., Burd, C. E., Liu, J., Marzluff, W. F., & Sharpless, N. E. (2013). Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA, 19*(2), 141–157.

68. Lu, D., & Xu, A. D. (2016). Mini review: Circular RNAs as potential clinical biomarkers for disorders in the central nervous system. *Frontiers in Genetics, 7*, 53.
69. Han, B., Zhang, Y., Zhang, Y., Bai, Y., Chen, X., Huang, R., Wu, F., Leng, S., Chao, J., Zhang, J. H., & Hu, G. (2018). Novel insight into circular RNA HECTD1 in astrocyte activation via autophagy by targeting MIR142-TIPARP: Implications for cerebral ischemic stroke. *Autophagy, 14*(7), 1164–1184.

Chapter 11
Therapeutic Aspects of Nanomedicines in Stroke Treatment

Lipika Ray

Abstract Stroke is one of the major causes of death worldwide, and the thrombolytic drug alteplase (tissue plasminogen activator or tPA) is the only treatment available for acute ischemic stroke; however, its use is limited by its short therapeutic window. Many potential therapeutic and diagnostic neuroprotectants to the brain are available, but, unfortunately, most of them are limited by the blood-brain barrier (BBB). Conversely, nanoparticles (NPs) easily cross the BBB with no undesired side effect and alteration of the integrity of BBB. Thus, NPs have created new facet in stroke therapy. The nanocarriers-based preclinical and clinical research in thrombolytic drug delivery is mentioned. Preclinical research carried out on different thrombolytic drug-loaded polymer, lipid, and magnetic nanoparticles showed an enhanced thrombolytic effect with least adverse effects. Targeted nanocarriers displayed an enhanced accumulation into thrombolytic area. NP-based drug delivery opens up new route for the management of thrombotic diseases.

Keywords Ischemic stroke · Nanomedicine · Blood-brain barrier · Drug delivery

11.1 Introduction

Ischemic stroke is a multifactorial pathophysiological disease and is positioned as the second deadliest disease. Worldwide, ~15 million people are affected by stroke event in every year, and 33% people ended up with a permanent disability and even death [1, 2] due to lack of the current diagnostic tools, before the event. Many etiological factors are involved in this disease and make much more difficult to determine in its early stages. According to the American Heart Association, about 87% cases are ischemic stroke among all categories of thrombosis, and the rest is caused by hemorrhage [1]. The brain tissue needs high quantity of oxygen and glucose for its survival and solely depends on oxidative phosphorylation for energy production. However, acute ischemic stroke leads to sudden loss of blood flow in cerebral

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arteries. Thus, ischemic stroke affected area shows a chain of pathological biochemical reactions, such as, rapid depletion of the intracellular ATP pool, anaerobic glycolysis, lactate acidosis and membrane depolarization, glutamate excitotoxicity, the entry of Ca^{2+}, Na^{+}, and H_2O into cells, activation of Ca-dependent proteins, mitochondrial dysfunction, overproduction of free radicals, activation of the immune system, gene over expression, and, finally, apoptosis of the brain cells [3–6]. Moreover, glucose transporter 1 (GLUT1) became upregulated due to lack of oxygen around the stroke-affected area of the endothelial cells [7]. Oxidative stress also amplifies blood-brain barrier dysfunction [8, 9], and vascular fluid components such as prostaglandins, white cells, amino acids, etc. cross to the abluminal side of the brain. Thus, they contribute toward the progression of a vasogenic edema [10]. Even though many complexities are involved during a stroke, secondary injury to penumbra tissue can be avoided if appropriate drugs are administered in an early time point of the event [11, 12]. Recent research in the stroke comprises advanced imaging for stroke diagnosis [13] and endovascular mechanical thrombectomy for reperfusion treatment, which has been validated in seven randomized controlled trials [13–19]. With the aid of new techniques, as well as intravenous tissue plasminogen activator, conventional stroke care has been reached in a new height. Currently, a few successful adjuvant neuroprotective therapies are available in the treatment of acute stroke where it reduces the secondary injury to penumbra tissue and minimizes the damage before and after reperfusion while promoting neural recovery and plasticity [13].

11.2 Secondary Neuronal Damage After Stroke and BBB Breakdown

It is evident that the BBB is severely compromised and impaired of re-establishing regular function for as long as 7 days post-trauma [20]. The longer period of BBB breakdown can affect neurons from both host and foreign components. Alongside, a complex cascade of events begins after stroke such as excitotoxicity, disruption of ion channels, inflammation, and activation of apoptotic pathways [21]. In the early stages of stroke, majority of these damages will not be detected, but neuronal injury and cell death can gradually continue even months after the initial event [22, 23]. The secondary damage can be up to motor impairments and neuropsychiatric illness [24].

11.3 Existing Treatment of Stroke

Time is the most important criteria of the event of a stroke because the activation cell death signaling happens within a minutes. Thus, drug administration to the stroke-affected area is essential. Though, less quantities drug reaches the area with

less blood covering area. The only commercially available pharmaceutical agent is recombinant tissue plasminogen activator (rt-PA) [25] which allows the oxygenation of the damaged area as well as partial restoration of the blood flow [26]. This therapy is also time dependent to be effective and not as beneficial if it exceeds a 3–4 h window [27]. Anticoagulants are the only other commercially available alternative to the patients, but anticoagulants cannot repair or protect the affected area from further damage [28].

11.4 Drug Delivery Through BBB During Stroke

The BBB has always been a major hurdle to efficient drug delivery. Thus, treatment needs to be target-specific where the bioavailability of drugs will be more and it will retain their molecular structure also while delivering into brain. Additionally, decomposition and chemical absorption by the host's metabolic system is another constrain toward the lack of systematic drug dosage toward the brain [29]. Increased rates of drug delivery can be achieved with vasodilators [2] by which stretching of the BBB junctional complex occurs, allowing higher molecular weight molecules or different surface charge molecules to cross to the abluminal side of the brain with minimal difficulties. However, complete dissociation of the BBB junctional complex also tolerates other toxic molecules to penetrate the brain which may cause more damage rather than protect during a cerebral edema [30, 31]. Thus, considering all aforementioned issues, drug for stroke must have high sustainability rates according to cut short against the host's metabolism and natural defenses. In this juncture, it can be assumed that safe drug delivery into the brain can be achieved by the use of nanoparticles (NPs) without disrupting BBB junctional complex.

11.5 NP-Based Drug Delivery

The sizes of NPs can range between 1 and 300 nm in spite of the field that is used. The thickness and size of the NP's capsule play a significant role for increased therapeutic abilities. With variation in sizes, the core to surface ratio changes [2]. For instance, NPs smaller in size have a smaller core to surface ratio, which permits a drug to be instantly released, while the membrane of NP is broken. On the contrary, uneven drug release may occur from larger NPs followed by inefficient drug delivery [32]. The time of release for a drug from NPs is also essential condition because fast release of drug from NPs into the blood-stream consequences it's decomposition and followed by clearance of drug from the host's system [33]. Increased specificity of the drug can be achieved through conjugation with antibodies, peptides, etc. as drug carriers.

11.6 NP-Based Chemical Agents

Different molecular weights PEG-based NPs are conjugated with glucose so that it is recognized by the brain endothelial cell transporter GLUT1 [34]. NP's usefulness is not limited in encapsulating drugs and conjugating with surface receptors. NPs can scavenge reactive oxygen species (ROS) and can be useful in case of stroke event as amount of free radicals increase due to cerebral ischemia, especially after reperfusion. Takamiya et al. reported that platinum nanoparticles (nPts) scavenge both superoxide anions and hydrogen peroxide from in vivo studies [35]. Similarly, ceria NPs also scavenge ROS by reduction—oxidization method (cerium's oxidation number) where oxygen binds in the same way like biological antioxidants. These NPs are in ultra-small sizes (i.e., 4 nm) and may be potential candidates for treatments against stroke [36].

11.7 A Hope: NPs as a Diagnostic Tool in Stroke

Efforts have been made toward designing NPs as a diagnostic tool in stroke. Statins or candesartan showed promising results in vitro and can be encapsulated in NPs for drug delivery into the brain by endocytotic or transcytotic route in order to cross the BBB [37, 38]. Jickling et al. [39] conjugated NPs with labeled antibodies of CNS injury biomarkers such as S100 calcium-binding protein B (S100B), vascular cell adhesion molecule (VCAM), glial fibrillary acidic protein (GFAP), etc., in order to easily detect them using a computed tomography (CAT) scan or magnetic resonance imaging (MRI). Circulating NPs, ability to abide degradation, can be conjugated with blood clotting biomarkers for the early diagnosis of stroke or the disruption of a molecular cascade that leads to stroke [20]. Another approach toward the diagnostic use of NPs has been made by Lin et al. where thrombin-sensitive peptide substrates have conjugated on the surface of the NPs and thrombin levels can be detected in the circulatory system with constant monitoring for coagulation [40].

11.8 Composition of Nanocarrier Used in Stroke Therapy

Several types of materials, such as polymeric, lipid, or metallic, are easy to functionalize and can deliver thrombolytic drugs with low toxicity. Both hydrophobic and hydrophilic drugs can be entrapped or conjugated with the materials depending on the composition. The NP-based formulations may be in different shapes (spheres, capsules, and vesicles), surface charge, and sizes. The size can be adjusted while the nanoparticles are prepared [41]. To enhance the circulation time of NPs in the body, poly(ethylene glycol) (PEG), a hydrophilic biocompatible and biodegradable polymer, has been often used as a coating [42]. Furthermore, PEG is easy to functionalize [22]; thus, different target ligands of interest can be attached on the surface of the carriers [43, 44].

11.9 Lipid Nanoparticles

Liposomes and few microbubbles are most popular lipid-based nano- and microcarriers in thrombolytic drug delivery because of their lack of toxicity and biodegradable nature [45]. Liposomes are bilayer phospholipid which resemblance with the cell membrane, encircling an watery core and it can be easily modified during preparation [46]. Although, the choice of the method and subsequent processing steps are vital as loading efficacy and the size of liposome depends on it. However, the therapeutic utility of liposome limit As the stability of liposomes in the blood compartment is very poor.

11.10 Polymer Carriers

Polymer nanocarrier includes both synthetic and natural polymers. Polymer nanocarriers are mainly used to encapsulate thrombolytic drugs. Importantly, hydrophobicity, size, and porosity of polymer-based carriers can be easily adjustable and can be functionalized with target groups for encapsulating the therapeutic drugs at target site. Dextran, a polysaccharide, is mainly built with α-1,4-glycose units through glycosidic bonds and is the mostly used polymer for coating the magnetic nanoparticles, especially iron oxide nanoparticles, as it has a large affinity for iron [47]. Moreover, polymer PEG is mostly used as a spacer to bind the therapeutic drug covalently in case of magnetic NPs. Polyvinyl alcohol (PVA) and poly(lactic-co-glycolic acid) (PLGA; FDA- and EMA-approved polymer), a biodegradable synthetic polymer, also have been used to encapsulate the drugs. Chitosan, is a natural water soluble polysaccharide, drug entrapment in chitosan-based nanoparticles are obtained by the ionic gelation method. Chitosan NPs possess overall cationic charge which assists to create hydrophilic properties of chitosan. Moreover, chitosan is biodegradable and biocompatible in nature with low toxicity and moderate immunogenicity [48]. In case of chitosan NPs, drug sometime forms electrostatic interaction with polymer chain and facilitates the drug entrapment. Gelatin is another natural and biodegradable polymer that has been used for drug entrapment purpose [49].

11.10.1 Inorganic-Based Nanocarriers

Inorganic nanocarriers, are mainly magnetic nanoparticles, were used to encapsulate thrombolytic drug. Magnetic nanocarrier has generally two parts: iron oxide core which is paramagnetic and a shell which is made of polymers. Inner core can be made of either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) or a mixture of both. On the other hand, the polymer surroundings improve colloidal stability of magnetic nanoparticles. Magnetic nanoparticles are mainly two types depending upon its size: (1) ultra-small superparamagnetic iron oxides (USPIO), having

hydrodynamic diameter >50 nm, and (2) superparamagnetic iron oxide (SPIO) particles, where hydrodynamic diameter falls between 50 and 100 nm. Magnetic nanoparticles are not only biodegradable but maintain the iron homeostasis in the body also [28]. Magnetic nanoparticles can be accumulated into a target site by applying external strong magnetic field. Thus, inorganic nanocarrier has essential role in brain imaging and the thrombolytic drug delivery in the brain [50–52].

11.11 Nanocarriers-Based Thrombolytic Therapy: Preclinical Development

Nanotechnology revolutionized the drug delivery field as nanocarriers not only protect the thrombolytic drugs from inhibitors present in the blood but enhance the effective concentration of thrombolytic drugs at the thrombus also. The size of the nanocarriers ranges between ~5 and 100 nm. A small number of thrombolytic drugs entrapped into nanoparticles or microparticles are known which are showed their promising therapeutic activity in in vivo.

11.12 Streptokinase

In 1933, Tillett and Garner discovered that few specific strains of *Streptococcus* can dissolve fibrin clots with the help of streptokinase (SK) [53, 54]. In fact, different strains of streptococcal bacteria produce streptokinase (SK) which is a single-chain protein [55]. SK contains 414 amino acids having 47 kDa MW. SK combines with free circulating plasminogen [56], and the resulting complex converts plasminogen to plasmin [57]. This protein demonstrates a biphasic half-life; an initial and short half-life of 16 min is due to the complexation of anti-SK antibodies, while the later longer half-life of 90 min is responsible for the elimination of the protein. Though, SK is cheap, it shows an immunogenic effect due to the presence of certain antibodies [22]. Sometime, SK induces serious bleeding difficulty since SK activates both fibrin-bound and circulating plasminogen [36].

11.13 Nanocarriers Loaded with SK

Vaidya et al. [58] prepared GPIIb/IIIa receptor, expressed at the surface of the activated platelets in thrombi, targeted SK entrapmented liposomes with 18% entrapment efficiency. Moreover, the liposomes were encapsulated with RGD (arginine-glycine-aspartic acid) peptide. When SK loaded liposomes were injected in rats having carotid thrombosis, generated by a human clot, a better thrombolytic

activity was observed just after 30 min compared with SK alone. This enhanced thrombolytic effect was obtained by SK liposome due to accumulation of SK by targeted liposomes into clot. Leach et al. encapsulated SK into naked liposomes, to reduce side effects of SK (Streptase®), into a water-soluble double emulsion polymer (PEG and PVA). However, it yielded only 30% of SK encapsulation and stability of liposome also was the issue. Higher SK loading of up to 82% was obtained by encapsulating SK into polymeric porous particles. The time for reperfusion was reduced significantly due to encapsulation of SK into polymer with ~7.3 min whereas 19.3 min for SK-liposome and 74.5 min for free SK in rabbit model with autologous carotid artery thrombosis [59]. Dog model with autologous coronary thrombus also showed similar results in reduction time required to achieve reperfusion by polymeric SK with less infarct size and hemorrhage [39, 22]. These results may be due to the increased supply of the thrombolytic drug into the specific site of thrombus [60, 61]. Thus, these preclinical results showed that nanocarriers loaded with SK shielded SK from early inactivation in the blood and enhances its concentration into the clot leading to an improved thrombolysis.

11.14 Urokinase (UK)

Urokinase (UK) is a serine protease and present in human urine but is currently produced from human renal cell lines. UK contains two polypeptide chains of 32 and 54 kDa. UK degrades fibrin clots by activating plasminogen into plasmin [62]. UK has a half-life from 15 to 20 min in case of human. To obtain a significant thrombolytic effect, high amounts of UK are necessary but may cause undesirable hemorrhages [38] as, alike SK, UK also activates both the fibrin-bound and free plasminogen [35].

11.15 Urokinase-Loaded Nanocarriers

UK-loaded CS nanoparticles (CS-UK-NPs) were prepared by Jin et al. with ~95% encapsulation efficiency (%EE). NPs are basically water-soluble as ionic cross-linking method was used to prepare this NPs, and the size of NPs is 236 nm. An increased amount of clot lysis in rabbit with jugular thrombosis was obtained by UK nanocarrier compared to free UK. UK nanocarrier is more active over free UK because release rate of UK from its NPs is slow. On the contrary, the half-life of free UK is only about 20 min and clears from the body easily [63].

Dextran-coated magnetic nanoparticles of UK were prepared by covalent conjugation via primary amine with the size of 116-nm. The magnetic UK NPs were applied into rats having left jugular vein thrombosis and autologous carotid artery. While the thrombus area is focused in front of permanent magnets, the magnetic nanoparticles accumulated specifically into the thrombotic area and showed a

fivefold enhanced thrombolytic activity than free UK [64]. Interestingly, Marsh et al. developed femoral thrombi-targeted perfluorocarbon (PFC) conjugated UK nanoparticles where antifibrin antibody was conjugated with the nanoparticles superficially. They found that dissolution was higher in animals treated with anti-febrin functionalized PFC-UK nanoparticles compared to control animals which are treated with non-specific IgG tagged UK PFC nanoparticles [65]. Thus, nanocarriers showed higher thrombolytic activity than the free kinases.

11.16 Tissue Plasminogen Recombinant (tPA, rtPA)

In humans, tPAs are produced by endothelial cells and encoded by a gene on chromosome 8. tPAs have PAI-1 and PAI-2 inhibitors in the serpin superfamily. It shows a half-life of about 4–6 min [22, 38]. On the other hand, recombinant tPA (rtPA, alteplase), having 68 kDa MW, is a serine protease. rtPA is produced by cDNA technology from Chinese hamster ovary (CHO) cell lines. Basically, rtPA breaks the Arg-Val bond during the activation of plasminogen to plasmin [35].

11.17 rtPA-Loaded Nanocarriers

rtPA (Cleactor®)-loaded PEG-grafted gelatin nanoparticles were prepared by Uesugi et al. and inserted into rabbits having balloon injury in the right femoral artery. The half-life of rtPA loaded gelatin NPs was three times greater than free rtPA [30] and a complete recanalization was observed. Similar results were reproduced in swine model with thrombotic occlusion in left coronary artery where nine out of ten swines showed high recanalization rate by NPs treatment. However, one out of ten swines demonstrated complete recanalization by rtPA administration [66].

Zhou et al. also developed rtPA (alteplase)-loaded Fe_3O_4-based PLGA nanocarriers which are coated with chitosan and targeted via cyclic RGD peptide. Fe_3O_4–PLGA-rtPA/CS-cRGD nanoparticles has twin role in the early detection and dynamic monitoring of thrombus using MRI. However, these NPs have few limitations such as low encapsulation efficiency of the rtPA (54.3–63.7%, lower than the early reported method 81.12%), the loss of enzyme activity, too positive value of the zeta potential of the nanoparticles due to the high concentration of the samples and slightly larger NPs for systemic delivery. The aforementioned drawbacks of the NPs need to be improved for avoiding the localization of the majority of the nanoparticles in the liver and spleen [67].

Magnetically targeted thrombolysis rtPA (alteplase)-loaded NPs were synthesized by coating magnetite on rtPA-loaded polyacrylic acid nanoparticles which easily respond to an external magnetic field and were concentrated into the thrombus area. The blood flow restoration was observed after 75 min with 0.2 mg/kg of rtPA magnetic NPs. Upon increasing the dose of rtPA (1 mg/kg), the blood flow was

restored faster (within 30 min) [68]. Ma et al. also developed magnetic rtPA-loaded poly(aniline-co-N-[1-one-butyric acid] aniline NPs and successfully obtained 50% higher rtPA loading inside the magnetic NPs than PAA-coated NPs.

Magnetic NPs are beneficial in terms of lowering active dose and thus side effect. Alongside, concentrations of the magnetic NPs at the target site are increased upon application of external magnetic field. However, the external magnetic field fails to target deeper tissue in the body as the intensity of magnetic field reduces with the tissue depth. To overcome this problem, ferromagnetic stents were implanted by Kempe et al. for the treatment of in-stent thrombosis. Actually, ferromagnetic stents with rtPA (alteplase)-loaded PEGylated magnetic nanoparticles were implanted in coronary artery of pigs. The re-establishment of blood flow was obtained with rtPA NPs compared with free rtPA [69].

The McCarthy group demonstrated that the synthesis of a FXIIIa thrombus-targeted nanocarrier (CLIOFXIII-PEG-tPA) for the efficient delivery of a rtPA. These thrombolytic NPs lower the overall amidolytic and fibrinolytic activity, as compared to free tPA in vitro, and bind to the thrombus edge in both arterial and venous thrombosis. Basically, it is thrombus-targeted fibrinolytic agent with theranostic capabilities [70].

The rtPA-loaded PEGylated liposomes showed 21-fold prolonged circulation time than free rtPA [23]. The main drawback of rtPA delivery at the target site is undesired hemorrhage which is partially tuned by Absar et al. They conjugated a peptide with PEGylated liposomes which targets fibrinogen gamma-chain GPIIb/IIIa. These nanoparticles showed 35% enhanced thrombolytic activity in rat model with inferior vena cava thrombosis induced with a $FeCl_3$ solution. Although, ex vivo experiment on human clot showed less thrombolytic activity of rtPA loaded NPs compared with native rtPA. This may be due to the incomplete release of the rtPA from liposomes [71].

Interesting to note that ultrasound is very much useful as adjuvant therapy for enhancing the thrombolytic effect. Thus, ultrasound increases the recanalization rate [72]. This enhancement could be enlightened by acoustic cavitation, thermal effects, or microstreaming [73–75]. Keeping mind this idea, echogenic liposomes (ELIP), built by a phospholipid bilayer enclosing both gas and liquid, were developed. ELIP not only detect thrombus before, during, and after thrombosis by echography but act as a potential vectors also for thrombosis. rtPA (alteplase)-loaded ELIP was developed by Laing et al. where rtPA is 15% in the core and 35% with the phospholipid bilayer. rtPA-loaded ELIP were injected into rabbits with abdominal aortic thrombi and exposed to ultrasound (pulsed 5.7 MHz for 2 min). The rate of recanalization was determined by Doppler flow measurement and showed similar thrombolytic efficacy of rtPA-loaded ELIP as free rtPA in vivo. However, saline (control), empty ELIP, or empty ELIP associated with ultrasound did not show any thrombolytic effect [76]. In this context, Hagisawa et al. also prepared perfluorocarbon-based targeted rtPA (monteplase)-loaded echogenic liposomes which are coupled with a RGD peptide. The study was done in rabbits with thrombus in iliofemoral arteries, and ELIP was applied intravenously. A higher recanalization rate (nine out of ten rabbits) was obtained with ultrasound application [77] (Table 11.1).

Table 11.1 List of thrombolytic drugs used clinically

Thrombolytic agent	Indication	Molecular weight (kDa)	Fibrino selective	Structure (domains)	Elimination	Half-life (min)	US FDA status
Urokinase (UK) (Abbokinase®, Abbot Laboratories, TX, USA)	AMI, PE	2 polypeptides chains (32/54)	No	P/K/ EGF	Kidney	15–20	Approved
Streptokinase (SK) (Streptase)	AMI, PE, DVT, PAO	47	No	α, β, γ	Kidney	10–16	Approved
Alteplase (tPA, rtPA) (Activase®, Genentech, CA, USA; Actilyse®, Boehringer Ingelheim, Germany)	AMI, PE, IS	68	Yes	F/EGF/ K1/K2/S	Liver	4–6	Approved
Tenecteplase (TNK-tPA) (TNKase®, Genentech, USA; Metalyse®, Boehringer Ingelheim, Germany)	AMI	70	Yes	F/EGF/K1	Kidney	20	Approve
Reteplase (rPA) (Retavase®, Chiesi Farmaceutici S.p.A, Italy; Rapilysin®, Actavis, NJ, USA)	AMI, DVT, PAO	40	Yes	K2/SP	Kidney	18	Phase II
Staphylokinase (SAK)	AMI	16.5	Yes	2 chains: a, b	Liver	6	Phase II
Lanoteplase (nPA)	AMI	53.5	Yes	F/K1/K2/ SP	Liver	37	Phase II
Desmoteplase (batPA)	IS	52	Yes	F/EGF/ K1/SP	Liver	240	Phase III

AMI Acute myocardial infarction, *DVT* Deep-vein thrombosis, *EGF* EGF domain, *F* Finger domain, *IS* Ischemic stroke, *K1* Kringle 1 domain, *K2* Kringle 2 domain, *PAO* Peripheral arterial occlusion, *PE* Pulmonary embolism, *SP* Serine protease domain

11.18 Conclusion

Stroke is a time-responsive disease where early diagnosis may decide the patient's outcome. In addition, most of the efficient thrombolytic drugs have many limitations, and the therapeutic window is also short. In this context, the NPs development may act as a diagnostic tool and a therapeutic tool or the combination of both to prevent ischemic stroke. However, preclinical research on the targeted thrombolytic drugs is necessary regarding nanomaterials efficiency and toxicity before their translation into clinical practice.

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Future Perspective The nanocarrier should encapsulate a maximum amount of thrombolytic drug and shield the drug from enzymatic degradation keeping the thrombolytic efficacy same. It should also release the drug in the site of thrombus. All these features are mandatory for a better patient care.

Conflict of Interests The authors declare that there is no conflict of interests regarding the publication of this book chapter.

References

1. Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Blaha, M. J., et al. (2014). Heart disease and stroke statistics – 2014 update: A report from the American Heart Association. *Circulation, 129*, e28–e292.
2. The European Stroke Initiave Executive Committee and the Eusi Writing Committee. (2003). European stroke initiative recommendations for stroke management – update 2003. *Cerebrovascular Diseases, 16*, 311–337.
3. Shcharbina, N., Shcharbin, D., & Bryszewska, M. (2013). Nanomaterials in stroke treatment perspectives. *Stroke, 44*, 2351–2355.
4. Rosamond, W., Flegal, K., Furie, K., Go, A., Greenlund, K., Haase, N., et al. (2008). American Heart Association statistics committee and stroke statistics subcommittee. Heart disease and stroke statistics–2008 update: A report from the American Heart Association statistics committee and stroke statistics subcommittee. *Circulation, 117*, e25–e146.
5. Deb, P., Sharma, S., & Hassan, K. M. (2010). Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. *Pathophysiology, 17*, 197–218.
6. Lee, J. M., Grabb, M. C., Zipfel, G. J., & Choi, D. W. (2000). Brain tissue responses to ischemia. *The Journal of Clinical Investigation, 106*, 723–731.
7. Yeh, W. L., Lin, C. J., & Fu, W. M. (2008). Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. *Molecular Pharmacology, 73*, 170–177.
8. Love, S. (1999). Oxidative stress in brain ischemia. *Brain Pathology, 9*, 119–131.
9. Kuroiwa, T., Ting, P., Martinez, H., & Klatzo, I. (1985). The biphasic opening of the blood-brain barrier to proteins following temporary middle cerebral artery occlusion. *Acta Neuropathologica, 68*, 122–129.

10. Pillai, D. R., Dittmar, M. S., Baldaranov, D., Heidemann, R. M., Henning, E. C., Schuierer, G., Bogdahn, U., & Schlachetzki, F. (2009). Cerebral ischemia - reperfusion injuryin rats – a 3 T MRI study on biphasic blood-brain barrier opening and the dynamics of edema formation. *Journal of Cerebral Blood Flow & Metabolism, 29*, 1846–1855.
11. Zhang, P. L., Wang, Y. X., Chen, Y., Zhang, C. H., Li, C. H., Dong, Z., Yin, H., Zhang, F. F., & Wang, J. H. (2015). Use of intravenous thrombolytic therapy in acute ischemic stroke patients: Evaluation of clinical outcomes. *Cell Biochemistry and Biophysics, 72*(1), 11–17.
12. Rajah, G. B., & Ding, Y. (2017). Experimental neuroprotection in ischemic stroke: A concise review. *Neurosurgical Focus, 42*, 1–8.
13. Campbell, B. C., Mitchell, P. J., Kleinig, T. J., Dewey, H. M., Churilov, L., Yassi, N., et al. (2015). Endovascular therapy for ischemic stroke with perfusion-imaging selection. *The New England Journal of Medicine, 372*, 1009–1018.
14. Berkhemer, O. A., Fransen, P. S., Beumer, D., van den Berg, L. A., Lingsma, H. F., Yoo, A. J., et al. (2015). A randomized trial of intraarterial treatment for acute ischemic stroke. *The New England Journal of Medicine, 372*, 11–20.
15. Bracard, S., Ducrocq, X., Mas, J. L., Soudant, M., Oppenheim, C., Moulin, T., et al. (2016). Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): A randomised controlled trial. *Lancet Neurology, 15*, 1138–1147.
16. Goyal, M., Demchuk, A. M., Menon, B. K., Eesa, M., Rempel, J. L., Thornton, J., et al. (2015). Randomized assessment of rapid endovascular treatment of ischemic stroke. *The New England Journal of Medicine, 372*, 1019–1030.
17. Jovin, T. G., Chamorro, A., Cobo, E., de Miquel, M. A., Molina, C. A., Rovira, A., et al. (2015). Thrombectomy within 8 hours after symptom onset in ischemic stroke. *The New England Journal of Medicine, 372*, 2296–2306.
18. Mocco, J., Zaidat, O., Von Kummer, R., Yoo, A., Gupta, R., Lopes, D., Frei, D., Sit, S. P., Bose, A., & Khatri, P. (2015). Results of the THERAPY trial: A prospective, randomized trial to define the role of mechanical thrombectomy as adjunctive treatment to IV rtPA in acute ischemic stroke. *International Journal of Stroke, 10*, 10.
19. Saver, J. L., Goyal, M., Bonafe, A., Diener, H. C., Levy, E. I., Pereira, V. M., et al. (2015). Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. *The New England Journal of Medicine, 372*, 2285–2295.
20. Lakhan, S. E., Kirchgessner, A., Tepper, D., & Leonard, A. (2013). Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Frontiers in Neurology, 4*, 32.
21. Chen, Y. (2012). Organophosphate-induced brain damage: Mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. *Neurotoxicology, 33*, 391–400.
22. Dihne, M., Grommes, C., Lutzenburg, M., Witte, O. W., & Block, F. (2002). Different mechanisms of secondary neuronal damage in thalamic nuclei after focal cerebralischemia in rats. *Stroke, 33*, 3006–3011.
23. Baron, J. C., Yamauchi, H., Fujioka, M., & Endres, M. (2014). Selective neuronal loss in ischemic stroke and cerebrovascular disease. *Journal of Cerebral Blood Flow and Metabolism, 34*, 2–18.
24. Chen, Y., Garcia, G. E., Huang, W., & Constantini, S. (2014). The involvement of secondary neuronal damage in the development of neuropsychiatric disorders following brain insults. *Frontiers in Neurology, 5*, 22.
25. Jahan, R., & Vinuela, F. (2009). Treatment of acute ischemic stroke: Intravenous and endovascular therapies. *Expert Review of Cardiovascular Therapy, 7*, 375–387.
26. Panagiotou, S., & Saha, S. (2015). Therapeutic benefits of nanoparticles in stroke. *Frontiers in Neuroscience, 9*, 182.
27. Messe, S. R., Fonarow, G. C., Smith, E. E., Kaltenbach, L., Olson, D. M., Kasner, S. E., & Schwamm, L. H. (2012). Use of tissue-type plasminogen activator before and after publication of the European Cooperative Acute Stroke Study III in Get With The Guidelines-Stroke. *Circulation. Cardiovascular Quality and Outcomes, 5*, 321–326.

28. Chen, Z. M., Sandercock, P., Pan, H. C., Counsell, C., Collins, R., & Liu, L. S. (2000). Indications for early aspirin use in acute ischemic stroke a combined analysis of 40,000 randomized patients from the Chinese acute stroke trial and the international stroke trial. *Stroke, 31*, 1240–1249.
29. Ghosh, S., Das, N., Mandal, A. K., Dungdung, S. R., & Sarkar, S. (2010). Mannosylated liposomal cytidine 5′ diphosphocholine prevent age related global moderate cerebral ischemia reperfusion induced mitochondrial cytochrome c release in aged rat brain. *Neuroscience, 171*, 1287–1299.
30. Sasaki, T., Kassell, N. F., Fujiwara, S., Torner, J. C., & Spallone, A. (1986). The effects of hyperosmolar solutions on cerebral arterial smooth muscle. *Stroke, 17*, 1266–1271.
31. Beletsi, A., Klepetsanis, L. L. P., Ithakissios, D. S., & Avgoustakis, K. (1999). Effect of preparative variables on the properties of poly (dl-lactide-co-glycolide)–methoxypoly(ethyleneglycol) copolymers related to their application in controlled drug delivery. *International Journal of Pharmaceutics, 182*, 187–197.
32. Singh, R., & Lillard, J. W., Jr. (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology, 86*, 215–223.
33. Desai, M. P., Labhasetwar, V., Walter, E., Levy, R. J., & Amidon, G. L. (1997). The mechanism of uptake of biodegradable microparticles in caco-2cells is size dependent. *Pharmaceutical Research, 14*, 1568–1573.
34. Xie, F., Yao, N., Qin, Y., Zhang, Q., Chen, H., Yuan, M., Tang, J., Li, X., Fan, W., Zhang, Q., Wu, Y., Hai, L., & He, Q. (2012). Investigation of glucose-modified liposomes using polyethyleneglycols with different chain lengths as the linkers for brain targeting. *International Journal of Nanomedicine, 7*, 163–175.
35. Takamiya, M., Miyamoto, Y., Yamashita, T., Deguchi, K., Ohta, Y., & Abe, K. (2012). Strong neuroprotection with a novel platinum nanoparticles against ischemic stroke-and tissue plasminogen activator-related brain damages in mice. *Neuroscience, 221*, 47–55.
36. Kim, C. K., Kim, T., Choi, I. Y., Soh, M., Kim, D., Kim, Y. J., Park, H. K., Park, S. P., Park, S., Yu, T., Yoon, B. W., Lee, S. H., & Hyeon, T. (2012). Ceria nanoparticles that can protect against ischemic stroke. *Angewandte Chemie (International Edition in English), 51*, 11039–11043.
37. Sierra, S., Ramos, M. C., Molina, P., Esteo, C., Vazquez, J. A., & Burgos, J. S. (2011). Statins as neuroprotectants: A comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. *Journal of Alzheimer's Disease, 23*, 307–318.
38. So, G., Nakagawa, S., Morofuji, Y., Hiu, T., Hayashi, K., Tanaka, K., Suyama, K., Deli, M. A., Nagata, I., Matsuo, T., & Niwa, M. (2014). Candesartan improves ischemia-induce dimpairment of the blood-brain barrier in vitro. *Cellular and Molecular Neurobiology, 35*, 563–572.
39. Jickling, G. C., & Sharp, F. R. (2011). Blood biomarkers of ischemic stroke. *Neurotherapeutics, 8*, 349–360.
40. Lin, K. Y., Kwong, G. A., Warren, A. D., Wood, D. K., & Bhatia, S. N. (2013). Nanoparticles that sense thrombin activity as synthetic urinary biomarkers of thrombosis. *ACS Nano, 7*, 9001–9009.
41. Varna, M., Juenet, M., Bayles, R., Mazighi, M., Chauvierre, C., & Letourneur, D. (2015). Nanomedicine as a strategy to fight thrombotic diseases. *Future Science OA, 1*, FSO46.
42. Kim, J. Y., Kim, J. K., Park, J. S., Byun, Y., & Kim, C. K. (2009). The use of pegylated liposomes to prolong circulation lifetimes of tissue plasminogen activator. *Biomaterials, 30*(29), 5751–5756.
43. Koshkaryev, A., Sawant, R., Deshpande, M., & Torchilin, V. (2013). Immunoconjugates and long circulating systems: Origins, current state of the art and future directions. *Advanced Drug Delivery Reviews, 65*(1), 24–35.
44. Rabanel, J. M., Hildgen, P., & Banquy, X. (2014). Assessment of peg on polymeric particles surface, a key step in drug carrier translation. *Journal of Controlled Release, 185*, 71–87.
45. Ruiz-Esparza, G. U., Flores-Arredondo, J. H., Segura-Ibarra, V., Torre-Amione, G., Ferrari, M., Blanco, E., & Serda, R. E. (2013). The physiology of cardiovascular disease and innovative liposomal platforms for therapy. *International Journal of Nanomedicine, 8*, 629–640.

46. Bowey, K., Tanguay, J. F., & Tabrizian, M. (2012). Liposome technology for cardiovascular disease treatment and diagnosis. *Expert Opinion on Drug Delivery, 9*(2), 249–265.
47. Tassa, C., Shaw, S. Y., & Weissleder, R. (2011). Dextran-coated iron oxide nanoparticles: A versatile platform for targeted molecular imaging, molecular diagnostics, and therapy. *Accounts of Chemical Research, 44*(10), 842–852.
48. Chen, J. P., Yang, P. C., Ma, Y. H., & Wu, T. (2011). Characterization of chitosan magnetic nanoparticles for in situ delivery of tissue plasminogen activator. *Carbohydrate Polymers, 84*(1), 364–372.
49. Uesugi, Y., Kawata, H., Jo, J., Saito, Y., & Tabata, Y. (2010). An ultrasoundresponsive nano delivery system of tissue-type plasminogen activator for thrombolytic therapy. *Journal of Controlled Release, 147*(2), 269–277.
50. Robert, D., Fayol, D., Le Visage, C., Frasca, G., Brulé, S., Ménager, C., Gazeau, F., Letourneur, D., & Wilhelm, C. (2010). Magnetic micromanipulations to probe the local physical properties of porous scaffolds and to confine stem cells. *Biomaterials, 31*(7), 1586–1595.
51. Cheng, K., Li, T. S., Malliaras, K., Davis, D. R., Zhang, Y., & Marban, E. (2010). Magnetic targeting enhances engraftment and functional benefit of iron-labeled cardiosphere-derived cells in myocardial infarction. *Circulation Research, 106*(10), 1570–1581.
52. Silva, A. K., Luciani, N., Gazeau, F., Aubertin, K., Bonneau, S., Chauvierre, C., Letourneur, D., & Wilhelm, C. (2015). Combining magnetic nanoparticles with cell derived microvesicles for drug loading and targeting. *Nanomedicine, 11*(3), 645–655.
53. Collen, D., & Lijnen, H. R. (2005). Thrombolytic agents. *Thrombosis and Haemostasis, 93*(4), 627–630.
54. Kotb, E. (2014). The biotechnological potential of fibrinolytic enzymes in the dissolution of endogenous blood thrombi. *Biotechnology Progress, 30*(3), 656–672.
55. Butcher, K., Shuaib, A., Saver, J., Donnan, G., Davis, S. M., Norrving, B., Wong, K. S., Abd-Allah, F., Bhatia, R., & Khan, A. (2013). Thrombolysis in the developing world: Is there a role for streptokinase? *International Journal of Stroke, 8*(7), 560–565.
56. Kunamneni, A., Abdelghani, T. T., & Ellaiah, P. (2007). Streptokinase – the drug of choice for thrombolytic therapy. *Journal of Thrombosis and Thrombolysis, 23*(1), 9–23.
57. Baruah, D. B., Dash, R. N., Chaudhari, M. R., & Kadam, S. S. (2006). Plasminogen activators: A comparison. *Vascular Pharmacology, 44*(1), 1–9.
58. Vaidya, B., Agrawal, G. P., & Vyas, S. P. (2011). Platelets directed liposomes for the delivery of streptokinase: Development and characterization. *European Journal of Pharmaceutical Sciences, 44*(5), 589–594.
59. Leach, J. K., O'Rear, E. A., Patterson, E., Miao, Y., & Johnson, A. E. (2003). Accelerated thrombolysis in a rabbit model of carotid artery thrombosis with liposome-encapsulated and microencapsulated streptokinase. *Thrombosis and Haemostasis, 90*(1), 64–70.
60. Leach, J. K., Patterson, E., & O'Rear, E. A. (2004). Encapsulation of a plasminogen activator speeds reperfusion, lessens infarct and reduces blood loss in a canine model of coronary artery thrombosis. *Thrombosis and Haemostasis, 91*(6), 1213–1218.
61. Leach, J. K., Patterson, E., & O'Rear, E. A. (2004). Distributed intraclot thrombolysis: Mechanism of accelerated thrombolysis with encapsulated plasminogen activators. *Journal of Thrombosis and Haemostasis, 2*(9), 1548–1555.
62. Kunamneni, A., Ravuri, B. D., Saisha, V., Ellaiah, P., & Prabhakhar, T. (2008). Urokinase – A very popular cardiovascular agent. *Recent Patents on Cardiovascular Drug Discovery, 3*(1), 45–58.
63. Jin, H. J., Zhang, H., Sun, M., Zhang, B. G., & Zhang, J. W. (2013). Urokinase-coated chitosan nanoparticles for thrombolytic therapy: Preparation and pharmacodynamics in vivo. *Journal of Thrombosis and Thrombolysis, 36*(4), 458–468.
64. Bi, F., Zhang, J., Su, Y., Tang, Y. C., & Liu, J. N. (2009). Chemical conjugation of urokinase to magnetic nanoparticles for targeted thrombolysis. *Biomaterials, 30*(28), 5125–5130.

65. Marsh, J. N., Hu, G., Scott, M. J., Zhang, H., Goette, M. J., Gaffney, P. J., Caruthers, S. D., Wickline, S. A., Abendschein, D., & Lanza, G. M. (2011). A fibrin-specific thrombolytic nanomedicine approach to acute ischemic stroke. *Nanomedicine (London), 6*(4), 605–615.
66. Kawata, H., Uesugi, Y., Soeda, T., Takemoto, Y., Sung, J. H., Umaki, K., Kato, K., Ogiwara, K., Nogami, K., Ishigami, K., Horii, M., Uemura, S., Shima, M., Tabata, Y., & Saito, Y. (2012). A new drug delivery system for intravenous coronary thrombolysis with thrombus targeting and stealth activity recoverable by ultrasound. *Journal of the American College of Cardiology, 60*(24), 2550–2557.
67. Zhou, J., Guo, D., Zhang, Y., Wu, W., Ran, H., & Wang, Z. (2014). Construction and evaluation of Fe(3)O(4)-based PLGA nanoparticles carrying rtPA used in the detection of thrombosis and in targeted thrombolysis. *ACS Applied Materials & Interfaces, 6*(8), 5566–5576.
68. Ma, Y. H., Wu, S. Y., Wu, T., Chang, Y. J., Hua, M. Y., & Chen, J. P. (2009). Magnetically targeted thrombolysis with recombinant tissue plasminogen activator bound to polyacrylic acid-coated nanoparticles. *Biomaterials, 30*(19), 3343–3351.
69. Kempe, M., Kempe, H., Snowball, I., Wallén, R., Arza, C. R., Götberg, M., & Olsson, T. (2010). The use of magnetite nanoparticles for implant-assisted magnetic drug targeting in thrombolytic therapy. *Biomaterials, 31*(36), 9499–9510.
70. Mccarthy, J. R., Sazonova, I. Y., Erdem, S. S., Hara, T., Thompson, B. D., Patel, P., Botnaru, I., Lin, C. P., Reed, G. L., Weissleder, R., & Jaffer, F. A. (2012). Multifunctional nanoagent for thrombus-targeted fibrinolytic therapy. *Nanomedicine (London, England), 7*(7), 1017–1028.
71. Absar, S., Nahar, K., Kwon, Y. M., & Ahsan, F. (2013). Thrombus-targeted nanocarrier attenuates bleeding complications associated with conventional thrombolytic therapy. *Pharmaceutical Research, 30*(6), 1663–1676.
72. Laing, S. T., Moody, M. R., Kim, H., Smulevitz, B., Huang, S. L., Holland, C. K., McPherson, D. D., & Klegerman, M. E. (2012). Thrombolytic efficacy of tissue plasminogen activator-loaded echogenic liposomes in a rabbit thrombus model. *Thrombosis Research, 130*(4), 629–635.
73. Brujan, E. A. (2009). Cardiovascular cavitation. *Medical Engineering & Physics, 31*(7), 742–751.
74. Unger, E., Porter, T., Lindner, J., & Grayburn, P. (2014). Cardiovascular drug delivery with ultrasound and microbubbles. *Advanced Drug Delivery Reviews, 72*, 110–126.
75. Chen, X., Leeman, J. E., Wang, J., Pacella, J. J., & Villanueva, F. S. (2014). New insights into mechanisms of sonothrombolysis using ultrahigh-speed imaging. *Ultrasound in Medicine & Biology, 40*(1), 258–262.
76. Laing, S. T., Moody, M., Smulevitz, B., Kim, H., Kee, P., Huang, S., Holland, C. K., & McPherson, D. D. (2011). Ultrasoundenhanced thrombolytic effect of tissue plasminogen activator-loaded echogenic liposomes in an in vivo rabbit aorta thrombus model – brief report. *Arteriosclerosis, Thrombosis, and Vascular Biology, 31*(6), 1357–1359.
77. Hagisawa, K., Nishioka, T., Suzuki, R., Maruyama, K., Takase, B., Ishihara, M., Kurita, A., Yoshimoto, N., Nishida, Y., Iida, K., Luo, H., & Siegel, R. J. (2013). Thrombustargeted perfluorocarbon-containing liposomal bubbles for enhancement of ultrasonic thrombolysis: In vitro and in vivo study. *Journal of Thrombosis and Haemostasis, 11*(8), 1565–1573.

Chapter 12
Neuroprotective Potential of Small Molecule Phytochemicals in Stroke Therapy

Sumedha Mukherjee, Amit Kumar Tripathi, Gaurav Kumar, Ranjana Patnaik, Nirav Dhanesha, and Divya Mishra

Abstract Stroke being the second largest reason for worldwide mortality requires specific neuroprotective approaches to combat the pathophysiological outcomes. Use of synthetic neuroprotectants has not proved fruitful in clinical trials. In such scenario, scientific research has been focused on various phytochemicals which turn out to be attractive neuroprotectants. Recent studies have demonstrated that different phytochemicals target receptors and marker proteins related to pathophysiological processes involved with stroke. The use of phytochemicals has ameliorated conditions associated with ischemic stroke such as cell death (both necrotic and apoptotic), inflammation, and oxidative stress. Administration of phytochemicals has also proved successful in controlled trials indicating the safety and efficacy of these natural products. Thus, the neuroprotective potential of phytochemicals can be further exploited to design future therapeutic strategies against stroke. In the present chapter, we corroborated the use of phytochemicals and their effects on signaling mechanisms involved in stroke pathology and subsequent cell death.

Keywords Neuroprotection · Phytochemicals · Apoptosis · Flavonoids · NMDA receptor

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Abbreviations

AChE	Acetylcholinesterase
AIF	Apoptosis-inducing factor
AKT	Protein kinase B (PKB)
ARE	Antioxidant response elements
BBB	Blood-brain barrier
Bcl-2	B-cell lymphoma 2
BDNF	Brain-derived neurotrophic factors
CAT	Catalase
ChAT	Choline acetyltransferase
CNS	Central nervous system
CREB	cAMP-response element binding protein
ERK	Extracellular signal-regulated kinases
FADD	Fas-associated protein with death domain
GCLC	Glutamate-cysteine ligase catalytic subunit
IL	Interleukin
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde-modified human albumin
NGF	Nerve growth factor
NMDAr	N-methyl-D-aspartate receptor
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
PI3K	Phosphoinositide 3-kinase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TGF	Transforming growth factor
TNF	Tumor necrosis factor

12.1 Introduction

Stroke is the reason behind more than six million deaths per year and has been reported as the second leading reason behind mortality throughout the world by the World Health Organization (WHO) [1]. Stroke, which can also lead to permanent disability, is one of the major economic health hazards of the urban world [2]. Various factors like obesity, hypertension, lack of physical activity, diabetes, cigarette smoking, and excessive alcohol consumption which have become part and parcel of urban lifestyle have been identified as risk factors contributing to the onset of stroke [3]. Aging is also a major cause behind incidence of stroke [2]. From statistical models, it is predicted that by the year 2030, there will be an annual increase in mortality rate due to stroke by 12% [4, 5]. This is an alarming situation and concern regarding public health. In order to combat such situation, extensive research has been undertaken in the field of stroke to develop novel strategies for treating and preventing stroke. Treatment of stroke majorly involves arterial

recanalization by surgical removal of blood clot [2] or by administration of recombinant tissue plasminogen activator (r-tPA), which is available globally by the name of alteplase [6–8]. Limitations associated with the administration of r-tPA in the treatment of ischemic stroke include a narrow therapeutic window (3–4.5 h), neuronal excitotoxicity, increased risk of incidence of intracranial hemorrhage, and little capability in rescuing degenerating neurons [9–11]. Besides arterial recanalization, neuroprotection is another therapeutic strategy devised to ameliorate stroke conditions [2]. Neuroprotection involves administration of pharmaceutical agents which can interfere the ischemic cascade and salvage the neuronal cells of ischemic penumbra from damage caused by excitotoxic assault [2]. Various synthetic neuroprotective agents such as nimodipine, a Ca^{2+} channel blocker [12]; fosphenytoin, a Na^{+} channel blocker [13]; and selfotel, an N-methyl-D-aspartic acid (NMDA) glutamate receptor antagonist [14] have proved useful in animal models of ischemic stroke but failed miserably in human clinical trials [15, 16].

Phytochemical, bacterial, and animal extracts have been of immense interest to researchers over the last few decades, and various studies reported abilities of such chemicals to modify outcomes of various human diseases [17–20]. Also, some common vegetables and fruits reportedly contain various biologically active compounds which can successfully reduce risks of cardiovascular diseases and stroke [21, 22]. Experimental models of animal stroke showed improved ischemic stroke conditions upon treatment with phytochemicals like dietary polyphenols [22, 23]. Also, epidemiological evidence [24] and quite a few controlled trials have indicated safety and efficacy of natural products for the treatment of ischemic stroke in human [25–30]. These studies establish natural products like phytochemicals as promising candidates in emerging stroke therapeutics. These products meddle with pathophysiological pathways involved in stroke and can reverse neuronal cell death (entirely or partially) caused due to stroke pathogenesis. So, this chapter focuses on the ability of various phytochemicals in combating neuronal cell death and thereby reversing damaging effects of ischemic stroke.

12.2 Role of Molecular Mediators in Apoptotic, Necrotic, and Necroptotic Neuronal Cell Death

The ability of nerve cells or neurons to successfully process information throughout the nervous system depends on their capability to manipulate the charges across their cell membranes. This information processing and transferring ability of nerve cells make them highly essential for proper functioning of the central nervous system (CNS) and brain. The death of nerve cells, more often termed as the neuronal loss, is a characteristic feature of the development of CNS and also neurodegenerative diseases and stroke [31]. Neuronal cell death can be of various types, namely, necrosis [32], apoptosis [33], and necroptosis [34]. Necrotic cell death features cell swelling, damage of cell organelles, membrane integrity loss, and uncontrolled cell lysis [35]. On the contrary, cell death by apoptosis shows features contrasting to necrosis, such as cell shrinkage and nuclear condensation finally leading to the

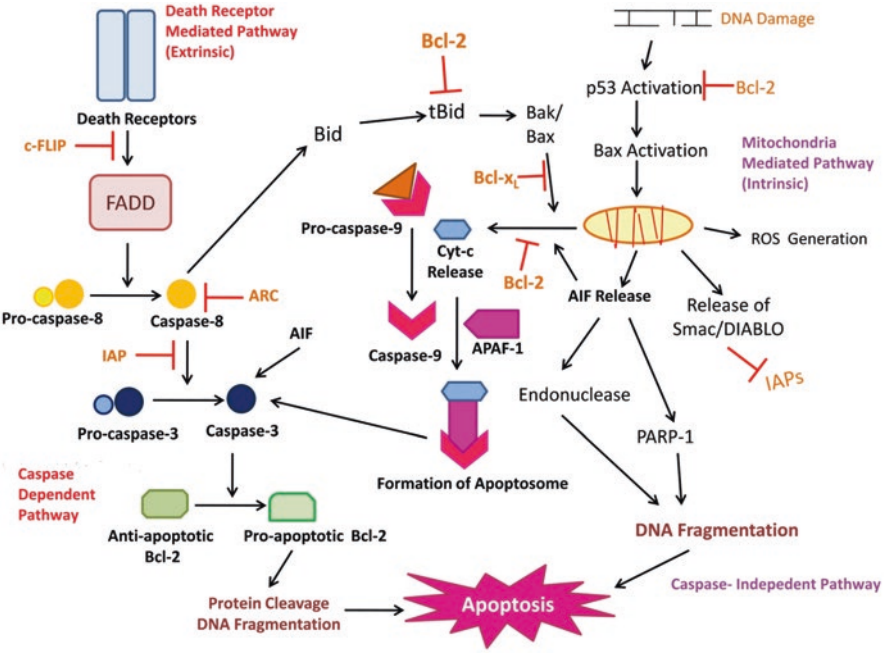

Fig. 12.1 Role of different molecular mediators in both extrinsic and intrinsic apoptotic cell death

formation of apoptotic bodies at the end stage of apoptosis [35]. Apoptosis has always been demonstrated to show programmed cell death, whereas necrosis is thought to be an abrupt and accidental event. But, recent research shows a particular type of necrotic cell death, which can be called programmed necrosis or necroptosis and is induced by death receptor stimulation [36]. So, there prevail multiple types of nerve cell death, each featuring its own characteristic molecular pathways and molecular mediators contributing to the process (Fig. 12.1). Caspases have been identified as prominent molecular factors leading to apoptotic events in the cell [31]. Caspases are cysteinyl-aspartic acid proteases [38, 39], which are activated by cleaving of caspase precursors at specific caspase recognition sites. Activated caspases, in turn, cleave and activate other pro-caspases and their precursors leading to a cascade of caspase activation. There are 14 identified caspases involved in neuronal apoptosis, of which caspase-3 has been recognized as the prime effector in an experiment involving caspase-3 knockout mice, where altered brain development was observed in the mice [40]. Activation of caspase-3 occurs via two processes: extrinsic and intrinsic [41]. While extrinsic pathway involves oligomerization of cell-surface receptors, also known as death-receptors [37], intrinsic pathway is triggered due to alterations in mitochondrial level and apoptosome formation [31]. The members of tumor necrosis factor (TNF) family, a soluble cytokine, regulate the extrinsic pathway by activating the death domains (DD), thereby cleaving procaspase-8 into activated caspase-8 [41]. Activated caspase-8 further activates caspase-3 and caspase-7, among which caspase-3 cleaves procaspase-8, causing amplification

of cell death [42]. Caspase-8 is also known to cleave the cytosolic protein Bid, a member of Bcl-2 family, thereby triggering intrinsic apoptotic pathway [31]. The pro-apoptotic genes of the Bcl-2 family are expressed, especially p53, which leads to the synthesis of cascades of protein, thus inducing intrinsic apoptotic pathway [42, 43]. Bax, another member of Bcl-2 family, translocates to mitochondria during early apoptotic stages [44], forms homo-oligomers and triggers chain of apoptotic events like mitochondrial membrane potential loss, and contributes to the release of cytochrome c (Cyt c), apoptosome formation, and activation of caspases [45]. Cyt c runs parallel pro-apoptotic events by involving mitochondrial proteins such as Smac/DIABLO and Omi/HtrA2, which after being released into cytosol promote activation of caspase-9 by removing inhibitory capabilities of inhibitors of apoptotic proteins (IAPs) [46–48]. Though apoptosis is mostly caspase dependent, scientific research suggests that blocking of caspase cascade attributes to temporary rescue of damaged neurons, because apoptotic features are observed in caspase-inhibited nerve cells [49]. Though mitochondrial proteins Smac/DIABLO and Omi/HtrA2 have been associated with caspase-9 activation, it is probable that they play a significant role in caspase-independent apoptosis [50]. But, the most important factors promoting caspase-independent apoptosis are apoptosis-inducing factor (AIF) and endonuclease G (Endo G) [50, 51]. AIF translocates to cytosol and nucleus from mitochondria, where it typically resides, after the cell is threatened with an apoptotic insult and induces chromatin condensation and DNA fragmentation [50, 52]. AIF translocates to the nucleus in cases of protein kinase C inhibition, when c-myc is overexpressed [50], and when ATP reservoir of the cell depletes, which inhibits apoptosis and leads cell to undergo necrotic death [52]. Translocation of AIF from mitochondria has been correlated to the promotion of DNA fragmentation and condensation of chromatin, even in cells that lack caspase-3 [53]. DNA degradation is also induced by Endo G, another mitochondrial protein which is translocated to the nucleus upon apoptotic assault and can generate inter-nucleosomal DNA fragmentation, even though inhibitors of caspases are present [54].

12.3 Impact of Neurodegeneration in Neurological Disorders Including Stroke

Neuronal cell death has always been associated with various dire diseases of the brain like stroke, Alzheimer's disease, Parkinson's disease, brain trauma, Huntington's disease, epilepsy, and much more [55]. Numerous studies have related intrinsic caspase-dependent apoptotic cell death pathway with brain trauma, cerebral ischemia, spinal cord injury, and other neurodegenerative diseases [31, 37, 41, 56]. Cerebral ischemia or stroke, which is an acute condition caused due to the deprivation of oxygen and glucose supply to brain tissue [57], is characterized by necrotic cell death in the ischemic core and apoptosis in the penumbra region [58, 59]. Also, beclin-1 upregulation has been associated with the ischemic insult of nerve cells, implying induction of autophagy in ischemic cell death [60, 61]. The

excitotoxic insult, which is a prevalent feature in ischemic stroke, is mediated via NMDA receptor leading to necrotic and apoptotic cell death, both of which can be blocked by NMDA receptor antagonists [62]. Besides cerebral stroke, another common neurological disorder, Alzheimer's disease (AD), also features loss of large and medium pyramidal neurons from the hippocampal area [62–64]. The presence of active caspases in postmortem tissues of AD brains suggests the occurrence of apoptosis leading to neuronal cell death [65–68]. In the case of the second most common neurodegenerative disease, Parkinson's disease or PD [57], increased levels of caspase-8 have been detected in substantia nigra neurons containing neuromelanin [69, 70]. Cell death by extrinsic pathway has also been related with neurodegeneration associated with PD. This is supported by the presence of increased TNF-α levels, soluble Fas, and Fas-associated protein with death domain (FADD) in the midbrain region of PD patients [71–73].

12.4 Small Molecule Bioactive Phytochemicals as Neuroprotective Agents in Ischemic Stroke

Various phytochemicals have been reported to display inhibitory roles against oxidative stress generated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) [74]. Small molecule phytochemicals combat pro-inflammatory conditions, such as generation of NF-κβ and cytokines like TNF-α, interleukin (IL)-1β, IL-6, and IFN-γ, due to hemorrhagic and ischemic insults of the brain [75, 76]. Among different phytochemicals, polyphenols such as curcumin, ginseng, resveratrol, quercetin, and rosmarinic acid and other beneficial compounds such as propolis, ω-3 polyunsaturated fatty acids (PUFAs), various alkaloids, and vitamin E have established their anti-oxidative and anti-inflammatory roles in both in vivo and in vitro models, simultaneously combating neurotoxic effects [77]. Effect of different phytochemicals and plant extracts in cerebral stroke is listed in Table 12.1.

12.4.1 Flavonoids

Flavonoids exhibit neuroprotection via anti-inflammatory, antioxidant, and various other mechanisms in several neurological disorders including cerebral stroke. Based on attached functional group, flavonoids are further classified into isoflavonoids, flavones, neoflavonoids, and phytoestrogens. Different categories of flavonoids and their examples are listed in Table 12.2.

Curcumin is long known for exerting neuroprotective effects in AD and PD by exercising control over inflammatory and oxidative mechanisms involved with these two diseases [85–87]. Curcumin (Fig. 12.2) is one of the main components of curcuminoids, polyphenolic compounds present in *Curcuma longa* [85]. Amyloid β (Aβ)-induced plaque formation, a characteristic feature of AD, was extensively

Table 12.1 Neuroprotective approaches of various phytochemicals in neurological disorders including stroke

Phytochemicals	Mechanism	Remark	References
Genistein (*Pisum sativum*)	Inhibition of mitochondria-dependent apoptosis	Inhibited ROS	[78]
Puerarin (*Radix puerariae*)	Inhibited inflammatory and apoptotic responses	Anti-apoptotic pathways	[79]
Curcumin (*Curcumin longa*)	Rescue BBB and anti-inflammatory	Anti-apoptotic pathways	[80]
Bacopa monnieri	Improve human cognitive function	Mechanism not known	[81]
P. dactylifera	Neuroprotective effect	Antioxidant	[82]
Ginkgo biloba	Protective effects against ischemic brain injury	Akt phosphorylation	[27]
Biochanin A	Decreased the neurological deficit scores	Antioxidant action	[83]
Apigenin	Inhibits production of NO and PGE2 in microglia	Inhibits cerebral stroke	[84]
Luteolin	Protects against neurodegenerative changes associated with cerebral ischemia	Novel targets for neuroprotection	[17]
Quercetin	Shows neuroprotection by antioxidant mechanism	Antioxidant	[96]

reduced by curcumin [85]. Curcumin also acts as an anti-inflammatory agent against inflammation in AD brain microglia [86, 87], and its regular consumption in the diet has led to the formation of lower concentrations of Aβ and tau protein (a hallmark of AD) [88]. This phytochemical has also exhibited protection against cytotoxicity induced by α-synuclein in SH-SY5Y neuroblastoma cell line by inhibiting activation of caspase-3, a regulator of extrinsic apoptosis, and by reduction of intracellular ROS levels [87]. Curcumin can impair phosphorylation of Bcl-2 proteins, leading to increased interaction between Bcl-2 and Bax, thus preventing the latter's translocation to the cytosol [89]. Furthermore, curcumin helps in maintaining the mitochondrial integrity and lowers sequestration of cytochrome c from mitochondria, an event of critical importance in apoptosis [89]. Activation of c-Jun N-terminal kinases (JNKs) mitochondrial pathway, a pro-apoptotic event, is also successfully inhibited by curcumin, thus establishing its role as anti-apoptotic [89]. Curcumin also offers protection in middle cerebral artery occlusion (MCAO) rat model for stroke by acting as an antioxidant [90].

Resveratrol (Fig. 12.2b), a non-flavonoid polyphenol present in various medicinal plants, berries, grapes, and peanuts [91, 92], is widely acknowledged for its antioxidant and anti-inflammatory activities [56]. In vitro and in vivo studies have revealed resveratrol's ability in preventing neuronal damage from amyloid-induced toxicity [93]. Most importantly, resveratrol exerts an anti-inflammatory and antioxidative effect by suppressing activated NF-κB, sirtuin 1, and MAPK pathways

Table 12.2 Classification of various flavonoids and isoflavonoids with relevant examples

Categories of flavonoid	Subcategories	Example
Anthocyanins		Aurantinidin, cyanidin, delphinidin, europinidin, luteolinidin, pelargonidin, malvidin, peonidin, petunidin, rosinidin, etc.
Flavonols		3-hydroxyflavone, azaleatin, fisetin, galangin, gossypetin, kaempferide, kaempferol, isorhamnetin, morin, myricetin, natsudaidain, pachypodol, quercetin, rhamnazin, rhamnetin, etc.
Flavones		Apigenin, luteolin, tangeritin, chrysin, 6-hydroxyflavone, baicalein, scutellarein, wogonin, diosmin, flavoxate, etc.
Flavanones	Isoflavones	Butin, eriodictyol, hesperetin, hesperidin, homoeriodictyol, isosakuranetin, naringenin, naringin, pinocembrin, poncirin, sakuranetin, sakuranin, sterubin, etc.
		Genistein, daidzein, lonchocarpane, laxiflorane, etc.
		Equol, etc.
Isoflavonoids	Monomers, oligomers, and polymers	Theaflavins, thearubigins, condensed tannins, proanthocyanidins, etc.
Phenolic acids	Derivatives of cinnamic acid	p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, etc.
Lignans	Derivatives of benzoic acid	Gallic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid, etc.
Stilbenes		Pinoresinol, podophyllotoxin, steganacin, etc.
		Resveratrol analogs, etc.

and reduces the release of pro-inflammatory TNF-α and IL-1 [94], establishing the potential of the compound in combating neurodegeneration. Besides suppression of TNF-α and NF-κB, resveratrol also promotes expression of IL-10, an anti-inflammatory molecule, in microglial cells [95].

Quercetin (Fig. 12.2c), a flavonol polyphenol present in various fruits, vegetables, and grains, exhibits anti-oxidative and neuroprotective properties by engaging in multiple signaling pathways in different neurodegenerative disease models [77]. This remarkable flavonoid acts as a neuroprotectant by counteracting oxidative stress generated by the accumulation of intracellular ROS [96]. Nuclear factor (erythroid-derived 2)-like 2 or Nrf2 regulates the defense mechanism of cells against oxidative stress [96]. Interaction of Nrf2 heterodimer with cis-acting antioxidant response elements (ARE) leads to transcription of antioxidant genes [97, 98]) which eventually provides neuroprotection against cellular death and also damage due to oxidative stress [96]. Various scientific studies confirm quercetin's ability to activate Nrf2-ARE pathway [99–101], establishing the phytochemical's ability to confer neuroprotection. Quercetin also offers protection toward neuronal cells from Aβ induced in AD model [102].

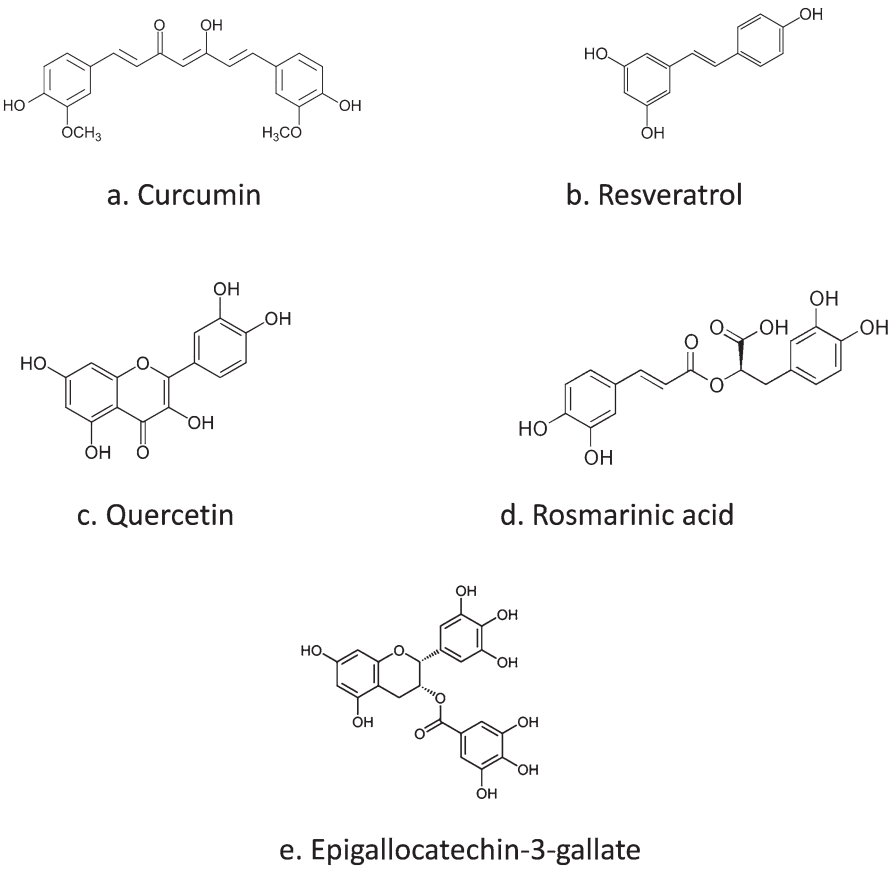

Fig. 12.2 (**a**–**e**) Structures of polyphenol flavonoids with neuroprotective ability

Rosmarinic acid (Fig. 12.2d), found in the plant *Rosmarinus officinalis*, displays neurotrophic effect in PC12 cells by induction of extracellular signal-regulated kinase-1/2 (ERK1/2)-mediated cell differentiation [103]. The compound is known to suppress the expression of hypoxia-inducible factor-1α (HIF-1α), thus protecting from neuronal damage due to hypoxia, mediated by caspase-3 activation and pro-inflammatory cytokines like IL-1β and TNF-α [104].

Epigallocatechin-3-gallate (EGCG) (Fig. 12.2e), a polyphenol found in natural green tea, is extracted from *Camellia sinensis* [105]. The compound exhibits neuroprotective effect by ameliorating cognitive defects in APP/PS1 mice by enhancing nerve growth factor (NGF) expression and aiding expression of cAMP-response element binding protein (CREB) expression [106]. EGCG induced neurite outgrowth by negating PI3K/AKT/GSK-3β-mediated caspase 3 activation and poly ADP-ribose polymerase (PARP) cleavage, ameliorating apoptotic conditions of PC12 cell line, induced by reduced hydrogen peroxide [107].

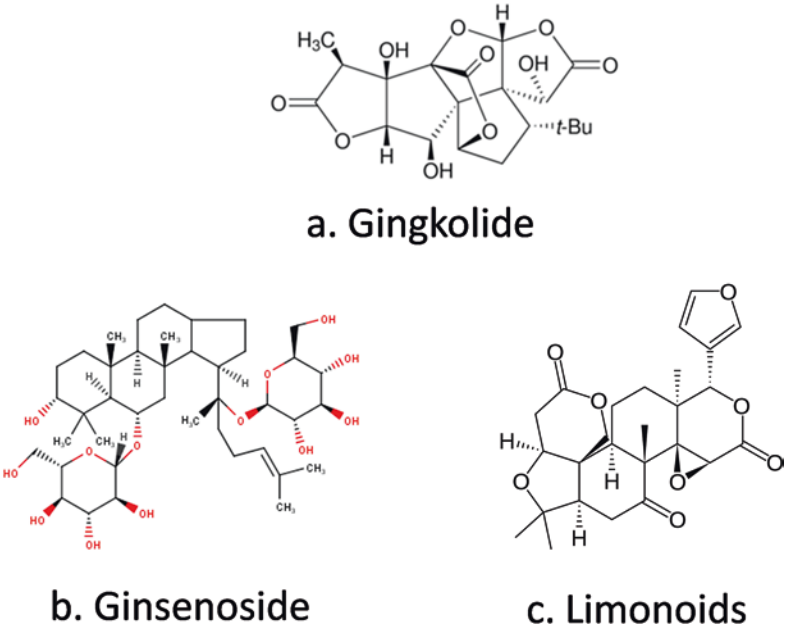

Fig. 12.3 (**a**–**c**) Structures of various terpenoids with ability to confer neuroprotection against stroke

12.4.2 Terpenoids

Ginkgolide, the active constituent of *Ginkgo biloba*, is widely known for its ability to confer neuroprotection in neurodegenerative disorders resulting from oxidative stress [106, 108]. Ginkgolide being BBB permeable becomes a promising candidate for neurodegenerative disease treatment [109]. Ginkgolide B reportedly increases expression of brain-derived neurotrophic factors or BDNF expression level by reduction of caspase-3 levels, lactate dehydrogenase (LDH), and K^+ ion level in primary hippocampal neuron cultures, treated with Aβ25–35 [110], thus conferring neuroprotection (Fig. 12.3).

Panax ginseng, a medicinal plant found in Asian countries such as China, Korea, Japan, and some parts of Vietnam [106], contains ginsenoside Rg3 [111], which stimulates TNF-α expression [68]. Induction of expression of TNF-α stimulates mononuclear phagocytic cells, which in turn clears Aβ plaques present in the brain [112, 113]. The compound is known to act as NGF mimetic and enhances cholinergic activities and promotes neuritogenesis [114]. Another compound present in *P. ginseng* is panaxynol, which also mimics the action of NGF and has been reported to stimulate neurite growth in PC12 cell line via MAPK signal pathway [115].

Limonoids, a family of triterpenoids found in the plant *Meliaceae*, which reportedly possess antibacterial, antimalarial, antiviral, and neuroprotective capacity [116]. Two mentionable compounds of this category, 1α, 3α-dihydroxyl-7α-tigloyloxy-12α-ethoxylnimbolinin and 12-O-ethyl-1-deacetyl-nimbolinin B, increase neurite growth and promote neuronal differentiation in PC12 cells (rat pheochromocytomous cell line), also demonstrating an increase in secretion of NGF [117].

12.4.3 Alkaloids

Huperzine A is an alkaloid compound present in *Huperzia serrata* and acts as a reversible acetylcholinesterase (AChE) inhibitor [118], alters the processing of Aβ peptide, relieves oxidative stress, and promotes NGF expression, thus conferring neuroprotection [119]. In mouse models of transient cerebral ischemia and reperfusion, huperzine A was reported to reverse memory deficits by increasing expression of neurotrophic factors like NGF, BDNF, and TGF-β through MAPK/ERK signaling pathway [115]. In an experimental model of H_2O_2-induced oxidative stress in SHSY5Y neuroblastoma cells, huperzine A restored NGF level, which was reduced due to this induced pathologic condition by activating the p75NTR and TrkA receptors and modulation of upstream MAP/ERK signaling pathway [118]. Huperzine A also promoted neurite outgrowth in rat cortical astrocyte cells and PC12 cell line by inhibition of AChE and mediated upregulation of NGF and p75NTR expression [90]. Huperzine A combats cognitive deficiency in streptozotocin-induced diabetic rats by enhancing expression of ChAT, BDNF, glutathione peroxidase, catalase, and SOD and by simultaneous inhibition of IL-1β, IL-6, AChE, MDA, TNF-α, CAT, NF-κB, and caspase-3 [120].

Berberine is an isoquinoline alkaloid exhibiting neuroprotective characters via neurotrophin-mediated pathway [106]. Berberine also ameliorates diabetic neuropathy conditions in neuroblastoma cells by inducing expression of heme oxygenase-1 and NGF [106]. Decreasing ROS levels and induction of NGF-mediated neurite outgrowth via the PI3K/Akt/Nrf2-dependent pathway were the significant features of the neuroprotective approach, which were also observed when the compound inhibited H_2O_2-induced neurotoxicity [121]. In another study, berberine is reported to significantly decrease expression of the pro-inflammatory cytokines, IL-1β, Cox-2, and TNF-α and to enhance restoration of BDNF and CREB levels, thus combating memory impairment induced by scopolamine in rat models [122]. Berberine pretreatment of primary microglial cells and BV2 cell lines prevented Aβ-induced production of IL-6 and MCP-1 and stimulated downregulation of Cox-2 and iNOS expression via phosphorylation of IκB-α and NF-κB by activated AKT/ERK1/2 [123] (Fig. 12.4).

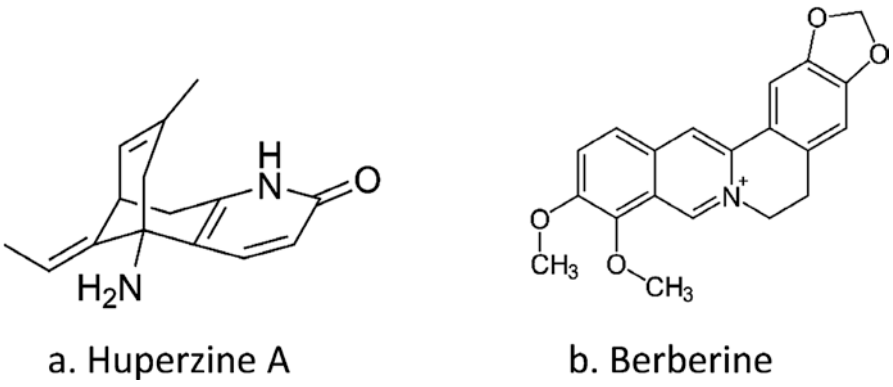

Fig. 12.4 (**a**, **b**) Structures of huperzine A and berberine, two highly efficient alkaloids reputed for their neuroprotective potential

12.4.4 Withanolides

Withanolides are found in root extracts of the plant *Withania somnifera*, which is a prominent Ayurvedic medicinal plant [124]. One of the prominent withanolides, withanolide A, successfully conferred neuroprotection in rats subjected to hypoxia by upregulating Nrf-2 expression [95]. The compound also enhanced expression of glutamate-cysteine ligase catalytic subunit (GCLC), which is downregulated in hypoxic exposure [124]. In a separate in silico study, withanolides such as anaferine, withaferin A, withanolide A, and withanolide B were reported to act as NMDA receptor antagonist, which might be a possible mechanism of combating glutamate-induced excitotoxicity by these phytochemicals [125]. NMDA receptor-mediated excitotoxic effects are standard features of cerebral ischemia, and withanolides' role as NMDAr antagonist makes them a suitable choice for combating stroke. Withanolides also reportedly bind to a catalytic domain of the enzyme matrix metalloproteinase-9 (MMP-9) and inhibit the enzyme with high affinity [126]. Upregulation of MMP-9 and MMP-2 is a hallmark of ischemic stroke [127] and is also observed in various neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, and brain trauma [126]. Inhibition of these gelatinases by the withanolides promotes these phytochemicals' ability for further therapeutic use in neurological disorders. Overproduction of nitric oxide by neuronal nitric oxide synthase (nNOS) or inducible isoform of NOS (iNOS) is another important feature associated with ischemic stroke [128]. Two phytochemicals of *W. somnifera*, withanolide M and stigmasterol, show dual selectivity toward nNOS and iNOS, thereby inhibiting both [129]. Five withanolides, namely, chlorogenic acid, withanolide B, withacnistin, calystegine B2, and elletierine, exhibit selective inhibition of nNOS [130] and can be used to design future therapeutics against ischemic stroke (Fig. 12.5).

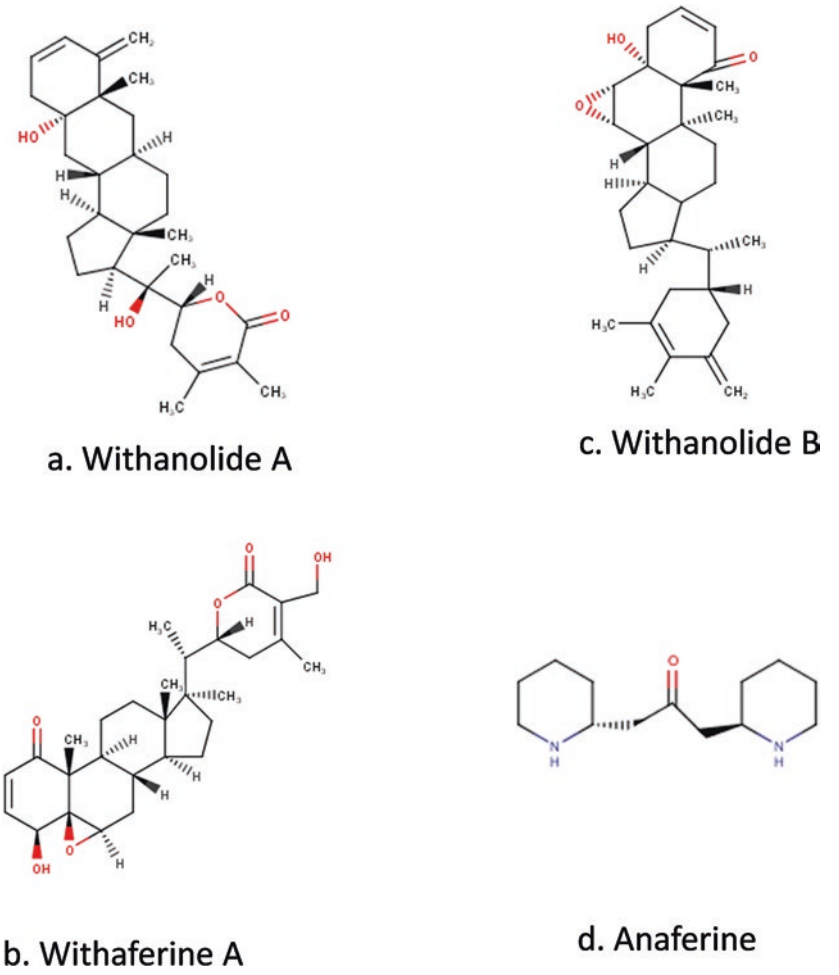

Fig. 12.5 (**a**–**d**) Structures of different withanolides known to combat NMDAr-mediated excitotoxicity

12.5 Conclusion

Phytochemicals have always been of immense interest to the scientific world due to their nontoxic nature and high efficacy, whereas synthetic compounds display toxic side effects. Various phytochemicals have been reported to show neuroprotective potential in models of neuropathological conditions. With increasing demand and scientific interest toward phytotherapeutic agents, proper validation of phytochemicals becomes extremely important before administration of plant extracts. A proper understanding of the mode of action of phytochemicals and their effects on molecular pathways involved in neurodegeneration might provide a new range of beneficial

neuropsychotropic drugs. This chapter tries to focus on different herbal compounds which have been established as potent neuroprotectants. Most of these plants have found uses in traditional medicine and therapy. Another critical criterion for the development of neuroprotective drug is the ability of a compound to cross the blood-brain barrier (BBB) so that it can reach its target sites present in the brain and central nervous system (CNS). Also, targeting a drug through the receptors or transporters present in brain tissue remains a favorable option for delivery of drugs into the brain. Phytochemicals for which multiple receptors are present will automatically become better choices as therapeutic agents. Flavonoids and polyphenols have been regarded as most beneficial among various phytochemicals [131], and oral administration till date remains the preferred mode of delivery [77]. Phytochemicals possess extraordinary ability to control oxidative stress and suppress chronic inflammation, all the while manifesting potential to modulate mitochondrial dysfunctions, events regarded as hallmarks of neurodegeneration [75]. Future scientific research should aim at establishing the neuroprotective claims of phytochemicals using in vitro and in vivo studies and human clinical trials. Studies regarding synergistic effects of different compounds should also be encouraged, along with an assessment of risk factors and toxicity, if any, involved. Incorporation of these phytochemicals in regular diet might be an effective strategy to protect the suffering of elderly from a sudden attack of cerebral stroke. Finally, it can be said that phytochemicals are gifts of nature which if utilized properly can provide human race with longer life span with lowered risk of onset of stroke and other neurodegenerative diseases, thus ensuring healthy aging.

References

1. World Health Organization, W.H., & World Health Organization (2014). The top 10 causes of death.
2. Kim, J., Fann, D. Y. W., Seet, R. C. S., Jo, D. G., Mattson, M. P., & Arumugam, T. V. (2016). Phytochemicals in ischemic stroke. *Neuromolecular Medicine, 18*(3), 283–305.
3. Goldstein, L. B., Bushnell, C. D., Adams, R. J., Appel, L. J., Braun, L. T., et al. (2011). Guidelines for the primary prevention of stroke. A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke, 42*(2), 517–584.
4. Mukherjee, D., & Patil, C. G. (2011). Epidemiology and the global burden of stroke. *World Neurosurgery, 76*(6), S85–S90.
5. Strong, K., Mathers, C., & Bonita, R. (2007). Preventing stroke: Saving lives around the world. *The Lancet Neurology, 6*(2), 182–187.
6. Caplan, L. R. (1999). Tissue plasminogen activator for acute ischemic stroke. *The New England Journal of Medicine, 341*(16), 1240–1241.
7. Smith, W. S., Sung, G., Saver, J., Budzik, R., Duckwiler, G., et al. (2008). Mechanical thrombectomy for acute ischemic stroke. *Stroke, 39*(4), 1205–1212.
8. Taschner, C. A., Treier, M., Schumacher, M., Berlis, A., Weber, J., & Niesen, W. (2011). Mechanical thrombectomy with the Penumbra recanalization device in acute ischemic stroke. *Journal of Neuroradiology, 38*(1), 47–52.

9. Hacke, W., Kaste, M., Bluhmki, E., Brozman, M., Dávalos, A., et al. (2008). Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *New England Journal of Medicine, 359*(13), 1317–1329.
10. Kelly, M. A., Shuaib, A., & Todd, K. G. (2006). Matrix metalloproteinase activation and blood–brain barrier breakdown following thrombolysis. *Experimental Neurology, 200*(1), 38–49.
11. Olivier, N., Docagne, F., Ali, C., Margaill, I., Carmeliet, P., MacKenzie, E. T., Denis, V., & Buisson, A. (2001). The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. *Nature Medicine, 7*(1), 59.
12. Chan, S. A., Reid, K. H., Schurr, A., Miller, J. J., Iyer, V., & Tseng, M. T. (1998). Fosphenytoin reduces hippocampal neuronal damage in rat following transient global ischemia. *Acta Neurochirurgica, 140*(2), 175–180.
13. Ahmed, N., Näsman, P., & Wahlgren, N. G. (2000). Effect of intravenous nimodipine on blood pressure and outcome after acute stroke. *Stroke, 31*(6), 1250–1255.
14. Davis, S. M., Lees, K. R., Albers, G. W., Diener, H. C., Markabi, S., Karlsson, G., & Norris, J. (2000). Selfotel in acute ischemic stroke. *Stroke, 31*(2), 347–354.
15. Cheng, Y. D., Al-Khoury, L., & Zivin, J. A. (2004). Neuroprotection for ischemic stroke: Two decades of success and failure. *NeuroRx, 1*(1), 36–45.
16. Green, A. R. (2002). Why do neuroprotective drugs that are so promising in animals fail in the clinic? An industry perspective. *Clinical and Experimental Pharmacology and Physiology, 29*(11), 1030–1034.
17. Zhang, Y. C., Gan, F. F., Shelar, S. B., Ng, K. Y., & Chew, E. H. (2013). Antioxidant and Nrf2 inducing activities of luteolin, a flavonoid constituent in Ixeris sonchifolia Hance, provide neuroprotective effects against ischemia-induced cellular injury. *Food and Chemical Toxicology, 59*, 272–280.
18. Zhang, A., Sun, H., & Wang, X. (2013). Recent advances in natural products from plants for treatment of liver diseases. *European Journal of Medicinal Chemistry, 63*, 570–577.
19. Salvador-Reyes, L. A., & Luesch, H. (2015). Biological targets and mechanisms of action of natural products from marine cyanobacteria. *Natural Product Reports, 32*(3), 478–503.
20. Huang, L., Su, T., & Li, X. (2013). Natural products as sources of new lead compounds for the treatment of Alzheimer's disease. *Current Topics in Medicinal Chemistry, 13*(15), 1864–1878.
21. Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J., Rimm, E. B., et al. (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. *Annals of Internal Medicine, 134*(12), 1106–1114.
22. Gaetano, G., Curtis, A., Castelnuovo, A., Donati, M. B., Iacoviello, L., & Rotondo, S. (2002). Antithrombotic effect of polyphenols in experimental models. *Annals of the New York Academy of Sciences, 957*(1), 174–188.
23. Moosavi, F., Hosseini, R., Saso, L., & Firuzi, O. (2016). Modulation of neurotrophic signaling pathways by polyphenols. *Drug Design, Development and Therapy, 10*, 23.
24. Larsson, S. C., Virtamo, J., & Wolk, A. (2013). Total and specific fruit and vegetable consumption and risk of stroke: A prospective study. *Atherosclerosis, 227*(1), 147–152.
25. Chen, G. C., Lv, D. B., Pang, Z., Dong, J. Y., & Liu, Q. F. (2013a). Dietary fiber intake and stroke risk: A meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition, 67*(1), 96.
26. Chen, C. L., Young, S. H., Gan, H. H., Singh, R., Lao, A. Y., Baroque, A. C., Chang, H. M., Hiyadan, J. H. B., Chua, C. L., Advincula, J. M., & Muengtaweepongsa, S. (2013b). Chinese medicine neuroaid efficacy on stroke recovery. *Stroke, 44*(8), 2093–2100.
27. Oskouei, D. S., Reza, R., Mazyar, H., Homayoun, S. B., et al. (2013). The effect of Ginkgo biloba on functional outcome of patients with acute ischemic stroke: A double-blind, placebo-controlled, randomized clinical trial. *Journal of Stroke and Cerebrovascular Diseases, 22*(8), e557–e563.

28. He, L., Chen, X., Zhou, M., Zhang, D., Yang, J., et al. (2011). Radix/rhizoma notoginseng extract (sanchitongtshu) for ischemic stroke: A randomized controlled study. *Phytomedicine, 18*(6), 437–442.
29. Poppitt, S. D., Howe, C. A., Lithander, F. E., Silvers, K. M., Lin, R. B., Croft, J., et al. (2009). Effects of moderate-dose omega-3 fish oil on cardiovascular risk factors and mood after ischemic stroke. *Stroke, 40*(11), 3485–3492.
30. Jung, W. S., Choi, D. J., Cho, K. H., Lee, K. S., Moon, S. K., Kim, Y. S., Bae, H. S., & Choi, B. O. (2003). Safety and efficacy assessment of Chungpyesagan-tang for acute ischemic stroke. *The American Journal of Chinese Medicine, 31*(02), 181–190.
31. Yakovlev, A. G., & Faden, A. I. (2004). Mechanisms of neural cell death: Implications for development of neuroprotective treatment strategies. *NeuroRx, 1*(1), 5–16.
32. Kitanaka, C., & Kuchino, Y. (1999). Caspase-independent programmed cell death with necrotic morphology. *Cell Death & Differentiation, 6*(6), 508–515.
33. Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. *British Journal of Cancer, 26*(4), 239.
34. Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., Cuny, G. D., Mitchison, T. J., Moskowitz, M. A., & Yuan, J. (2005). Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chemical Biology, 1*(2), 112.
35. Bredesen, D. E. (1995). Neural apoptosis. *Annals of Neurology, 38*(6), 839–851.
36. Wu, W., Liu, P., & Li, J. (2012). Necroptosis: An emerging form of programmed cell death. *Critical Reviews in Oncology/Hematology, 82*(3), 249–258.
37. Yakovlev, A. G., & Faden, A. I. (2001). Caspase-dependent apoptotic pathways in CNS injury. *Molecular Neurobiology, 24*(1–3), 131–144.
38. Alnemri, E. S., Livingston, D. J., Nicholson, D. W., Salvesen, G., Thornberry, N. A., Wong, W. W., & Yuan, J. (1996). Human ICE/CED-3 protease nomenclature. *Cell, 87*(2), 171.
39. Christofferson, D. E., & Yuan, J. (2010). Necroptosis as an alternative form of programmed cell death. *Current Opinion in Cell Biology, 22*(2), 263–268.
40. Kuida, K., Zheng, T. S., Na, S., & Kuan, C. Y. (1996). Deceased apoptosis in the brain and premature lethality in CPP32-deficient mice. *Nature, 384*(6607), 368.
41. Eldadah, B. A., & Faden, A. I. (2000). Caspase pathways, neuronal apoptosis, and CNS injury. *Journal of Neurotrauma, 17*(10), 811–829.
42. Slee, E. A., Harte, M. T., Kluck, R. M., Wolf, B. B., Casiano, C. A., Newmeyer, D. D., Wang, H. G., Reed, J. C., Nicholson, D. W., Alnemri, E. S., & Green, D. R. (1999). Ordering the cytochrome c–initiated caspase cascade: Hierarchical activation of caspases-2,-3,-6,-7,-8, and-10 in a caspase-9–dependent manner. *The Journal of Cell Biology, 144*(2), 281–292.
43. Cory, S., & Adams, J. M. (2002). The Bcl2 family: Regulators of the cellular life-or-death switch. *Nature Reviews Cancer, 2*(9), 647.
44. Hsu, Y. T., Wolter, K. G., & Youle, R. J. (1997). Cytosol-to-membrane redistribution of Bax and Bcl-XL during apoptosis. *Proceedings of the National Academy of Sciences, 94*(8), 3668–3672.
45. Gross, A., Jockel, J., Wei, M. C., & Korsmeyer, S. J. (1998). Enforced dimerization of BAX results in its translocation, mitochondrial dysfunction and apoptosis. *The EMBO Journal, 17*(14), 3878–3885.
46. Hay, B. A. (2000). Understanding IAP function and regulation: A view from Drosophila. *Cell Death and Differentiation, 7*(11), 1045.
47. Du, C., Fang, M., Li, Y., Li, L., & Wang, X. (2000). Smac, a mitochondrial protein that promotes cytochrome c–dependent caspase activation by eliminating IAP inhibition. *Cell, 102*(1), 33–42.
48. Hegde, R., Srinivasula, S. M., Zhang, Z., Wassell, R., Mukattash, R., et al. (2002). Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *Journal of Biological Chemistry, 277*(1), 432–438.
49. Volbracht, C., Leist, M., Kolb, S. A., & Nicotera, P. (2001). Apoptosis in caspase-inhibited neurons. *Molecular Medicine, 7*(1), 36.

50. Van Loo, G., Saelens, X., Van Gurp, M., MacFarlane, M., Martin, S. J., & Vandenabeele, P. (2002). The role of mitochondrial factors in apoptosis: A Russian roulette with more than one bullet. *Cell Death and Differentiation, 9*(10), 1031.
51. Ravagnan, L., Roumier, T., & Kroemer, G. (2002). Mitochondria, the killer organelles and their weapons. *Journal of Cellular Physiology, 192*(2), 131–137.
52. Daugas, E., Susin, S. A., Zamzami, N., Ferri, K. F., Irinopoulou, T., et al. (2000). Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis. *The FASEB Journal, 14*(5), 729–739.
53. Loeffler, M., Daugas, E., Susin, S. A., Zamzami, N., Métivier, D., et al. (2001). Dominant cell death induction by extramitochondrially targeted apoptosis-inducing factor. *The FASEB Journal, 15*(3), 758–767.
54. Li, L. Y., Luo, X., & Wang, X. (2001). Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature, 412*(6842), 95–100.
55. Hutchins, J. B., & Barger, S. W. (1998). Why neurons die: Cell death in the nervous system. *The Anatomical Record, 253*(3), 79–90.
56. Honig, L. S., & Rosenberg, R. N. (2000). Apoptosis and neurologic disease. *The American Journal of Medicine, 108*(4), 317–330.
57. Gorman, A. M. (2008). Neuronal cell death in neurodegenerative diseases: Recurring themes around protein handling. *Journal of Cellular and Molecular Medicine, 12*(6a), 2263–2280.
58. Nitatori, T., Sato, N., Waguri, S., Karasawa, Y., Araki, H., et al. (1995). Delayed neuronal death in the CA1 pyramidal cell layer of the gerbil hippocampus following transient ischemia is apoptosis. *Journal of Neuroscience, 15*(2), 1001–1011.
59. Du, C., Hu, R., Csernansky, C. A., Hsu, C. Y., & Choi, D. W. (1996). Very delayed infarction after mild focal cerebral ischemia: A role for apoptosis? *Journal of Cerebral Blood Flow & Metabolism, 16*(2), 195–201.
60. Rami, A. (2008). Upregulation of Beclin 1 in the ischemic penumbra. *Autophagy, 4*(2), 227–229.
61. Sauer, D., Allegrini, P. R., Cosenti, A., Pataki, A., Amacker, H., & Fagg, G. E. (1993). Characterization of the cerebroprotective efficacy of the competitive NMDA receptor antagonist CGP40116 in a rat model of focal cerebral ischemia: An in vivo magnetic resonance imaging study. *Journal of Cerebral Blood Flow & Metabolism, 13*(4), 595–602.
62. West, M. J., Coleman, P. D., Flood, D. G., & Troncoso, J. C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *The Lancet, 344*(8925), 769–772.
63. Price, J. L., Ko, A. I., Wade, M. J., Tsou, S. K., McKeel, D. W., & Morris, J. C. (2001). Neuron number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. *Archives of Neurology, 58*(9), 1395–1402.
64. Gómez-Isla, T., Price, J. L., McKeel, D. W., Jr., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *Journal of Neuroscience, 16*(14), 4491–4500.
65. Stadelmann, C., Deckwerth, T. L., Srinivasan, A., Bancher, C., Brück, W., et al. (1999). Activation of caspase-3 in single neurons and autophagic granules of granulovacuolar degeneration in Alzheimer's disease: Evidence for apoptotic cell death. *The American Journal of Pathology, 155*(5), 1459–1466.
66. Rohn, T. T., Head, E., Nesse, W. H., Cotman, C. W., & Cribbs, D. H. (2001). Activation of caspase-8 in the Alzheimer's disease brain. *Neurobiology of Disease, 8*(6), 1006–1016.
67. Rohn, T. T., Rissman, R. A., Davis, M. C., Kim, Y. E., Cotman, C. W., & Head, E. (2002). Caspase-9 activation and caspase cleavage of tau in the Alzheimer's disease brain. *Neurobiology of Disease, 11*(2), 341–354.
68. Colurso, G. J., Nilson, J. E., & Vervoort, L. G. (2003). Quantitative assessment of DNA fragmentation and beta-amyloid deposition in insular cortex and midfrontal gyrus from patients with Alzheimer's disease. *Life Sciences, 73*(14), 1795–1803.

69. Hartmann, A., Troadec, J. D., Hunot, S., Kikly, K., Faucheux, B. A., Mouatt-Prigent, A., Ruberg, M., Agid, Y., & Hirsch, E. C. (2001). Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *Journal of Neuroscience, 21*(7), 2247–2255.
70. Viswanath, V., Wu, Y., Boonplueang, R., Chen, S., Stevenson, F. F., Yantiri, F., Yang, L., Beal, M. F., & Andersen, J. K. (2001). Caspase-9 activation results in downstream caspase-8 activation and bid cleavage in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced Parkinson's disease. *Journal of Neuroscience, 21*(24), 9519–9528.
71. Mogi, M., Harada, M., Riederer, P., Narabayashi, H., Fujita, K., & Nagatsu, T. (1994). Tumor necrosis factor-a (TNF-a) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. *Neuroscience Letters, 165*(1), 208–210.
72. Mogi, M., Harada, M., Kondo, T., Mizuno, Y., Narabayashi, H., Riederer, P., & Nagatsu, T. (1996). The soluble form of Fas molecule is elevated in parkinsonian brain tissues. *Neuroscience Letters, 220*(3), 195–198.
73. Hartmann, A., Mouatt-Prigent, A., Faucheux, B. A., Agid, Y., & Hirsch, E. C. (2002). FADD: A link between TNF family receptors and caspases in Parkinson's disease. *Neurology, 58*(2), 308–310.
74. Bhullar, K. S., & Rupasinghe, H. P. (2013). Polyphenols: Multipotent therapeutic agents in neurodegenerative diseases. *Oxidative Medicine and Cellular Longevity, 2013*, 891748.
75. Reale, M., Iarlori, C., Thomas, A., Gambi, D., Perfetti, B., Di Nicola, M., & Onofrj, M. (2009). Peripheral cytokines profile in Parkinson's disease. *Brain, Behavior, and Immunity, 23*(1), 55–63.
76. Menza, M., Dobkin, R. D., Marin, H., Mark, M. H., Gara, M., Bienfait, K., Dicke, A., & Kusnekov, A. (2010). The role of inflammatory cytokines in cognition and other non-motor symptoms of Parkinson's disease. *Psychosomatics, 51*(6), 474–479.
77. Wang, J., Song, Y., Gao, M., Bai, X., & Chen, Z. (2016). Neuroprotective effect of several phytochemicals and its potential application in the prevention of neurodegenerative diseases. *Geriatrics, 1*(4), 29.
78. Xia, J., Cheng, L., Mei, C., Ma, J., Shi, Y., et al. (2014). Genistein inhibits cell growth and invasion through regulation of miR-27a in pancreatic cancer cells. *Current Pharmaceutical Design, 20*(33), 5348–5353.
79. Chang, Y., Hsieh, C. Y., Peng, Z. A., Yen, T. L., Hsiao, G., et al. (2009). Neuroprotective mechanisms of puerarin in middle cerebral artery occlusion-induced brain infarction in rats. *Journal of Biomedical Science, 16*(1), 9.
80. Dohare, P., Garg, P., Jain, V., Nath, C., & Ray, M. (2008). Dose dependence and therapeutic window for the neuroprotective effects of curcumin in thromboembolic model of rat. *Behavioural Brain Research, 193*(2), 289–297.
81. Saraf, M. K., Prabhakar, S., & Anand, A. (2010). Neuroprotective effect of Bacopa monnieri on ischemia induced brain injury. *Pharmacology Biochemistry and Behavior, 97*(2), 192–197.
82. Pujari, R. R., Vyawahare, N. S., & Kagathara, V. G. (2011). Evaluation of antioxidant and neuroprotective effect of date palm (*Phoenix dactylifera* L.) against bilateral common carotid artery occlusion in rats. *Indian Journal of Experimental Biology, 49*(8), 627–633.
83. An, G., & Morris, M. E. (2010). Effects of the isoflavonoid biochanin A on the transport of mitoxantrone in vitro and in vivo. *Biopharmaceutics & Drug Disposition, 31*(5–6), 340–350.
84. Yamagata, K., Kitazawa, T., Shinoda, M., Tagawa, C., Chino, M., & Matsufuji, H. (2009). Stroke status evoked adhesion molecule genetic alterations in astrocytes isolated from stroke-prone spontaneously hypertensive rats and the apigenin inhibition of their expression. *Stroke Research and Treatment, 2010*.
85. Lim, G. P., Chu, T., Yang, F., Beech, W., Frautschy, S. A., & Cole, G. M. (2001). The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *Journal of Neuroscience, 21*(21), 8370–8377.

86. Frautschy, S. A., Hu, W., Kim, P., Miller, S. A., Chu, T., Harris-White, M. E., & Cole, G. M. (2001). Phenolic anti-inflammatory antioxidant reversal of Aß-induced cognitive deficits and neuropathology. *Neurobiology of Aging, 22*(6), 993–1005.
87. Wang, M. S., Boddapati, S., Emadi, S., & Sierks, M. R. (2010). Curcumin reduces a-synuclein induced cytotoxicity in Parkinson's disease cell model. *BMC Neuroscience, 11*(1), 57.
88. Yang, F., Lim, G. P., Begum, A. N., Ubeda, O. J., Simmons, M. R., Ambegaokar, S. S., Chen, P. P., Kayed, R., Glabe, C. G., Frautschy, S. A., & Cole, G. M. (2005). Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *Journal of Biological Chemistry, 280*(7), 5892–5901.
89. Pan, J., Li, H., Ma, J. F., Tan, Y. Y., Xiao, Q., Ding, J. Q., & Chen, S. D. (2012). Curcumin inhibition of JNKs prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease through suppressing mitochondria dysfunction. *Translational Neurodegeneration, 1*(1), 16.
90. Wang, Q., Sun, A. Y., Simonyi, A., Jensen, M. D., Shelat, P. B., et al. (2005). Neuroprotective mechanisms of curcumin against cerebral ischemia-induced neuronal apoptosis and behavioral deficits. *Journal of Neuroscience Research, 82*(1), 138–148.
91. Bigford, G. E., & Del Rossi, G. (2014). Supplemental substances derived from foods as adjunctive therapeutic agents for treatment of neurodegenerative diseases and disorders. *Advances in Nutrition: An International Review Journal, 5*(4), 394–403.
92. Baur, J. A., & Sinclair, D. A. (2006). Therapeutic potential of resveratrol: The in vivo evidence. Nature reviews. *Drug Discovery, 5*(6), 493.
93. Han, Y. S., Zheng, W. H., Bastianetto, S., Chabot, J. G., & Quirion, R. (2004). Neuroprotective effects of resveratrol against ß-amyloid-induced neurotoxicity in rat hippocampal neurons: Involvement of protein kinase C. *British Journal of Pharmacology, 141*(6), 997–1005.
94. Zhang, F., Liu, J., & Shi, J. S. (2010). Anti-inflammatory activities of resveratrol in the brain: Role of resveratrol in microglial activation. *European Journal of Pharmacology, 636*(1), 1–7.
95. Song, J., Cheon, S. Y., Jung, W., Lee, W. T., & Lee, J. E. (2014). Resveratrol induces the expression of interleukin-10 and brain-derived neurotrophic factor in BV2 microglia under hypoxia. *International Journal of Molecular Sciences, 15*(9), 15512–15529.
96. Costa, L. G., Garrick, J. M., Roquè, P. J., & Pellacani, C. (2016). Mechanisms of neuroprotection by quercetin: Counteracting oxidative stress and more. *Oxidative Medicine and Cellular Longevity, 2016*, 2986796.
97. Liang, L., Gao, C., Luo, M., Wang, W., Zhao, C., Zu, Y., Efferth, T., & Fu, Y. (2013). Dihydroquercetin (DHQ) induced HO-1 and NQO1 expression against oxidative stress through the Nrf2-dependent antioxidant pathway. *Journal of Agricultural and Food Chemistry, 61*(11), 2755–2761.
98. Gan, L., & Johnson, J. A. (2014). Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1842*(8), 1208–1218.
99. Arredondo, F., Echeverry, C., Abin-Carriquiry, J. A., Blasina, F., Antúnez, K., Jones, D. P., Go, Y. M., Liang, Y. L., & Dajas, F. (2010). After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult. *Free Radical Biology and Medicine, 49*(5), 738–747.
100. Saw, C. L. L., Guo, Y., Yang, A. Y., Paredes-Gonzalez, X., Ramirez, C., Pung, D., & Kong, A. N. T. (2014). The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: Involvement of the Nrf2-ARE signaling pathway. *Food and Chemical Toxicology, 72*, 303–311.
101. Granado-Serrano, A. B., Martín, M. A., Bravo, L., Goya, L., & Ramos, S. (2012). Quercetin modulates Nrf2 and glutathione-related defenses in HepG2 cells: Involvement of p38. *Chemico-Biological Interactions, 195*(2), 154.

102. Ansari, M. A., Abdul, H. M., Joshi, G., Opii, W. O., & Butterfield, D. A. (2009). Protective effect of quercetin in primary neurons against Aß (1–42): Relevance to Alzheimer's disease. *The Journal of Nutritional Biochemistry, 20*(4), 269–275.
103. El Omri, A., Han, J., Yamada, P., Kawada, K., Abdrabbah, M. B., & Isoda, H. (2010). *Rosmarinus officinalis* polyphenols activate cholinergic activities in PC12 cells through phosphorylation of ERK1/2. *Journal of Ethnopharmacology, 131*(2), 451–458.
104. Bayat, M., Tameh, A. A., Ghahremani, M. H., Akbari, M., Mehr, S. E., Khanavi, M., & Hassanzadeh, G. (2012). Neuroprotective properties of *Melissa officinalis* after hypoxic-ischemic injury both in vitro and in vivo. *DARU Journal of Pharmaceutical Sciences, 20*(1), 42.
105. Gramza-Michalowska, A., & Regula, J. (2007). Use of tea extracts (Camelia sinensis) in jelly candies as polyphenols sources in human diet. *Asia Pacific Journal of Clinical Nutrition, 16*(S1), 43–46.
106. Venkatesan, R., Ji, E., & Kim, S. Y. (2015). Phytochemicals that regulate neurodegenerative disease by targeting neurotrophins: A comprehensive review. *BioMed Research International, 2015*, 814068.
107. Koh, S. H., Kim, S. H., Kwon, H., Park, Y., Kim, K. S., Song, C. W., Kim, J., Kim, M. H., Yu, H. J., Henkel, J. S., & Jung, H. K. (2003). Epigallocatechin gallate protects nerve growth factor differentiated PC12 cells from oxidative-radical-stress-induced apoptosis through its effect on phosphoinositide 3-kinase/Akt and glycogen synthase kinase-3. *Molecular Brain Research, 118*(1), 72–81.
108. Yang, Y. H., Hsieh, T. J., Tsai, M. L., Chen, C. H., Lin, H. T., & Wu, S. J. (2014). Neuroprotective effects of Hu-Yi-Neng, a diet supplement, on SH-SY5Y human neuroblastoma cells. *The Journal of Nutrition, Health & Aging, 18*(2), 184–190.
109. Xiao, Q., Wang, C., Li, J., Hou, Q., Li, J., Ma, J., Wang, W., & Wang, Z. (2010). Ginkgolide B protects hippocampal neurons from apoptosis induced by beta-amyloid 25–35 partly via up-regulation of brain-derived neurotrophic factor. *European Journal of Pharmacology, 647*(1), 48–54.
110. Kim, C. S., Kwon, O. W., Kim, S. Y., & Lee, K. R. (2013). Bioactive lignans from the trunk of Abies holophylla. *Journal of Natural Products, 76*(11), 2131–2135.
111. Chung, H. S., Lee, Y. C., Kyung Rhee, Y., & Lee, S. Y. (2011). Consumer acceptance of ginseng food products. *Journal of Food Science, 76*(9), S516–S522.
112. Joo, S. S., Yoo, Y. M., Ahn, B. W., Nam, S. Y., Kim, Y. B., Hwang, K. W., & Lee, D. I. (2008). Prevention of inflammation-mediated neurotoxicity by Rg3 and its role in microglial activation. *Biological and Pharmaceutical Bulletin, 31*(7), 1392–1396.
113. Kang, K. A., Kang, J. H., & Yang, M. P. (2008). Ginseng total saponin enhances the phagocytic capacity of canine peripheral blood phagocytes in vitro. *The American Journal of Chinese Medicine, 36*(02), 329–341.
114. Kim, M. S., Yu, J. M., Kim, H. J., Kim, H. B., Kim, S. T., Jang, S. K., Choi, Y. W., Lee, D. I., & Joo, S. S. (2014). Ginsenoside Re and Rd enhance the expression of cholinergic markers and neuronal differentiation in Neuro-2a cells. *Biological and Pharmaceutical Bulletin, 37*(5), 826–833.
115. Wang, Z. J., Nie, B. M., Chen, H. Z., & Lu, Y. (2006). Panaxynol induces neurite outgrowth in PC12D cells via cAMP-and MAP kinase-dependent mechanisms. *Chemico-Biological Interactions, 159*(1), 58–64.
116. Roy, A., & Saraf, S. (2006). Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. *Biological and Pharmaceutical Bulletin, 29*(2), 191–201.
117. Zhang, Q., Li, J. K., Ge, R., Liang, J. Y., Li, Q. S., & Min, Z. D. (2013). Novel NGF-potentiating limonoids from the fruits of Melia toosendan. *Fitoterapia, 90*, 192–198.
118. Zu Zhu, X., Li, X. Y., & Liu, J. (2004). Recent pharmacological studies on natural products in China. *European Journal of Pharmacology, 500*(1), 221–230.
119. Zhang, H. Y., & Tang, X. C. (2006). Neuroprotective effects of huperzine A: New therapeutic targets for neurodegenerative disease. *Trends in Pharmacological Sciences, 27*(12), 619–625.

120. Mao, X. Y., Cao, D. F., Li, X., Yin, J. Y., Wang, Z. B., Zhang, Y., Mao, C. X., Zhou, H. H., & Liu, Z. Q. (2014). Huperzine A ameliorates cognitive deficits in streptozotocin-induced diabetic rats. *International Journal of Molecular Sciences, 15*(5), 7667–7683.
121. Hsu, Y. Y., Tseng, Y. T., & Lo, Y. C. (2013). Berberine, a natural antidiabetes drug, attenuates glucose neurotoxicity and promotes Nrf2-related neurite outgrowth. *Toxicology and Applied Pharmacology, 272*(3), 787–796.
122. Lee, B., Sur, B., Shim, I., Lee, H., & Hahm, D. H. (2012). Phellodendron amurense and its major alkaloid compound, berberine ameliorates scopolamine-induced neuronal impairment and memory dysfunction in rats. *The Korean Journal of Physiology & Pharmacology, 16*(2), 79–89.
123. Jia, L., Liu, J., Song, Z., Pan, X., Chen, L., Cui, X., & Wang, M. (2012). Berberine suppresses amyloid-beta-induced inflammatory response in microglia by inhibiting nuclear factor-kappaB and mitogen-activated protein kinase signalling pathways. *Journal of Pharmacy and Pharmacology, 64*(10), 1510–1521.
124. Baitharu, I., Jain, V., Deep, S. N., Shroff, S., Sahu, J. K., Naik, P. K., & Ilavazhagan, G. (2014). Withanolide A prevents neurodegeneration by modulating hippocampal glutathione biosynthesis during hypoxia. *PLoS One, 9*(10), e105311.
125. Kumar, G., & Patnaik, R. (2016). Exploring neuroprotective potential of *Withania somnifera* phytochemicals by inhibition of GluN2B-containing NMDA receptors: An in silico study. *Medical Hypotheses, 92*, 35–43.
126. Kumar, G., Paliwal, P., & Patnaik, R. (2017). *Withania somnifera* phytochemicals confer neuroprotection by inhibition of the catalytic domain of human matrix metalloproteinase-9. *Letters in Drug Design & Discovery, 14*(6), 718–726.
127. Kumar, G., & Patnaik, R. (2017). Inhibition of gelatinases (MMP-2 and MMP-9) by *Withania somnifera* phytochemicals confers neuroprotection in stroke: An in silico analysis. *Interdisciplinary Sciences: Computational Life Sciences, 9*, 1–2. https://doi.org/10.1007/s12539-017-0231-x.
128. Eliasson, M. J., Huang, Z., Ferrante, R. J., Sasamata, M., Molliver, M. E., Snyder, S. H., & Moskowitz, M. A. (1999). Neuronal nitric oxide synthase activation and peroxynitrite formation in ischemic stroke linked to neural damage. *Journal of Neuroscience, 19*(14), 5910–5918.
129. Kumar, G., Mukherjee, S., & Patnaik, R. (2017). Identification of withanolide-M and stigmasterol as potent neuroprotectant and dual inhibitor of inducible/neuronal nitric oxide synthase by structure-based virtual screening. *Journal of Biological Engineering Research and Review, 4*(1), 09–13.
130. Kumar, G., Paliwal, P., Patnaik, N., & Patnaik, R. (2017). *Withania somnifera* phytochemicals confer neuroprotection by selective inhibition of nNos: An in silico study to search potent and selective inhibitors for human nNOS. *Journal of Theoretical and Computational Chemistry*. https://doi.org/10.1142/S0219633617500420.
131. Darvesh, A. S., Carroll, R. T., Bishayee, A., Geldenhuys, W. J., & Van der Schyf, C. J. (2010). Oxidative stress and Alzheimer's disease: Dietary polyphenols as potential therapeutic agents. *Expert Review of Neurotherapeutics, 10*(5), 729–745.

Chapter 13
Post-Stroke Treatment Strategies, Management, and Rehabilitation: Where We Stand?

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Abstract Stroke is a medical condition occurring due to the deprivation of blood supply temporarily/permanently to specific area of the brain leading to hypoxia to the brain cells, which eventually causes mortality of the patients. Stroke is categorized into three different types, i.e., transient ischemic attack (shows stroke-like symptoms), acute ischemic strokes (hiccups, nausea, troubles in walking and speaking, numbness of the face, arm, and leg mainly on one side of the body), and hemorrhagic strokes (loss of consciousness and balance, nausea, vomiting, seizures, dizziness). Over the past 20 years, the inhospital and post-discharge management for stroke has been deeply transformed. Their early detection and inhospital therapies can deteriorate short- and long-term outcomes of strokes. Inhospital treatments include thrombolysis, aspirin treatment, antithrombotic therapy, therapeutic hypothermia, blood pressure management, antiplatelet strategies, and surgery for cerebral edema. There are several important aspects which are discussed below in this chapter that should be taken care in order to decrease mortality rate in stroke patients.

Keywords Stroke · Acute ischemic strokes · Transient ischemic attack · Cerebral edema · Treatments

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Abbreviations

AA	Arachidonic acid
ACE	Angiotensin-converting enzyme
ADP	Adenosine diphosphate
AF	Atrial fibrillation
AIS	Acute ischemic strokes
APA	Antiplatelet agent
APP	Amyloid precursor protein
ASA	American Stroke Association
BBB	Blood-brain barrier
BP	Blood pressure
cAMP	Cyclic adenosine monophosphate
CBF	Cerebral blood flow
CNS	Central nervous system
COX	Cyclooxygenase
CSF	Cerebrospinal fluid
DAPT	Dual antiplatelet therapy
GP	Glycoprotein
HS	Hemorrhagic strokes
I/R	Ischemia/reperfusion
MI	Myocardial infarction
NOACs	Novel oral anticoagulants
PDE	Phosphodiesterase
PROACT II	Prolyse in acute cerebral thromboembolism II
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rtPA	Recombinant tissue plasminogen activator
T×A2	Thromboxane A2
TCM	Traditional Chinese medicine

13.1 Introduction

Stroke, i.e., brain attack is a medical emergency, which occurs due to the temporary/permanent seizure of vascularity to certain areas of the brain leading to the depletion of oxygen to the brain cells that may eventually cause the death of the patients. This sudden oxygen deprivation in the area leading to cell death can cause severe consequences affecting the memory and motor control. The consequences of the stroke mainly depend on the area of the brain affected and percentage it has been affected. These strokes may be major or minor; the former leads to temporary impairments, while the latter leads to paralysis and death. Around 25% of the population suffers a further stroke and recurrent strokes. Most of the patients with strokes will end up with some disabilities. In 2013, globally 6.5 million stroke deaths

occurred with 795,000 deaths in the USA itself [1], and by 2030 almost 70 million will be stroke survivors [2]. Keeping this in mind, this chapter focuses on the several therapeutic measures utilized by the physicians to deal with the stroke victims.

13.2 Types of Stroke

The strokes have been mainly categorized into three different types, i.e., transient ischemic attack, acute ischemic strokes (AIS), and hemorrhagic strokes (HS). When the blood supply to the brain gets stopped for some time, a transient ischemic attack occurs, and symptoms related to stroke are displayed by it, which generally retain for a short period, even less than a day. Acute ischemic stroke occurs due to the thrombotic or embolic blockage/closing of a cerebral artery leading to loss of blood circulation in the brain with symptoms such as hiccups or nausea; troubles in movement and speech; senselessness in the face, arm, and leg mainly on one side of the body; etc. The hemorrhagic strokes occur due to the breach in the blood vessel supplying the brain with symptoms such cessation of consciousness and balance, vomiting, nausea, seizures, dizziness, etc.

13.3 Treatments

13.3.1 In hospital

Over the past 20 years, the inhospital management for stroke has been deeply transformed. Their early detection and inhospital therapies can deteriorate short- and long-term outcomes of strokes. Inhospital treatments include thrombolysis, aspirin treatment, antithrombotic therapy, therapeutic hypothermia, blood pressure management, antiplatelet strategies, and surgery for cerebral edema.

13.3.1.1 Thrombolysis

13.3.1.1.1 Intravenous Thrombolytic Therapy

Recombinant tissue plasminogen activator (rtPA), also known as alteplase, has been generally administered intravenously for the chemical thrombolysis therapy around the world within 3 (the USA)/4.5 (other countries) hours of acute ischemic onset [3]. The efficacy of intravenous thrombolytic therapy was published in 1995 and has been licensed since 1997 in the USA and 2002 in the UK. Several randomized controlled trials besides several observational and meta-analysis studies showed recovery from the acute ischemic stroke [4–7]. A combined meta-analysis of 2775

patients from the crucial thrombolysis records, for treatment within 90 min, showed 2.8 of the odds of a favorable outcome with no disability followed by 1.6 for treatment between 91 and 180 min and 1.4 treated between 181 and 270 min [8]. Hence the chances of being free from disability with the stroke enhanced by threefold when treated with rtPA within 90 min of onset. At up to 4.5 h, thrombolytic treatment provided smaller and significant benefits. The number that was required to treat was seven to arrive at a perfect outcome, while three was required to achieve reduction in disability with rtPA [8, 9].

13.3.1.1.2 Intra-arterial Thrombolysis

Intra-arterial thrombolysis involves the incorporation of thrombolytic agents, i.e., rtPA or urokinase, post the direct catheterization of a blocked artery. This has been a productive therapy for occlusions in the middle cerebral artery or basilar artery. Trials such as Prolyse in Acute Cerebral Thromboembolism II (PROACT II) stand as an example for this therapy [10]. This trial showed that on treatment within 6 h of stroke with urokinase and intravenous heparin, the middle cerebral artery occlusion showed significantly higher reperfusion rates alongside with patients living independently at 90th day with relative risk reduction rate of 58%. While basilar artery occlusion patients showed a high mortality rate >70% [11], several case studies showed declined mortality rate after intra-arterial thrombolysis treatment [12, 13]. A structured analysis of both intravenous treatment and intra-arterial thrombolysis treatment showcased similar reperfusion rates and clinical outcomes showing the fact that these two forms of thrombolysis will be of great need [13].

13.3.1.2 Aspirin Treatment

It has been proved that subsequent to the occurrence of first stroke, aspirin decreases the likelihood of having another stroke [14]. It was also reported that aspirin can prevent myocardial infarction (MI) with a dose of 325 mg five times a week. Patients with the first occurrence of hemispheric ischemic stroke hospitalized within 24 h of stroke onset displayed the presence of glycine and glutamate in cerebrospinal fluid (CSF) [15]. It was found that group of patients who were not taking aspirin have higher concentrations of glutamate than those who were taking it [16]. It led to a conclusion that aspirin decreases the risk of early neurological deterioration up to 97% at the time of stroke onset.

Aspirin (acetylsalicylic acid) has been used for the past 25 years for the avoidance of MI and acute ischemic stroke [17]. It is cost-effective, widely available, and used for the prevention of cardiovascular events (both primary and secondary) with very little side effects. Apart from possessing antithrombotic properties by inhibition of cyclooxygenase (COX) enzyme, which metabolizes arachidonic acid into thromboxane A2 as a potent vasoconstrictor and platelet agonist [18], aspirin also has neuroprotective effects [19]. The antiplatelet activity of aspirin clearly indicates

that the major risk associated with its consumption is bleeding complications as it prolongs bleeding time.

13.3.1.3 Therapeutic Hypothermia

Therapeutic hypothermia also referred as targeted temperature treatment or protective hypothermia involves intentional reduction of the core body temperature (37 °C) of a patient in the range of 32–34 °C for a specific duration of time in an effort to improve health outcomes during recovery after a period of obstructed blood flow to the brain [20]. Conditions like cardiac arrest or the blockage of an artery by the formation of the clot as seen in case of stroke can cause poor blood flow to the brain. Therapeutic hypothermia is performed in an attempt to minimize the risk of injury to the tissue following lack of blood flow. The role of this therapy in case of cardiac arrest-induced cerebral injury is nicely documented, while in case of stroke, extensive amount of investigation is still required to have an idea of the optimal duration of therapeutic hypothermia, rates of cooling and rewarming, and optimum target temperature. The different levels of hypothermia include mild (32 °C), moderate (28–32 °C), deep (20–28 °C), profound (5–20 °C), and ultraprofound (<5 °C) hypothermia [21].

There is ample amount of research that indicates the potent neuroprotective role of induced hypothermia in patients with neurological injury [21–23]. Hypothermia safeguards the brain tissue in several different ways, and thus it may be an ideal choice for stroke therapy. The benefit of this treatment will be greatest if initiated in the early stage, mostly within several hours of beginning of symptoms. Hypothermia given for a longer duration came out to be the more effective strategy of neuroprotection after an ischemic event in clinical trials [21]. Investigations related to ultraprofound resuscitative hypothermia are under process in trauma patients for quick cooling using very cold fluids [21].

13.3.1.4 Blood Pressure Management

The connection between hypotension and hypertension with stroke is constantly progressing and multifaceted. The patients with acute ischemic stroke show higher incidence of hypertension (75% or more) and are associated with poor outcomes [24]. It has already been investigated that high blood pressure can lead to elevated cerebral edema, hematoma expansion, or hemorrhagic transformation, while low blood pressure can cause an increase in cerebral infarction or perihematomal ischemia [25]. More than 60% of patients with acute ischemic stroke (AIS) showed an elevation in the BP within an hour of symptom onset. Numerous published evidence suggests that, in patients with intracerebral hemorrhage, BP should be brought down rapidly because it affects thrombolytic eligibility and has been associated with a delay in administration of IV tissue plasminogen activator [26, 27]. This suggestion could be fatal because most of the stroke patients are septuagenarians and

cannot withstand the rapid lowering in BP and it also decreases cerebral blood flow (CBF). But neurologically stable patients have shown improved functional outcome. The modulation of hypertension in stroke is determined by the type of stroke, timing, use of thrombolysis, existing medical conditions, and pharmacologic changes.

13.3.1.5 Antiplatelet Strategies

For the prevention of secondary stroke, antiplatelet therapy is extensively studied. Monotherapy with aspirin was used for secondary prevention of ischemic events in cardiovascular patients for several years, and it was believed to subside the risk of such events 25% compared to a placebo [28]. The recent trials have found out the competence of new antiplatelet agent strategies. Several agents have been evaluated in this setting, both in isolation and combination for reducing the risk of cardiovascular events specifically on cardioembolic strokes. The most commonly used antiplatelet agents approved by FDA are aspirin, thienopyridines (ticlopidine and clopidogrel), and phosphodiesterase (PDE) inhibitors (dipyridamole and cilostazol) [29]. The glycoprotein (GP) IIb/IIIa antagonists specifically prevent aggregation by inhibiting the fibrinogen receptor αIIbβ3 [30].Clopidogrel is a precious alternative to aspirin in case of monotherapy. The combination of clopidogrel and aspirin proves useful in the treatment of patients with high risks of vascular blockage, but it is generally prescribed for acute coronary artery injuries like myocardial infarction (heart attack), coronary stenting, as well as peripheral stenting [31]. But it is seen that stable patients of cardiovascular diseases prefer monotherapy. The combination of dipyridamole and aspirin was found effective in ischemic stroke than monotreatment with mild doses of aspirin, but its use is limited due to certain side effects. The research for new APA strategies is still under process.

13.3.1.6 Surgery for Cerebral Edema

Cerebral edema is the most common reason of death due to AIS. It begins soon after the onset of ischemic stroke and attains peak at 24–96 h [32]. Initially, it is cytotoxic because the cell membranes are disturbed, and later disturbance in the blood-brain barrier (BBB) led to the occurrence of vasogenic edema. The larger the infarct, the more severe will be the edema. Brain herniation due to symptomatic cerebral edema is seen in case of 5–10% patients. It is so problematic that even minute quantities of edema from a stroke can elevate the intracranial pressure in the posterior fossa.

In order to relieve high intracranial pressure due to edema, decompressive surgical techniques are used. But there is no cemented evidence that these surgical procedures have improved outcome after massive stroke. These surgeries involve the removal of some of the skull bones lying above the region of swelling which may

reduce the risk of death and disability, but it may become risky in patients who are acutely ill after a stroke [32, 33].

13.3.1.7 Antithrombotic Therapy

The basic pathophysiological process involved in acute coronary syndromes is thrombosis. Thus the use of antithrombotic therapy becomes quite evident in these processes, and it includes proper selection of antithrombotic drugs to reduce platelet aggregation or interference with the clotting process. Antithrombotic drugs include antiplatelet as well as anticoagulants and are responsible for the reduction of first-ever and recurrent ischemic stroke in patients with AF [34]. The administration of regulated dose of warfarin (anticoagulant) reduces the occurrence of stroke by approximately 60% compared with no treatment similarly 20% by aspirin compared with no treatment and about 40% by warfarin compared to aspirin [35, 36]. Meta-analysis revealed that warfarin reduces ischemic stroke by 65% [37]. But the long-term use of warfarin (anticoagulant therapy) may not be as effective in case of elderly persons [38]. Atrial fibrillation (AF) is a potent risk factor for stroke, but its threat can be gradually reduced by proper use of antithrombotic agents.

13.3.2 Post-discharge

Sixteen to 17 million people suffer stroke each year, 6 million of whom do not survive. Fifteen percent ischemic stroke patients die within a month of being hospitalized. Over 30% of ischemic stroke patients die within a year of being hospitalized. More than 60% of ischemic stroke patients are either readmitted or die within 12 months post-discharge [39]. There are several important aspects which are discussed below that should be taken care in order to decrease mortality rate in post-discharge stroke patients.

13.3.2.1 Lifestyle Physical Activity

13.3.2.1.1 Smoking

Smoking elevates the chances of ischemic and hemorrhagic stroke due to blood vessel obstruction and alteration in blood dynamicity. To overcome from a subsequent stroke in post-discharge patients, several therapies are recommended for smoking cessation. Nicotine replacement therapy, the use of antidepressants (like bupropion, nortriptyline, varenicline), and behavioral therapies (telephone counseling) improve smoking cessation [40].

13.3.2.1.2 Alcohol

Excessive alcohol abuse increases the incidence of stroke, but no study has been done specifically to secondary stroke prevention. National guideline recommends limited alcohol consumption to two standard drink/day [41].

13.3.2.1.3 Diet

Dietary modification is important to maintain an optimal level of blood glucose, lipid level, and blood pressure that help to prevent stroke. Diet with low-sodium intake, avoidance of excessive sugar, low fat but high in fruit and vegetable, and high in oily fish is beneficial in controlling dyslipidemia, obesity, and hypertension and reduces recurrence of stroke in post-discharge stroke patients [42].

13.3.2.1.4 Physical Activity

Physical inactivity after stroke is highly prevalent. Regular exercise improves the functional capacity, ability to perform mundane activities, and lifestyle, and it lowers the risk for subsequent cardiovascular events. The inclusion of physiotherapy in stroke survivors emphasizes on moderate aerobic activity, cardio and muscle-strengthening exercises, and reduction of sedentary behavior [43].

Other therapies like occupational therapy for recuperation from physical and mental illness involve reacquiring the skills needed for everyday living that include taking food, using bathroom, grooming, dressing, etc. On the contrary, speech therapy includes techniques to mitigate problems in communication, thinking, or swallowing of food [44].

13.3.2.2 Blood Glucose Management

In 25% patients, glucose intolerance after stroke is common and is linked to high stroke recurrence [45]. Hyperglycemia may worsen brain injury during acute cerebral infarct. The intravenous insulin protocol corrects hyperglycemia in post-discharge patients or during acute cerebral infarction [46].

13.3.2.3 Cholesterol Management

High cholesterol level may increase the risk of stroke recurrence by forming a clot in the arteries. Several drugs, including a class of drug statins, may help in lowering cholesterol levels. Unless cholesterol levels are already low, taking a statin is generally beneficial. Diet plays an important role in maintaining blood serum lipid

profile, therefore avoiding oily food; increase fiber-rich diet helps in lowering cholesterol level [47].

13.3.2.4 Blood Pressure Management

High BP is the threat for upcoming stroke in post-discharge patients. Therefore the consumption of antihypertensive agents (except beta-blocker) and ACE inhibitor (alone or with a diuretic) is effective in lowering blood pressure. Other drugs like calcium channel blockers, angiotensin receptor antagonists, clonidine, and glyceryl trinitrate lower blood pressure, while phenylephrine appeared to increase blood pressure, but there is no proof that therapy declines mortality or improves functional outcomes [25].

13.3.2.5 Anticoagulation and Antiplatelet Therapy

Anticoagulant and antiplatelet both prevent clot, but anticoagulant requires regular monitoring by healthcare [48]. Main antiplatelet like ASA can be given 81 mg daily or combination of ASA, and Plavix can be given in the first 21 days to 3 months after stroke. Anticoagulant like Coumadin and NOACs are beneficial in blood clotting situation. In comparison with placebo, aspirin, or DAPT, oral anticoagulants with dose-adjusted warfarin are associated with reduction in risk of stroke [49].

13.3.2.6 Herbal Neuroprotective Intervention

Stroke is a major cause of several disabilities and patient develops dementia within 3 months after a stroke. One of the world's oldest documented medical systems, i.e., traditional Chinese medicine (TCM) system through herbal medicines, has been used for the treatment of stroke subjects [50]. Cognitive dysfunction and dementia have been reduced by the leaf extract of *Ginkgo biloba*, which can be helpful for treatment of stroke [51]. *G. biloba* leaf extract contains several derivatives such as ginkgolides A, B, C, J, and M and bilobalide that have some helpful results against cognitive dysfunction. Ginkgolides, which is an antagonist for platelet-activating factor, have shown to reduce activation of platelet and their aggregation, which finally helps in improving the blood circulation. Additionally, bilobalide, one of the constituent Egb 761, has shown to mitigate the repercussions associated with brain ischemia and neuronal death.

Another neuroprotective medicinal plant, i.e., *Scutellaria baicalensis*, shows anti-inflammatory and antioxidant activity and has been widely used in the oriental (Korean and Chinese) areas of the world. In Korea, several neuro-inflammatory diseases have been treated with root extract rich in bioactive components, which have shown some pretty good results against inflammation. Additionally, flavonoids, baicalein, and baicalin act as antioxidant agents, in different in vitro cultures

and in vivo models by quenching the ROS that literally helps in protecting the neurons from ROS/RNS damage in cerebral ischemia/reperfusion (I/R) injury [52].

13.4 Limitations of Treatment

Post-stroke participants experience several challenges including paucity of medium of transport and topography in the village areas not suitable to wheelchair use. Because of restricted participation and lack of accomplishment, only approximately 40% of patients receive physiotherapy [53]. There are limitations in walking, personal care, and household activities. The stroke patients who have been discharged from hospital have reduced social interactions, incompetence to resume to the prior job, and shiftlessness in participating in activities related to religion [54].

13.5 Conclusion

The medical care for stroke survivors is often patchy and incompatible. Once admitted, patients are confronted with a baffling range of tests, people, and places, as well as confusion about treatment and services. This chapter takes into account the various strategies that could be employed to treat patients suffering from both ischemic and hemorrhagic stroke. The advances in acute treatment are saving the lives of people who would not have survived in the past. As a result post-stroke therapy is more important than ever. The inhospital treatments include the administration of thrombolytic agents like rtPA or urokinase as a part of thrombolysis therapy; aspirin (acetylsalicylic acid) for the prevention of MI and AIS; targeted temperature treatment to clear blood obstructions; management of blood pressure; the use of antiplatelet agents such as aspirin, clopidogrel, and PDE inhibitors; surgery of cerebral edema; and antithrombotic agents. The post-discharge therapies in stroke rehabilitation are mostly noninvasive and include lifestyle restrictions such as limiting the daily alcohol intake, abstaining from smoking, and resorting to physical exercise and herbal medications. Many trials are currently underway, which, in time, may impact on future rehabilitative practice.

References

1. Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., Deo, R., et al. (2017). Heart disease and stroke statistics—2017 update: A report from the American Heart Association. *Circulation, 135*(10), e146–e603.
2. Langhorne, P., Bernhardt, J., & Kwakkel, G. (2011). Stroke rehabilitation. *The Lancet, 377*(9778), 1693–1702.

3. Rabinstein, A. A. (2017). Treatment of acute ischemic stroke. *CONTINUUM: Lifelong Learning in Neurology, 23*(1, Cerebrovascular Disease), 62–81.
4. Wardlaw, J. M., Murray, V., Berge, E., & Del Zoppo, G. J. (2009). Thrombolysis for acute ischaemic stroke. *Cochrane Database of Systematic Reviews, 4*, CD000213.
5. Group, S. S. (1995). Tissue plasminogen activator for acute ischemic stroke. *The New England Journal of Medicine, 333*, 1581–1587.
6. Hacke, W., Kaste, M., Bluhmki, E., Brozman, M., Dávalos, A., Guidetti, D., et al. (2008). Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *The New England Journal of Medicine, 359*(13), 1317–1329.
7. Wahlgren, N., Ahmed, N., Dávalos, A., Ford, G. A., Grond, M., Hacke, W., et al. (2007). Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): An observational study. *The Lancet, 369*(9558), 275–282.
8. ATLANTIS T. (2004). Association of outcome with early stroke treatment: Pooled analysis of ATLANTIS, ECASS, and NINDS rt-PA stroke trials. *The Lancet, 363*(9411), 768–774.
9. Saver, J. L. (2004). Number needed to treat estimates incorporating effects over the entire range of clinical outcomes: Novel derivation method and application to thrombolytic therapy for acute stroke. *Archives of Neurology, 61*(7), 1066–1070.
10. Furlan, A., Higashida, R., Wechsler, L., Gent, M., Rowley, H., Kase, C., et al. (1999). Intra-arterial prourokinase for acute ischemic stroke: The PROACT II study: A randomized controlled trial. *Journal of the American Medical Association, 282*(21), 2003–2011.
11. Dawson, J. (2009). *Prevention of stroke: Risk stratification and targeted and novel therapies*. University of Glasgow.
12. Arnold, M., Nedeltchev, K., Schroth, G., Baumgartner, R., Remonda, L., Loher, T., et al. (2004). Clinical and radiological predictors of recanalisation and outcome of 40 patients with acute basilar artery occlusion treated with intra-arterial thrombolysis. *Journal of Neurology, Neurosurgery & Psychiatry, 75*(6), 857–862.
13. Lindsberg, P., & Mattle, H. (2006). Therapy of basilar artery occlusion: A systematic analysis comparing intra-arterial and intravenous thrombolysis. *Stroke, 37*(3), 922–928.
14. Dalen, J. E. (2006). Aspirin to prevent heart attack and stroke: What's the right dose? *The American Journal of Medicine, 119*(3), 198–202.
15. Castillo, J., Davalos, A., Naveiro, J., & Noya, M. (1996). Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. *Stroke, 27*(6), 1060–1065.
16. Castillo, J., Leira, R., Moro, M. Á., Lizasoain, I., Serena, J. N., & Dávalos, A. (2003). Neuroprotective effects of aspirin in patients with acute cerebral infarction. *Neuroscience Letters, 339*(3), 248–250.
17. Ansara, A. J., Nisly, S. A., Arif, S. A., Koehler, J. M., & Nordmeyer, S. T. (2010). Aspirin dosing for the prevention and treatment of ischemic stroke: An indication-specific review of the literature. *The Annals of Pharmacotherapy, 44*(5), 851–862.
18. Warner, T. D., Nylander, S., & Whatling, C. (2011). Anti-platelet therapy: Cyclo-oxygenase inhibition and the use of aspirin with particular regard to dual anti-platelet therapy. *British Journal of Clinical Pharmacology, 72*(4), 619–633.
19. Diener, H.-C., Sacco, R. L., Yusuf, S., Cotton, D., Ôunpuu, S., Lawton, W. A., et al. (2008). Effects of aspirin plus extended-release dipyridamole versus clopidogrel and telmisartan on disability and cognitive function after recurrent stroke in patients with ischaemic stroke in the Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial: A double-blind, active and placebo-controlled study. *The Lancet Neurology, 7*(10), 875–884.
20. Andresen, M., Gazmuri, J. T., Marín, A., Regueira, T., & Rovegno, M. (2015). Therapeutic hypothermia for acute brain injuries. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine, 23*(1), 42.
21. Liu, L., & Yenari, M. A. (2009). Clinical application of therapeutic hypothermia in stroke. *Neurological Research, 31*(4), 331–335.

22. Karnatovskaia, L. V., Wartenberg, K. E., & Freeman, W. D. (2014). Therapeutic hypothermia for neuroprotection: History, mechanisms, risks, and clinical applications. *The Neurohospitalist, 4*(3), 153–163.
23. Yokobori, S., Frantzen, J., Bullock, R., Gajavelli, S., Burks, S., Bramlett, H., et al. (2011). The use of hypothermia therapy in traumatic ischemic/reperfusional brain injury: Review of the literatures. *Therapeutic Hypothermia and Temperature Management, 1*(4), 185–192.
24. McManus, M., & Liebeskind, D. S. (2016). Blood pressure in acute ischemic stroke. *Journal of Clinical Neurology, 12*(2), 137–146.
25. Appleton, J. P., Sprigg, N., & Bath, P. M. (2016). Blood pressure management in acute stroke. *Stroke and Vascular Neurology, 1*, e000020.
26. Bowry, R., Navalkele, D. D., & Gonzales, N. R. (2014). Blood pressure management in stroke five new things. *Neurology: Clinical Practice, 4*(5), 419–426.
27. Martin-Schild, S., Hallevi, H., Albright, K. C., Khaja, A. M., Barreto, A. D., Gonzales, N. R., et al. (2008). Aggressive blood pressure–lowering treatment before intravenous tissue plasminogen activator therapy in acute ischemic stroke. *Archives of Neurology, 65*(9), 1174–1178.
28. Fontana, P., & Reny, J.-L. (2007). New antiplatelet strategies in atherothrombosis and their indications. *European Journal of Vascular and Endovascular Surgery, 34*(1), 10–17.
29. Gurbel, P. A., & Tantry, U. S. (2010). Combination antithrombotic therapies. *Circulation, 121*(4), 569–583.
30. Schneider, D. J. (2011). Anti-platelet therapy: Glycoprotein IIb-IIIa antagonists. *British Journal of Clinical Pharmacology, 72*(4), 672–682.
31. Yip, S., & Benavente, O. (2011). Antiplatelet agents for stroke prevention. *Neurotherapeutics, 8*(3), 475.
32. Jha, S. (2003). Cerebral edema and its management. *Medical Journal, Armed Forces India, 59*(4), 326.
33. Park, J.-O., Park, D.-H., Kim, S.-D., Lim, D.-J., & Park, J.-Y. (2007). Surgical treatment for acute, severe brain infarction. *Journal of Korean Neurosurgical Society, 42*(4), 326–330.
34. Broderick, J. P., Bonomo, J. B., Kissela, B. M., Khoury, J. C., Moomaw, C. J., Alwell, K., et al. (2011). Withdrawal of antithrombotic agents and its impact on ischemic stroke occurrence. *Stroke, 42*(9), 2509–2514.
35. Guo, Y., Wang, H., Tian, Y., Wang, Y., & Lip, G. Y. (2015). Time trends of aspirin and warfarin use on stroke and bleeding events in Chinese patients with new-onset atrial fibrillation. *CHEST Journal, 148*(1), 62–72.
36. Rybak, I., Ehle, M., Buckley, L., & Fanikos, J. (2011). Efficacy and safety of novel anticoagulants compared with established agents. *Therapeutic Advances in Hematology, 2*(3), 175–195.
37. Molteni, M., & Cimminiello, C. (2014). Warfarin and atrial fibrillation: From ideal to real the warfarin affaire. *Thrombosis Journal, 12*(1), 5.
38. Bajorek, B. (2011). A review of the safety of anticoagulants in older people using the medicines management pathway: Weighing the benefits against the risks. *Therapeutic Advances in Drug Safety, 2*(2), 45–58.
39. Fonarow, G. C., Smith, E. E., Reeves, M. J., Pan, W., Olson, D., Hernandez, A. F., et al. (2011). Hospital-level variation in mortality and rehospitalization for medicare beneficiaries with acute ischemic stroke. *Stroke, 42*(1), 159–166.
40. Casella, G., Caponnetto, P., & Polosa, R. (2010). Therapeutic advances in the treatment of nicotine addiction: Present and future. *Therapeutic Advances in Chronic Disease, 1*(3), 95–106.
41. Goldstein, L. B., Bushnell, C. D., Adams, R. J., Appel, L. J., Braun, L. T., & Chaturvedi, S., et al. (2010). Guidelines for the primary prevention of stroke. A guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*.
42. Ding, E. L., & Mozaffarian, D. (Eds.). (2006). *Optimal dietary habits for the prevention of stroke. Seminars in neurology*. New York: Copyright© 2006 by Thieme Medical Publishers.
43. Billinger, S. A., Arena, R., Bernhardt, J., Eng, J. J., Franklin, B. A., Johnson, C. M., et al. (2014). Physical activity and exercise recommendations for stroke survivors. *Stroke, 45*(8), 2532–2553.

44. Freire, F. R., Coelho, F., Lacerda, J. R., Silva, M. F. D., Gonçalves, V. T., Machado, S., et al. (2011). Cognitive rehabilitation following traumatic brain injury. *Dementia & Neuropsychologia, 5*(1), 17–25.
45. Kernan, W. N., Viscoli, C. M., Inzucchi, S. E., Brass, L. M., Bravata, D. M., Shulman, G. I., et al. (2005). Prevalence of abnormal glucose tolerance following a transient ischemic attack or ischemic stroke. *Archives of Internal Medicine, 165*(2), 227–233.
46. Bruno, A., Kent, T. A., Coull, B. M., Shankar, R. R., Saha, C., Becker, K. J., et al. (2008). Treatment of hyperglycemia in ischemic stroke (THIS). *Stroke, 39*(2), 384–389.
47. Foroughi, M., Akhavanzanjani, M., Maghsoudi, Z., Ghiasvand, R., Khorvash, F., & Askari, G. (2013). Stroke and nutrition: A review of studies. *International Journal of Preventive Medicine, 4*(Suppl 2), S165.
48. Michota, F. (2013). Transitions of care in anticoagulated patients. *Journal of Multidisciplinary Healthcare, 6*, 215.
49. Goto, K., Nakai, K., Shizuta, S., Morimoto, T., Shiomi, H., Natsuaki, M., et al. (2014). Anticoagulant and antiplatelet therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention. *The American Journal of Cardiology, 114*(1), 70–78.
50. Fung, F. Y., & Linn, Y. C. (2015). Developing traditional Chinese medicine in the era of evidence-based medicine: Current evidences and challenges. *Evidence-Based Complementary and Alternative Medicine, 2015*, 425037.
51. Birks, J., & Grimley, E. J. (2007). *Ginkgo biloba* for cognitive impairment and dementia.
52. Iriti, M., Vitalini, S., Fico, G., & Faoro, F. (2010). Neuroprotective herbs and foods from different traditional medicines and diets. *Molecules (Basel, Switzerland), 15*(5), 3517–3555.
53. Rhoda, A., Cunningham, N., Azaria, S., & Urimubenshi, G. (2015). Provision of inpatient rehabilitation and challenges experienced with participation post discharge: Quantitative and qualitative inquiry of African stroke patients. *BMC Health Services Research, 15*(1), 423.
54. Urimubenshi, G. (2015). Activity limitations and participation restrictions experienced by people with stroke in Musanze district in Rwanda. *African Health Sciences, 15*(3), 917–924.