Stroke

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R. M. Touyz, C. Delles (eds.), *Textbook of Vascular Medicine*,
https://doi.org/10.1007/978-3-030-16481-2_43

Key Concepts

- Stroke is a cerebrovascular incident resulting in neurological dysfunction; most commonly caused by an ischaemic stroke whereby a thrombus occludes a major vessel within the brain.
- Stroke is one of the leading causes of death and disability in the world, resulting in a large economic burden; however, treatment options are currently limited to the use of a tissue plasminogen activator (tPA) and/or a mechanical thrombectomy procedure to recanalize the blood vessel.
- The pathophysiology of ischaemic stroke is complex with numerous pathways converging to result in cell death and the development of an area of irreversibly damaged brain tissue called an infarct.
- Numerous elements of the pathophysiology of ischaemic stroke have been previously targeted as neuroprotective strategies in preclinical stroke models but have failed to translate clinically.
- The preclinical stroke research community has learned from previous failures and strives to improve the translational potential and quality of stroke research with improved study design, rigour and reproducibility which is the benchmark for other preclinical disease models.

43.1 Introduction: What Is Stroke?

Stroke was defined by the World Health Organisation in the 1970s as a clinical syndrome of "rapidly developing clinical signs of focal and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin". However, due to the advances in modern medicine, this definition has been updated in recent years to include more specific definitions based on the cause or clinical presentation and also to distinguish transient ischaemic attack (TIA) from ischaemic stroke. Briefly, ischaemic stroke is defined as "an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction" with an infarction being defined by imaging or clinical symptoms persisting longer than 24 hours. Alternatively, TIA is defined as "a transient episode of neurological dysfunction caused by focal brain, spinal cord, or retinal ischemia *without acute infarction*" [1]. Haemorrhagic stroke is defined as "rapidly developing clinical signs of neurological dysfunction attributable to a focal collection of blood within the brain parenchyma or ventricular system that is not caused by trauma," or in the case of subarachnoid haemorrhage, this may also include headache as a symptom and is caused by bleeding into the subarachnoid space (the space between the arachnoid membrane and the pia mater) [1]. Ischaemic stroke accounts for approximately 87% of stroke incidences with cerebral haemorrhage or subarachnoid haemorrhage accounting for around 10% and 3%, respectively [2].

43.1.1 Epidemiology

Stroke is the second leading cause of death worldwide accounting for around 10% of all deaths. It is also the third leading cause of loss of disability-adjusted life years (DALYs) accounting for nearly 5% of DALYs lost. The most recently available figures tell us that in 2013 there were an estimated 10.3 million new incidences of stroke worldwide with higher incidence in developed countries [3]. More specifically, in the United States (US), someone has a stroke every 40 seconds, 1 in 20 deaths are due to stroke, and the cost to the US economy is estimated at around $34 billion each year [2]. Meanwhile, in the United Kingdom (UK), the incidence is lower but still substantial, with a stroke occurring once every 5 minutes, accounting for around 1 in 14 deaths and costing the UK economy around £25.6 billion each year [4]. In both the United Kingdom and United States, stroke is one of the leading causes of disability contributing greatly to this economic burden. The worldwide occurrence of stroke is slightly higher in females than males with stroke affecting one in every five women ages 55–75 years and approximately one in six men [2]. Race is also a factor in stroke prevalence, with a higher prevalence of stroke in black individuals than white, Hispanic or Asian [2].

43.1.2 Classification and Causes of Stroke

As previously mentioned, the two major subtypes of stroke are ischaemic or haemorrhagic, a classification based on their pathology. The Bamford (or Oxford) classification system, however, uses the clinical presentation to divide stroke into four types: total anterior circulation, partial anterior circulation, lacunar circulation and posterior circulation strokes [5]. It provides important prognostic information, and the relationship with outcome reflects the link between the clinical syndrome and stroke topography on brain imaging, which in turn is indicative of potential aetiological mechanisms.

Ischaemic stroke is typically caused by either cardiac thromboembolism or atherothrombotic arterial occlusion, which can in turn be due to large artery atherosclerotic disease or small-vessel arteriosclerotic disease. Clinicians may use different classification systems to aetiologically categorise ischaemic stroke. The TOAST classification system for ischaemic stroke incorporates clinical, radiological, cardiac and laboratory investigations and assigns the cause of ischaemic stroke to one of five aetiological categories: large artery atherosclerosis (LAA), small artery occlusion (SVD), cardioembolic (CE), other determined pathology or undetermined pathology. Using this classification, LAA, SVD and CE account for 13.4–16.7%, 15.9–22.6% and 18.6–29.1% of cases, respectively [6]. Alternatively, the Causative Classification System (CCS-TOAST) allows for a more rapid, computer-assisted classification of the TOAST system. The ASCOD phenotyping system assigns a degree of likelihood that the stroke was caused by five categories of disease: A, atherosclerosis; S, small-vessel disease; C, cardiac pathology; O, other causes; and D, dissection. This allows for appreciation of the overlap between different aetiologies and the importance of dissection as a cause of ischaemic stroke in younger people [7].

Haemorrhagic stroke is classified based on its location and is usually caused by hypertension, amyloid angiopathy or structural brain disease such as arteriovenous malformation [5]. Haemorrhagic stroke, however, is not a focus of this chapter, and the following sections will focus on ischaemic stroke.

43.1.3 Symptoms of Ischaemic Stroke

The most common symptoms of stroke are sudden speech disturbances, sudden arm or leg weakness or numbness (in particular on just one side of the body), sudden facial weakness, sudden visual disturbance and sudden loss of balance/coordination [5]. In an effort to increase public awareness of stroke and increase hospital admissions early after stroke onset, the Department of Health in England introduced the "Stroke—Act FAST (Face, Arms, Speech: Time to call Emergency Medical Services)" campaign in 2009 which has been successful in increasing public education on stroke [8] and has since been introduced in various other countries.

Various scales are used in the hospital to grade patients' severity of stroke symptoms. The most commonly used is the National Institute of Health Stroke Scale (NIHSS) which gives patients a score between 0 and 42 (the greater the score, the more severe the stroke) based on various symptoms such as consciousness, facial weakness, speech disturbance, eye movement and vision and limb weakness or ataxia [9]. Similarly, various scales are used to grade patients' level of disability in the weeks or months following a stroke, the most common of which is the modified Rankin Scale (mRS). This gives patients a score between 0 and 6, where 0 means no symptoms present and 6 means dead. Scales like these are useful for clinicians to provide prognostic information or to monitor patient recovery, but they are also of particular use in clinical stroke studies. For example, the NIHSS can be used for trial inclusion criteria or for subgroup analysis, while the mRS is a common outcome measure to measure patients' level of disability at 90 days [10].

43.1.4 Risk Factors and Current Treatments for Ischaemic Stroke

Ischaemic stroke has a number of risk factors such as hypertension, atrial fibrillation, lack of regular physical activity, poor diet, diabetes and smoking [11]. As with many diseases, prevention is preferable to a cure, and therefore management of these risk factors is the first line of stroke prevention. For example, management of hypertension can

result in a 41% reduction of stroke risk with just a 10 mmHg reduction in systolic blood pressure [2], while the introduction of smoking ban legislation in numerous countries worldwide has been associated with a reduced incidence of stroke [12].

Immediately following an ischaemic stroke, the current most commonly available treatment is intravenous alteplase which is a tissue plasminogen activator (tPA), a clot-busting drug. This dissolves the occluding blood clot with the aim to restore blood flow and reduce the period of ischaemia in order to salvage non-infarcted brain tissue with the concept that "time is brain". This drug has a very narrow effective treatment window and is licensed for use only up to 4.5 hours from the onset of symptoms resulting in low numbers of patients being eligible for treatment. Additionally, alteplase can increase the risk of haemorrhagic stroke [11]. Tenecteplase is a genetically modified tPA which is commonly used in the treatment of myocardial infarction and may have some favourable properties in comparison to alteplase. A procedure known as thrombectomy, where the blood clot is mechanically removed from the a large blood vessel within in the brain, has also shown great promise in recent trials, with improvements in revascularization at 24 hours (76% vs 34%, thrombectomy vs standard care) and of 90-day functional outcome compared to standard medical treatment [13]. More recently, this procedure has proven effective with an extended treatment window up to 24 hours (since the onset of stroke symptoms) [14], and as a result, the American Heart Association Guidelines for the management of acute ischaemic stroke were updated to recommend the use of mechanical thrombectomy in patients between 6 and 24 hours, provided they meet appropriate selection criteria [15].

43.2 Pathophysiology of Ischaemic Stroke

This section will describe the molecular processes that take place following ischaemic stroke, rather than the processes that cause arterial occlusion, and is summarised in Fig. 43.1. For reviews providing further detail on the pathophysiology, see [16–19]. Although stroke refers to a collection of heterogeneous diseases, at the molecular level there is considerable consistency with regards to the cell signalling pathways involved.

43.2.1 Infarct Core and Penumbra

After the onset of ischaemic stroke, a core infarct (area of dead tissue due to lack of blood supply) develops locally to the obstructed vessel. While the blood supply in the surrounding brain tissue is significantly reduced, cell metabolism is maintained by collateral flow from other blood vessels within the brain. This region of the brain, known as the ischaemic penumbra, is potentially still viable and salvageable after a stroke [20]. Its viability, however, depends on the severity and duration of ischaemia, and, during the time lag to reperfusion, a physiological cascade occurs which places this penumbral tissue at further risk. If the time lag is too long, the penumbra may eventually be subsumed into the infarct core resulting in a larger infarct and thus more severe neurological symptoms [21].

43.2.2 Ischaemic Injury

The brain receives around 20% of the body's total oxygen despite accounting for only approximately 2% of body weight [22]. This high metabolic demand consequently makes the brain very susceptible to ischaemic injury.

43.2.3 Energy Failure

In the acute phase of ischaemic injury, the lack of oxygen and glucose results in a depletion of cellular stores of adenosine triphosphate (ATP) – the energy currency of the cell which is required for many key cellular processes. As a result of the lack of oxygen, cells switch to anaerobic respiration resulting in the production of lactate. This leads to an accumulation of lactic acid called metabolic acidosis and results in cell death through necrosis [17].

Mitochondria are responsible for aerobic respiration in cells, that is, they catalyse the conversion of adenosine diphosphate (ADP) to ATP using oxidative phosphorylation (OXPHOS). Pyruvate is produced through glycolysis of glucose in the cytosol but is then oxidised within the mitochondria generating NADH and $FADH_2$, which are then oxidised by the electron transport chain (ETC) in the mitochondrial membrane. OXPHOS produces a proton gradient across the mitochondrial membrane which then powers

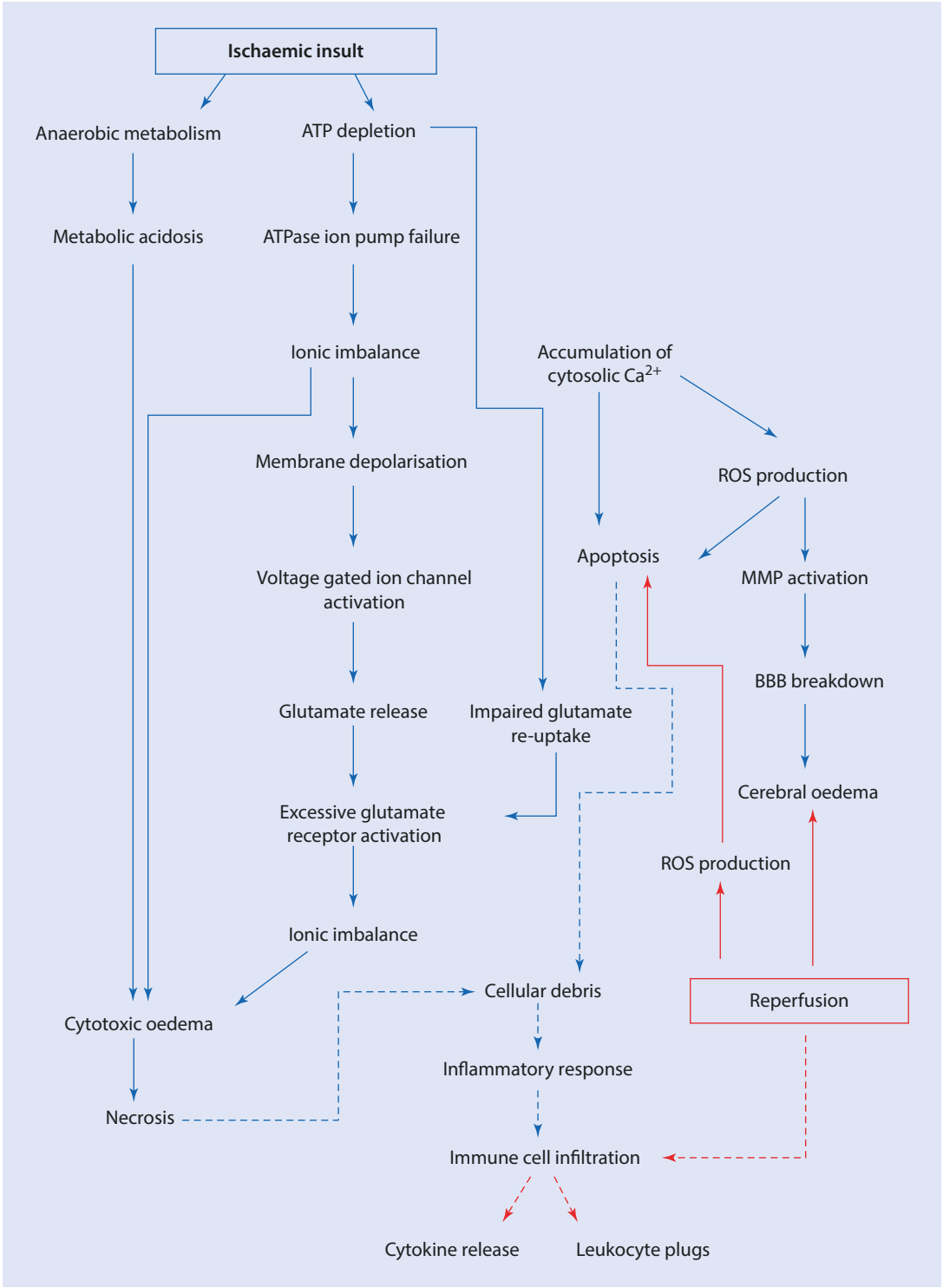

Fig. 43.1 The pathophysiology of ischaemic stroke. Schematic demonstrating the causes of cell death through necrosis or apoptosis resulting from ischaemia (blue arrows) and as a result of recanalization and reperfusion (red arrows); *ATP* adenosine triphosphate, *ROS* reactive oxygen species, *MMP* matrix metalloproteinases, *BBB* blood-brain barrier

ATP synthase. This proton gradient, however, is also important for the maintenance of low intracellular calcium ions (Ca^{2+}) by aiding Ca^{2+} uptake into the mitochondria, through the Ca^{2+} uniporter on the mitochondrial membrane. Therefore, reduction of respiration causes a rise in intracellular Ca^{2+} levels, and cells will utilise any remaining ATP stores to try to maintain Ca^{2+} homeostasis, resulting in more rapid ATP depletion.

43.2.4 Ionic Imbalance and Calcium Dysregulation

The depletion of ATP also affects the function of ATP-driven membrane ion pumps (ATPases), namely, sodium-potassium (Na^+-K^+) ATPase, Ca^{2+} ATPase and synaptic proton (H^+) ATPase. Low intracellular calcium levels are tightly controlled by Na^+-Ca^{2+} exchangers, on the mitochondrial and cell membrane, and Ca^{2+} ATPases, on the endoplasmic reticulum (ER), mitochondrial and cell membrane, in order to maintain a 10,000-fold gradient across the cell membrane. During ischaemia, failure of Na^+-K^+ ATPase results in K^+ efflux and Na^+ influx, which in turn causes reversal of the Na^+-Ca^{2+} exchanger. Coupled with the failure of Ca^{2+} ATPases, this leads to Ca^{2+} accumulation in the cytosol [17].

43.2.5 Excitotoxicity

Disruption in cellular ionic homeostasis causes the loss of membrane potential and membrane depolarisation. This in turn causes the activation of voltage-gated ion channels resulting in glutamate efflux. In normal neuronal electrical signalling, glutamate, a potent neurotransmitter, is released into the synapse and activates a family of receptors on the postsynaptic membrane (of the adjacent neuron): the NMDA, AMPA, kainite receptors or mGluRs. Activation of these receptors results in influx of Ca^{2+} and Na^+ and subsequent loss of K^+ which causes depolarisation and glutamate release so that this signal propagates from neuron to neuron. In the ischaemic cascade, ionic imbalance results in excess glutamate release, and the normal reuptake mechanisms for glutamate are impaired due to the energy failure state of the cell, and so this results in an extracellular accumulation of glutamate. This causes overactivation of the glutamate receptors and excess depolarisation in surrounding neurons, in an insult known as excitotoxicity. Severe excitotoxicity and ATPase failure ultimately lead to necrotic cell death due to cell ionic imbalance causing a passive influx of water into the cells known as cytotoxic oedema [17].

43.2.6 Apoptosis Initiation

Accumulation of intracellular Ca^{2+} also activates proteases, lipases and nucleases leading to degradation of key cellular proteins, the plasma membrane and nucleic acids. This causes irreversible cellular damage leading to the initiation of apoptosis (programmed cell death). Furthermore, calcium activates Ca^{2+}-dependent enzymes (calpains) which bind with pro-apoptotic proteins at the mitochondrial membrane. This results in the opening of mitochondrial transition pores (MTP) and the subsequent release of cytochrome C, from the mitochondria, leading to activation of caspase enzymes which are integral in the execution of apoptosis [23]. Opening of the MTP also further depletes cellular ATP by disrupting the mitochondrial membrane potential and therefore preventing the action of ATP synthase.

43.2.7 Reactive Oxygen Species (ROS)

Calcium dysregulation has further implications, in the ischaemic cascade, with the production of reactive oxygen species (ROS) (Fig. 43.2) [24]. As previously described, the ETC is responsible for the majority of cellular ATP; however, the ETC is also a major source of basal levels of cellular ROS: superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Increased mitochondrial Ca^{2+} promotes oxidation via the ETC and thus results in a greater production of ROS. Additionally, however, high mitochondrial levels of Ca^{2+} can inhibit enzymes of the ETC, for example, by causing disassociation of cytochrome C from the mitochondrial membrane and thus inhibiting complex III or by inhibiting complex I in combination with nitric oxide (NO). Ca^{2+} also activates additional enzymes contributing to ROS production, namely NADPH oxidases (NOX2, NOX3 and NOX4) and neuronal nitric oxide synthase

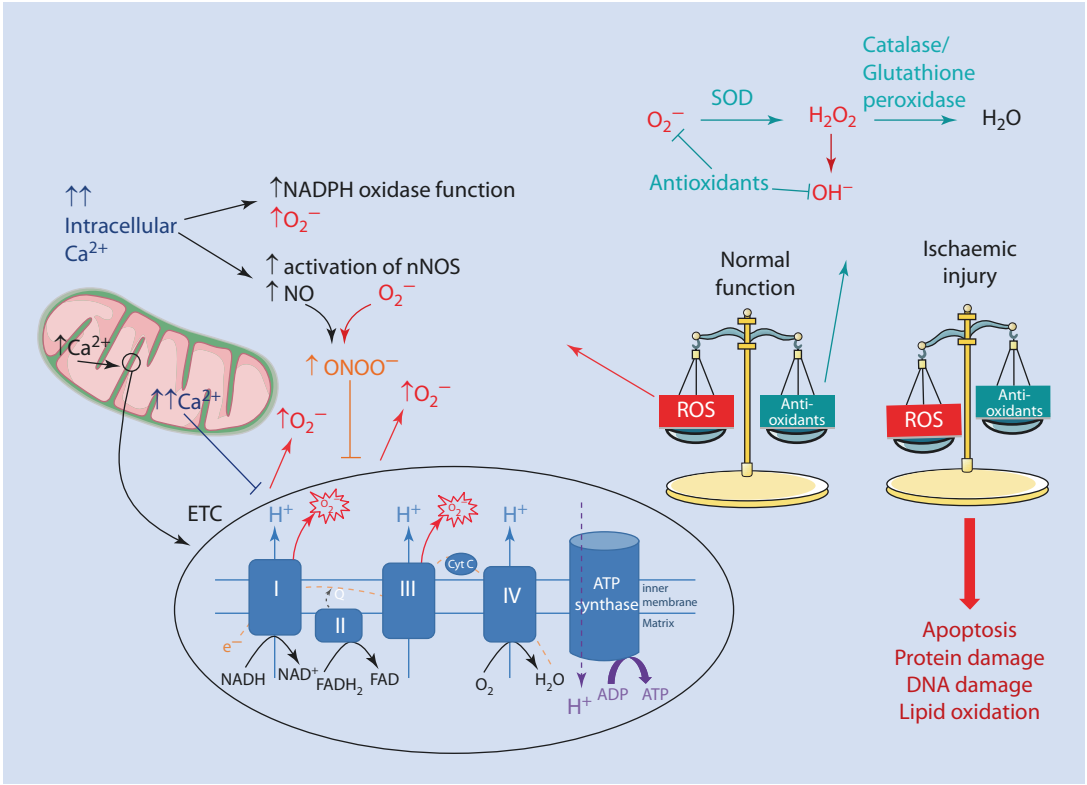

Fig. 43.2 ROS production in ischaemic stroke. Schematic representation of the sources of ROS resulting from increased calcium levels in ischaemic injury through NADPH oxidase activation or ETC stimulation/inhibition. Antioxidant strategies (top right, teal) exist within cells and manage basal levels of ROS under normal conditions. Ischaemic injury disrupts this balance leading to increased levels of ROS resulting in cellular damage and death. *ETC* electron transport chain, $O_{2\_}$ superoxide ion, *OH_* hydroxyl radical, *ONOO_* peroxynitrite, *nNOS* neuronal nitric oxide synthase, *SOD* superoxide dismutase, *ROS* reactive oxygen species

(nNOS). nNOS activation causes an increased production of NO, and although some studies have suggested a neuroprotective role of NO in the brain, through inhibition of NMDA receptors or increased vasodilation and therefore increased neuronal blood supply, this molecule is also considered neurotoxic. This is because, at high concentrations, NO combines with superoxide to form the highly reactive oxygen radical, peroxynitrite ($ONOO^-$). Cells have endogenous antioxidant enzymes and compounds, such as superoxide dismutase (SOD), catalase or α-tocopherol, to deal with excess ROS. If these antioxidant mechanisms are overloaded by too much ROS production, this leads to oxidative stress where ROS can damage proteins, lipids and nucleic acids leading to cellular damage which can result in the initiation of apoptosis. The brain is particularly vulnerable to oxidative stress because of its high respiration rate, its antioxidant defences are not high enough to deal with excess levels of ROS and also because of high levels of lipids, which are very vulnerable to ROS damage, and iron, which promotes free-radical reactions/damage [18].

43.2.8 Reperfusion Injury

Although ischaemia results in cell damage and death, recanalization of cerebral vasculature also causes further damage known as reperfusion injury. ROS generation, due to a combination of the reintroduction of oxygen and dysfunction of the ETC due to ischaemic damage, plays a key role in this injury. Moreover, an ischaemic accumulation of succinate within the mitochondria has been shown to drive reverse election transport (RET) which generates large amounts of ROS [25]. The damaging effects of ROS and oxidative stress

within the brain have already been discussed; however, coupling this with the physical restoration of blood flow and the brain is left open to further damage with inflammation and an immune response (discussed below). Matrix metalloproteinases (MMPs) are activated by ROS, pro-inflammatory cytokines and also by tPA (the clot-busting drug used for reperfusion). MMPs play a part in tissue remodelling (discussed later), but they are also involved in degradation of the blood-brain barrier (BBB). The BBB protects the brain by limiting the compounds which can pass from the bloodstream into the brain, and it is compromised following ischaemic injury due to MMP activity and damage to endothelial cells causing them to detach from the basal membrane. As a result, the BBB becomes more permeable, and increased BBB permeability causes cerebral oedema by allowing excess water, from the blood, into the brain. It can also increase the risk of intracerebral haemorrhage.

43.2.9 Inflammation and Immune Response

In the later stages of ischaemic injury and following reperfusion, an inflammatory response causes additional damage to the already compromised penumbra. This inflammatory response is considered sterile as it is not caused by infiltrating microorganisms.

Increased levels of intracellular Ca^{2+} and the cell stress response initiate activation of inflammatory transcription factors such as nuclear factor-kappaB (NF-κB) or mitogen-activated protein kinases (MAPK) signalling pathways. In addition, necrotic and apoptotic cell debris such as protein or nucleic acids, known as danger/damage-associated molecular pattern molecules (DAMPs), bind to and activate toll-like receptors (TLRs) on microglia, macrophages and endothelial cells inducing the release of pro-inflammatory signalling molecules. This inflammatory response induced during ischaemia results in the infiltration of leukocytes such as lymphocytes, neutrophils and monocytes, coupled with increased expression of endothelial adhesion molecules (such as p-selectin, ICAM-1 or VCAM-1). Increased BBB permeability following ischaemia assists this by allowing infiltrating immune cells' easier access to the brain parenchyma. The infiltrating leukocytes then accumulate within ischaemic tissue and adhere to endothelial cells, further exacerbating damage by releasing pro-inflammatory (e.g. IL-1β, TNFα) or cytotoxic (e.g. NO, ROS) molecules. Endothelial or platelet-derived p-selectin can also cause leukocytes to adhere to each other forming "leukocyte plugs" causing further obstruction within the blood vessel, worsening damage [19].

Although infiltrating neutrophils and monocytes play a role in the phagocytosis of damaged neurons or other cells, microglia are the resident macrophages of the brain. These cells are normally present in the brain in a resting or ramified state but are activated in response to insult or injury. Activated microglia contribute to the inflammatory response by releasing pro-inflammatory molecules; however, they also contribute to repair mechanisms by clearing dead cells and releasing anti-inflammatory cytokines [26].

43.2.10 Repair Mechanisms

Due to the damage induced by ischaemia and reperfusion injury, the brain responds with endogenous repair mechanisms in an effort to retain and restore function (Fig. 43.3).

As previously mentioned, collateral flow maintains the ischaemic penumbra to some extent during ischaemia; however, neovascularisation takes place following ischaemia to help improve blood flow compromised by ischaemic damage. This is via angiogenesis (the formation of new capillaries from existing blood vessels), vasculogenesis (the formation of new blood vessels) and arteriogenesis (the growth of new collateral arteries from existing arterioles) primarily driven by the upregulation of vascular endothelial growth factor (VEGF), induced by hypoxia and aided by production of NO, which dilates blood vessels improving flow and aids in the induction of vessel remodelling, for example, by increasing MMP9 activity. Protease enzymes such as MMPs are upregulated to aid in this remodelling process, whereby the MMP degrades the extracellular matrix proteins making space for new blood vessel growth.

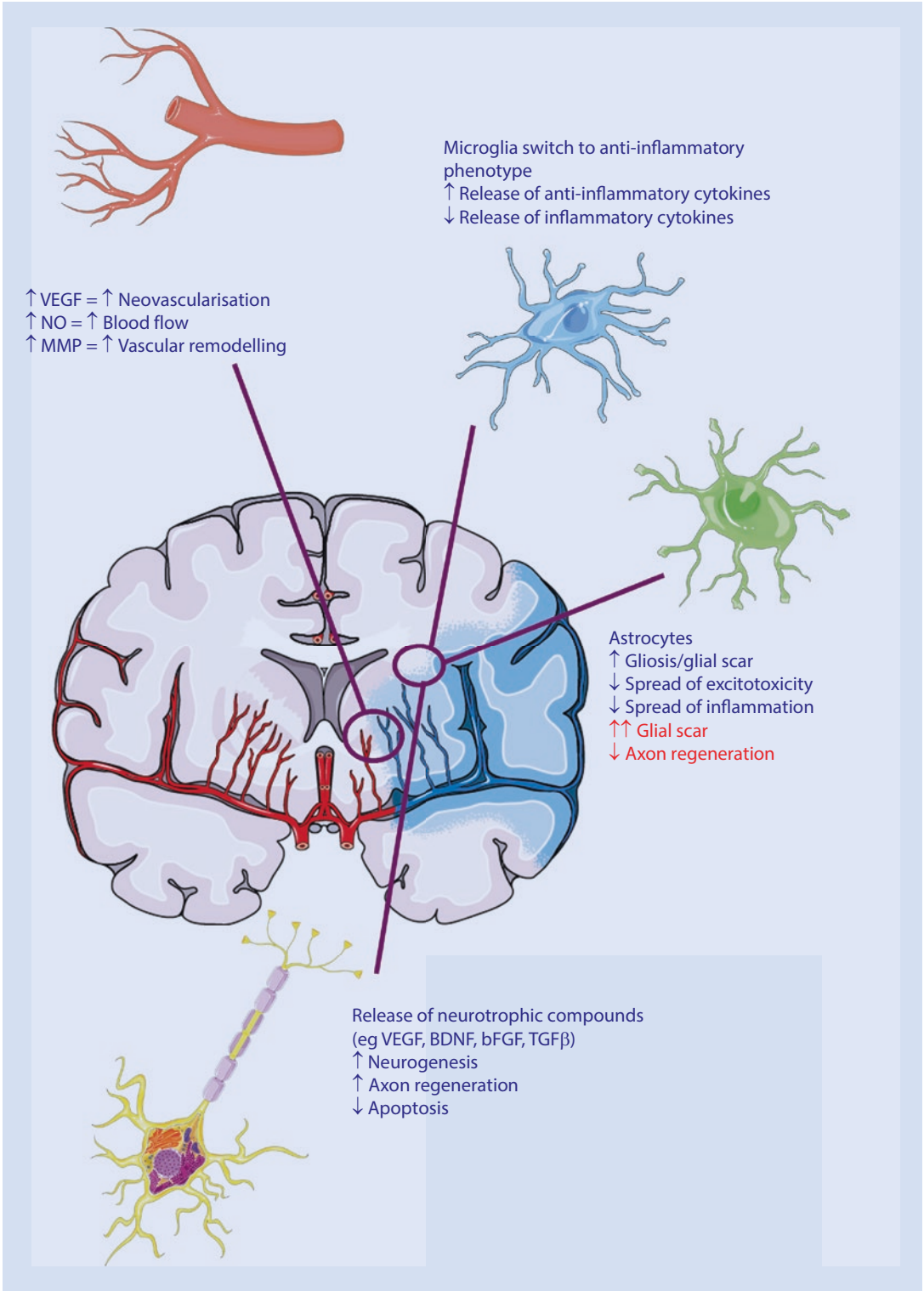

Fig. 43.3 Repair mechanisms in ischaemic stroke. Diagram summarising vascular, neuronal, microglial and astrocytic specific repair mechanisms following ischaemic injury

VEGF has also been shown to act upon neurons and promote neuronal repair. Production of this and other neurotrophic compounds, such as brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF), is upregulated following cerebral ischaemia to promote axon regeneration and neurogenesis. The subventricular zone (SVZ) of the brain contains neural stem cells which can proliferate and differentiate into new neurons (neurogenesis). This is stimulated by transforming growth factor β (TGFβ) superfamily signalling and activation of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Neurotrophic compounds also promote repair and reduce injury following ischaemia by supressing apoptosis [27].

Ischaemic injury also stimulates astrocytes to initiate gliosis and lay down a glial scar. This creates a physical barrier and "cordons off" the damaged tissue to reduce its spread, for example, preventing the spread of inflammation or excitotoxicity. The glial scar also, however, prevents axon regeneration, and so if the scar is too large, it can become detrimental to brain repair.

Anti-inflammatory cytokines, such as interleukin-10 (IL-10) or TGF-β, are also upregulated, particularly in microglia, in response to ischaemic injury. IL-10 acts by inhibiting the actions of pro-inflammatory cytokines, while TGF-β induces the anti-inflammatory phenotype of microglia by preventing their release of pro-inflammatory molecules.

43.2.11 Pathophysiology of Haemorrhagic Stroke

Although not the focus of this chapter, an overview of stroke would not be complete without a comment on the pathophysiology of haemorrhagic stroke. Being caused by the rupture of a blood vessel rather than occlusion, haemorrhagic stroke does not suffer the ischaemic pathophysiology described above. It does, however, result in excitotoxicity, cytotoxicity, oxidative stress and inflammation. This is because the components of blood are directly exposed to the brain resulting in an inflammatory response and ROS production. In addition, the breakdown of red blood cells releases haemoglobin which is cytotoxic [28].

43.2.12 The Quest for Neuroprotective Therapies

As previously mentioned, the current treatments for ischaemic stroke are focussed on clot removal either pharmacologically, with the clot buster tPA, or mechanically, using the thrombectomy procedure.

Although reducing the period of ischaemia can improve patient prognosis, currently there are no neuroprotective drugs or strategies in use to minimise damage due to ischaemia or reperfusion. Over the years, many different aspects of ischaemia-reperfusion injury pathophysiology have been targeted as potential treatments for stroke but with little success in clinical trials. For example, excitotoxicity was targeted with NMDA receptor antagonists such as selfotel or dizocilpine [29]; calcium overload, which is integral to ischaemia-reperfusion-induced cell death, was targeted with calcium channel blockers (CCBs) such as nimidopine or verapamil [30]; immune response was targeted with anti-inflammatories such as minocycline or drugs to prevent leukocyte adhesion such as enlimomab [31]; and excess ROS production was targeted with ROS scavengers such as tirilazad mesylate or NXY-059 (also known as Cerovive) [18]. Similarly, other treatments targeting multiple pathways of ischaemic injury have been assessed, such as physical or pharmacological hypothermia. Hypothermia has been shown to have a neuroprotective effect through reducing cellular metabolic demand, preventing apoptosis, reducing ROS production and reducing inflammation, but so far, the beneficial effects seen in preclinical stroke models have not yet consistently translated clinically, and further work is required [32].

There have been various animal models of stroke, mostly rats or mice, developed in order to study stroke pathophysiology and search for neuroprotective strategies. These models involve the induction of focal cerebral ischaemia and may be permanent, to study the mechanism of and treat the ischaemic injury, or transient, to study and target reperfusion injury. Some examples of the most common models are the intraluminal filament model of middle cerebral artery occlusion (MCAO), where a filament is inserted into the internal carotid artery (ICA) at the neck and advanced into the circle of Willis to occlude the origin of the middle cerebral artery (MCA) and

either left in place (permanent, pMCAO) or removed after a period of time (transient, tMCAO); the electrocoagulation model of pMCAO, where electrocoagulation forceps are used to permanently occlude the MCA via a craniectomy; the embolic model, where a blood clot is formed outside the body and injected via a catheter into the ICA to occlude the origin of the MCA which can then be dissolved with tPA; and the endothelin-1 (ET-1) model, where ET-1 is injected via stereotactic injection into the tissue surrounding the MCA; ET-1 is a potent vasoconstrictor and causes temporary occlusion of the MCA which then gradually relaxes allowing for reperfusion [20].

The failure of many neuroprotective strategies to translate clinically is thought to be due to the poor quality of preclinical trials. For this reason, the stroke community came together in 1999 to produce the stroke therapy academic industry roundtable (STAIR) recommendations, for advancing preclinical research, which have since been updated in the 2009 STAIR report and the newer RIGOR [33] and IMPROVE [34] guidelines. These guidelines focus on improving the quality and validity of preclinical studies with emphasis on the use of randomisation and investigator blinding to minimise bias, the use of power calculations to define group sizes, the use of functional outcome measures rather than infarct volume alone, the use of comorbidity rather than healthy animal models and the replication of trials in different animal models and/or laboratories to demonstrate reproducibility and robustness [33]. These guidelines aim to improve the quality of preclinical studies so that drugs or therapies are more likely to succeed in clinical trials where the population is more heterogeneous and displays multi-morbidities. The stroke research community is committed to resolving the issues relating to translating preclinical research to successful clinical treatments for stroke. This is evidenced by the continuously improving guidelines for stroke research but also by the creation of the MULTIPART (Multicentre Animal Research Team) network (► http://www.dcn.ed.ac.uk/multipart/default.htm). This international network was established with a vision to perform large multicentre preclinical trials, similar to phase III clinical human trials, to combat the translational stroke research crisis.

Other potential confounders limiting efficacy in clinical trials are unsuccessful recanalization with tPA and the variety of stroke subtypes included in trials. The recent successes of numerous mechanical thrombectomy trials, in improving patient outcome following stroke and extending the length of the stroke treatment window, mark the beginning of an exciting new era of stroke research. This is because the imaging techniques, used prior to thrombectomy, allow for reduction in patient heterogeneity, by the inclusion of only specific types of vessel occlusion in clinical trials, and allow treatments to be given when salvageable brain tissue is apparent. Meanwhile, the procedure itself improves recanalization success and also offers a local, intraarterial treatment opportunity which may be considered too invasive otherwise [35].

Conclusions and Clinical Perspectives

- Stroke remains a substantial cardiovascular and neurological problem with complex aetiology and pathology.
- Recent research advances have provided a new treatment option for some patients, with the introduction of mechanical thrombectomy procedures which can be utilised as late as 24 hours after stroke onset.
- Although no neuroprotective therapies have yet been successful, advances in brain imaging techniques may provide better opportunities for successful clinical trials by reducing patient heterogeneity, while improvements in the quality of preclinical research may result in more promising drug targets.

Gaps in Knowledge

- Further research is required to better understand the failures of previous neuroprotective trials to produce successful neuroprotective therapies.
- Although the basic pathophysiology of ischaemia-reperfusion injury in stroke is well understood, the roles and interactions of specific cell types in the neurovascular unit are less well understood and may provide insight for the direct targeting of drugs.
- The use of large multicentre preclinical trials in comorbid or multi-morbid animal models may help to improve the success of translation of stroke therapeutics.

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