Chapter 20
Oxidative Stress in White Matter Injury

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Abbreviations

4-HNE	4-Hydroxynonenal
APP	Amyloid precursor protein
eNOS	Endothelium nitric oxide synthase
H_2O_2	Hydrogen peroxide
iNOS	Inducible nitric oxide synthase
MBP	Myelin basic protein
MCA	Middle cerebral artery
MDA	Malondialdehyde
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide

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NOS	Nitric oxide synthase
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
$O_2^{\bullet-}$	Superoxide anions
$^{\bullet}OH$	Hydroxyl radicals
$ONOO^-$	Peroxynitrite
PWMI	Periventricular white matter injury
RIP	Receptor-interacting protein
ROS	Reactive oxygen species
SMI-32	An antibody against a non-phosphorylated neurofilament epitope
SOD	Superoxide dismutase
SOD1	Copper/zinc superoxide dismutase
SOD2	Manganese superoxide dismutase

20.1 Introduction

White matter is the region of the brain underlying gray matter and is primarily composed of axonal bundles ensheathed with myelin. The cells forming these sheaths are oligodendrocytes, which tend to be arranged in rows parallel to axonal tracts. White matter comprises over half the human brain, a far greater proportion than in other animals (Fields 2008). Because white matter is at risk for ischemic injury throughout life, from periventricular white matter injury (PWMI) in neonates to stroke and vascular dementia in later life, this injury is of great clinical interest. The failure to ameliorate ischemic white matter injury is a major factor in the failure to translate preclinical studies into therapy (Dewar et al. 1999). Accordingly, ischemic white matter injury has been the focus of much attention in recent years.

White matter is extremely vulnerable to ischemic stress. First, blood flow in white matter is lower than in gray matter and there is little collateral blood supply in deep white matter (Xing et al. 2012). Second, once injured by ischemic stress, the structural integrity of axons and the myelin sheath is weakened and demyelinated axons become more susceptible to ischemic injury due to their heightened metabolic requirements caused by loss of energy-efficient saltatory conduction and leaky sodium channels (Lo et al. 2003; Trapp and Stys 2009). Third, axons, oligodendrocytes, and their progenitors are highly sensitive to oxidative stress (Dewar et al. 2003).

Oxidative stress is a condition under which reactive oxygen species (ROS) generation is enhanced and ROS metabolism is impaired. Axons are exceedingly vulnerable to oxidative stress, since axons contain abundant mitochondria, organelles that are a main source of ROS (Xing et al. 2012). In addition, the myelin sheath contains numerous lipids, which can be peroxidized after oxidative stress (Ueno et al. 2009). Oligodendrocytes are also vulnerable to oxidative stress. One of the reasons for this vulnerability is that they generate ROS for production and maintenance of myelin. Oligodendrocytes have been estimated to have the highest metabolic rate of any cell in the brain for production and maintenance of the myelin sheath (Connor and Menzies 1996). Because myelin production is energy dependent,

large amounts of adenosine triphosphate are consumed in the process and toxic oxidants are produced as byproducts of adenosine triphosphate synthesis (McTigue and Tripathi 2008). Another reason for the vulnerability is that many metabolic and myelin synthetic enzymes require iron as a cofactor (Connor and Menzies 1996). Accordingly, oligodendrocytes and oligodendrocyte progenitors have the largest intracellular stores of iron in the brain (20-fold greater than astrocytes under baseline culture conditions) (Thorburne and Juurlink 1996; Cheepsunthorn et al. 1998). Iron is necessary for myelin production, while it is also highly reactive and can evoke free radical formation and lipid peroxidation. In addition, low concentrations of glutathione (a robust antioxidative enzyme) in oligodendrocytes contribute to the vulnerability to oxidative stress (Thorburne and Juurlink 1996). Oxidative stress in white matter injury is deeply involved in a number of pathological conditions, including trauma, stroke, and multiple sclerosis.

20.2 Generation of Oxygen Free Radicals

Free radicals are molecular species that contain one or more unpaired valence electrons not contributing to intramolecular bonding. Free radicals are highly interactive with other molecules, such as DNA and lipids, pairing with their single electrons and causing oxidation of those molecules (Dröge 2002). Several oxygen free radicals (oxidants) and their derivatives are generated after ischemia, including superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). $O_2^{\bullet -}$ are formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron. A major source of $O_2^{\bullet -}$ is mitochondria where approximately 2–5 % of the molecular oxygen consumed during normal physiological respiration is converted into $O_2^{\bullet -}$ and H_2O_2 (Boveris and Chance 1973). Pro-oxidant enzymes, such as cyclooxygenase, xanthine dehydrogenase, xanthine oxidase, myeloperoxidase, monoamine oxidase, and nicotinamide adenine dinucleotide phosphate oxidase (NOX), also catalyze the generation of $O_2^{\bullet -}$. Among them, much attention has recently been focused on the role of NOX in cerebral ischemia. NOX is a multi-subunit enzyme that transfers an electron from nicotinamide adenine dinucleotide phosphate to molecular oxygen to generate $O_2^{\bullet -}$. Originally discovered in phagocytic cells, expression and distribution of NOX have subsequently been found in many other cell types, such as neurons, astrocytes, and microglia, in the cortex, hippocampus, and cerebellum (Kim et al. 2005; Tejada-Simon et al. 2005; Infanger et al. 2006; Bedard and Krause 2007). It is becoming clear that overactivation of NOX plays a role in many neurodegenerative diseases, such as stroke and Alzheimer's disease (Walder et al. 1997; Bedard and Krause 2007; Chen et al. 2009; Kim et al. 2009; Yoshioka et al. 2011a). NOX may also play an important role in the pathogenesis of white matter injury.

Peroxynitrite ($ONOO^-$) generated by inducible nitric oxide synthase (iNOS) and NOX in activated microglia injures oligodendrocytes (Li et al. 2005). $O_2^{\bullet -}$ can react with nitric oxide (NO) to produce $ONOO^-$ ($NO + O_2^{\bullet -} \rightarrow ONOO^-$), which is a strong oxidative radical that causes protein nitration and dysfunction (Beckman et al. 1990).

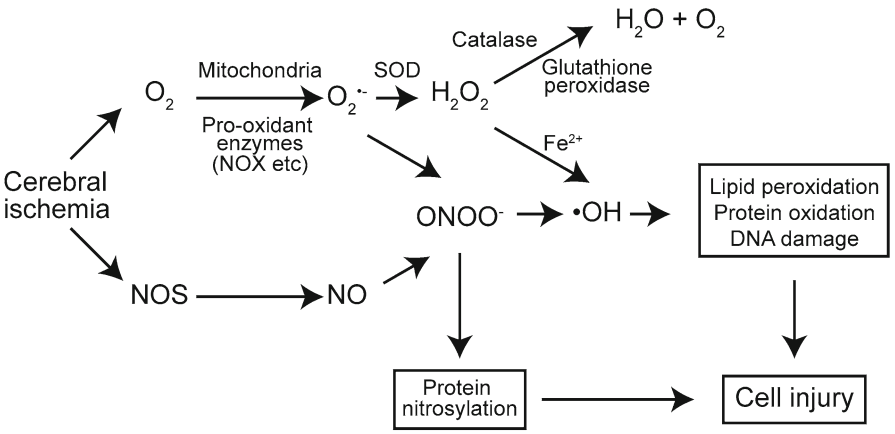

Fig. 20.1 Oxidants and NO generated after ischemia induce lipid peroxidation, protein oxidation, DNA damage, and protein nitrosylation, all of which cause cell injury

NO is produced by nitric oxide synthase (NOS) using arginine and O_2 as substrates. Three isoforms of NOS exist in central nervous system parenchyma: neuronal nitric oxide synthase (nNOS), a constitutive isoform that is localized in neurons; iNOS, an isoform that is induced in microglia/macrophages and astrocytes and endothelial cells; and eNOS, a constitutive form that is localized in the endothelium. nNOS and eNOS activity is Ca^{2+}-dependent, whereas iNOS is Ca^{2+}-independent. NO produced by nNOS and iNOS has been implicated in both in vitro cell culture injury and in vivo ischemic brain damage. NO produced by eNOS is known to be neuroprotective because of its vasodilative effects (Chan 2001).

Superoxide dismutases (SODs) are specific enzymes and have three isoforms: copper/zinc SOD (SOD1), manganese SOD (SOD2), and extracellular SOD. All three SOD isoforms detoxify $O_2^{\bullet-}$ to H_2O_2 ($O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2$), which is further converted to H_2O by catalase or glutathione peroxidase ($2H_2O_2 \rightarrow 2H_2O + O_2$) (Chan 2001). $^{\bullet}OH$ are extremely reactive oxidants produced by H_2O_2 through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow OH^- + Fe^{3+} + {}^{\bullet}OH$) and the Haber-Weiss reaction ($O_2^{\bullet-} + H_2O_2 \rightarrow {}^{\bullet}OH + HO^- + O_2$) or by $ONOO^-$ (Beckman et al. 1990; Chan 1996). Experimental studies using transgenic or knockout animals have shown that both SOD1 and SOD2 are neuroprotective against ischemic injury (Kinouchi et al. 1991; Chan 2001; Niizuma et al. 2010; Chen et al. 2011). In addition to antioxidant enzymes, other antioxidants, including glutathione, ascorbic acid, and vitamin E, are also involved in the detoxification of oxidants (Chan 1996, 2001). A constitutively low concentration of oxidants is necessary; they act as signaling molecules for various functions, such as regulation of vascular tone, monitoring of oxygen tension, and erythropoietin production (Dröge 2002). However, excessive oxidants may irreversibly oxidize macromolecules such as DNA, lipids, and protein, and cause severe cell injury. Figure 20.1 summarizes the generation of oxidants in vivo that leads to brain cell damage under ischemic conditions.

20.3 Oxidative Stress in Ischemic White Matter Injury

Three pathologic conditions are deeply connected to ischemic oxidative injury of white matter, namely, PWMI, acute cerebral ischemia, and chronic cerebral hypoperfusion.

20.3.1 Oxidative Stress in Ischemic Injury of Oligodendrocyte Progenitors

Oligodendroglial lineages are vulnerable to ischemic conditions. Among them, oligodendrocyte progenitors are much more vulnerable to ischemic insults than mature oligodendrocytes (Husain and Juurlink 1995; Fern and Möller 2000). PWMI is related to the ischemic vulnerability of oligodendrocyte progenitors and is the major pathological finding underlying cerebral palsy, subsequently observed in survivors of premature birth. Cerebral palsy is a motor disorder affecting 10 % of very low-birth-weight (<1,500 g) premature infants who survive the intensive care nursery. The pathogenesis of PWMI is multifactorial and likely involves damage related to ischemic injury in the critically ill premature infant with impaired regulation of cerebral blood flow, as well as inflammation-induced brain injury associated with maternal and/or fetal infection (Haynes et al. 2005).

PWMI includes a spectrum of cerebral injuries that ranges from focal cystic necrotic lesions (periventricular leukomalacia) to extensive white matter lesions (diffuse PWMI). Periventricular leukomalacia commonly occurs in the subventricular zone adjacent to the lateral ventricle and involves injury to all cellular elements. In contrast, diffuse PWMI is characterized by deep cerebral white matter that contains extensive regions of numerous reactive astrocytes (diffuse gliosis) and diffuse myelination disturbances. Although diffuse gliosis is believed to arise in response to extensive white matter damage, the types of injured cells that provoke this gliosis remain unknown, but are hypothesized to be oligodendrocyte progenitors because such lesions occur in a similar distribution in regions with myelination disturbance (Back and Rivkees 2004).

Oligodendrocytes develop a well-established lineage, precisely defined by antibodies that are stage-specific for sequentially expressed oligodendrocyte cell-surface and myelin-specific epitope (Back and Rivkees 2004). Since the major period of vulnerability for PWMI (23–32 weeks gestation) occurs before the onset of myelination, Back and Volpe (1997) first proposed that the myelination disturbances in PWMI might arise from targeted death of oligodendrocyte progenitors. Several lines of evidence support a role for targeted death of oligodendrocyte progenitors in the pathogenesis of PWMI, derived from in vitro and in vivo experimental models (Back and Rivkees 2004).

Ischemic oxidative stress is thought to be closely connected to the targeted death of oligodendrocyte progenitors. In human brain samples, immunohistochemical studies showed evidence of oxidative and nitrative stress in PWMI. Expression of the markers of lipid peroxidation (4-hydroxynonenal [4-HNE] and malondialdehyde

[MDA]) and nitrative stress (nitrotyrosine) increases in premyelinating oligodendrocytes within PWMI. These cells are also positive for staining with terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end labeling, which indicates the vulnerability of premyelinating oligodendrocytes. Oxidative and nitrative stress markers are also expressed in reactive astrocytes within the lesion. However, astrocytes are expected to protect surrounding oligodendrocytes because they do not undergo cell death (Haynes et al. 2003).

The release of ROS is related to ischemia that leads to a decrease in the cellular antioxidant glutathione, failure of membrane channels that regulate ionic and osmotic homeostasis of the cells, and excessive release of glutamate from injured axons, which results in a subsequent release of ROS. Ischemia also induces the upregulation of iNOS in reactive astrocytes (Haynes et al. 2003). In addition to ischemia, inflammatory response associated with infection is another source of ROS. Upregulation of cytokines, including tumor necrosis factor-α, interleukin-2, interleukin-6, and interferon-γ (Deguchi et al. 1996; Yoon et al. 1997; Kadhim et al. 2001, 2002; Folkerth et al. 2004b), activates astrocytes and microglia in the surrounding lesions, which releases ROS and reactive nitrogen species (Haynes et al. 2003). Recent evidence has revealed that the vulnerability of oligodendrocyte progenitors to oxidative stress results, in part, from developmental mismatch in antioxidant capacity in white matter of the human fetus. During the period of greatest risk for PWMI, expression of the peroxide-generating enzymes SOD1 and SOD2 significantly lags behind that of peroxide-degrading catalase and glutathione peroxidase, which alters a crucial balance in oxidant metabolism (Folkerth et al. 2004a).

Several experimental models have been developed to study the mechanism of PWMI in rodents, rabbits, sheep, and nonhuman primates. These models include ischemia, injection of endotoxin, administration of excitotoxic agents, and chronic sublethal hypoxia. Although rodent acute hypoxia–ischemia models (usually achieved through bilateral or unilateral carotid artery occlusion followed by exposure to hypoxia) generate extensive cortical and subcortical neuronal death and do not reproduce the many distinct physiological features unique to the premature human infant, these models have been widely used to study the cellular and molecular mechanisms of the injury. At postnatal day 2 (P2), white matter of rodents contains predominantly oligodendrocyte progenitors as in premature infants (Craig et al. 2003). The vulnerable period for white matter injury in rodents is around P2, then declines thereafter, coincident with the onset of oligodendrocyte differentiation and myelination between P7 and P14. In rodents, oligodendrocyte progenitors are also more vulnerable to ischemic stress than mature oligodendrocytes (Back et al. 2002), and developmental mismatch in the antioxidant capacity in rat oligodendrocyte progenitors is also reported in human fetuses. Expression and activity of the antioxidant SOD2 in oligodendrocyte progenitors are lower than in mature oligodendrocytes (Baud et al. 2004). Poor capability of oligodendrocyte progenitors to treat free iron is another mechanism of this vulnerability. Normal development of the brain requires iron as a cofactor for many enzymes; however, free iron can enhance oxidative damage by the Fenton reaction (Halliwell 1989). Because oligodendroglial lineage, especially oligodendroglia progenitors, has high iron stores but

low glutathione, peroxide remains at dangerously high levels if iron is released from iron stores (Thorburne and Juurlink 1996; Juurlink et al. 1998). In addition, the delayed appearance of ferritin in oligodendrocytes contributes to the vulnerability of oligodendrocyte progenitors. Ferritin not only makes iron available within cells, but also provides protection from iron-induced oxidative damage (Harrison and Arosio 1996), and there is a shift in ferritin-containing cell types during development from predominantly microglia at P5 to predominantly oligodendrocytes by P30 (Cheepsunthorn et al. 1998). This delayed appearance of ferritin also makes oligodendrocyte progenitors susceptible to ischemic oxidative stress (Cheepsunthorn et al. 2001).

These studies indicate that oxidative stress is implicated in white matter injury in the developing brain, and some studies using neonatal rodent models have revealed that free radical scavengers are protective against this injury. The concentrations of 4-HNE and MDA in the brain increased 1–24 h after hypoxia–ischemia, and 4-HNE was expressed in pyknotic O_4-positive oligodendrocyte progenitors. α-Phenyl-*n-tert*-butyl-nitrone, a spin-trapping agent, attenuated this oxidative stress and injury in axons and oligodendrocyte progenitors after hypoxia–ischemia (Lin et al. 2004). Edaravone, a free radical scavenger clinically used in Japan, also protected white mater after mouse neonatal hypoxia–ischemia (Shen et al. 2012). In addition to free radical scavengers, some reagents were reported to reduce oxidative stress and alleviate ischemic white matter injury. Minocycline is a bacteriostatic agent with broad-spectrum antimicrobial activity shown to provide neuroprotection against ischemic brain injury in adult and neonatal rodents (Yrjänheikki et al. 1998; Arvin et al. 2002; Xu et al. 2004). Cai et al. (2006) reported that minocycline alleviated hypoxia–ischemia injury to developing oligodendrocytes due to reduction in oxidative damage. They showed that minocycline reduced hypoxia–ischemia-induced oxidative and nitrosative stress, as indicated by 4-HNE- and nitrotyrosine-positive oligodendrocytes. They also showed a decrease in 8-isoprostane, an oxidative stress marker, in minocycline-treated rats compared with vehicle-treated rats. Administration of 17β-estradiol, which plays an important function in the developing brain, protected oligodendrocyte progenitors against oxidative stress induced by cysteine depletion and alleviated white matter ischemic injury in a neonatal rat unilateral carotid ligation model (Gerstner et al. 2009). Although no clinically effective therapy for PWMI has been established, reduction in oxidative stress is a strategy for treatment.

20.3.2 Oxidative Stress in Ischemic Injury of Mature Oligodendrocytes

Susceptibility to oxidative stress is not restricted to oligodendrocyte progenitors. Mature oligodendrocytes in culture are also susceptible to oxidative stress. H_2O_2 induced cell death in bovine oligodendrocytes in culture (Kim and Kim 1991). Adult rat oligodendrocytes were damaged by H_2O_2 formed by redox cycling of catecholamines (Noble et al. 1994). NO also damaged oligodendrocytes in vitro

(Mitrovic et al. 1995, 1996). Compared with other cell types tested under similar hypoxic conditions, oligodendrocytes are less vulnerable than neurons, but are much more quickly injured than astrocytes, microglia, or endothelial cells (Lyons and Kettenmann 1998; Xu et al. 2000; Dewar et al. 2003).

Several factors related to the susceptibility of oligodendrocytes have been reported. Juurlink et al. (1998) found that not only oligodendrocyte progenitors but also mature oligodendrocytes had low levels of reduced glutathione and high levels of iron content. Hence, mature oligodendrocytes have a poor ability to scavenge peroxides in a way similar to oligodendrocyte progenitors. Bernardo et al. (2003) reported that this susceptibility was related to the low expression of SOD2 and catalase in mature oligodendrocytes. In the next sections, acute cerebral ischemia and chronic hypoperfusion, in which ischemic injury of mature oligodendrocytes participates, will be discussed.

20.3.2.1 Acute Ischemic Injury

Much attention has been focused on gray matter in ischemic stroke, while white matter has been considered less vulnerable than gray matter to ischemic injury. However, almost all cases of ischemic stroke involve white matter, and ischemia sometimes primarily involves white matter (Bogousslavsky and Regli 1992). Therefore, white matter is now being recognized as highly vulnerable to the effects of ischemia (Pantoni et al. 1996).

White matter injury after acute ischemia has been studied in both focal and global ischemia rodent models. In a rat permanent middle cerebral artery (MCA) occlusion model, morphologic changes in oligodendrocytes and myelinated axons in white matter occurred as rapidly as in neuronal perikarya. After 3 h of ischemia most oligodendrocytes were lethally injured and preceded the appearance of necrotic neurons in the cortex and basal ganglia by several hours. Vacuolation and pallor of white matter were very marked after 24 h and reflected the segmental swelling of myelinated axons, the formation of spaces between myelin sheaths, and axolemma (Pantoni et al. 1996). Rapid injury of white matter oligodendrocytes was confirmed by immunostaining with tau, a microtubule-associated protein (Dewar and Dawson 1995; Irving et al. 1997). For detection of axonal injury after ischemia, immunostaining with amyloid precursor protein (APP) has been widely used. APP is conveyed by fast anterograde axonal transport, and the presence of APP within axons at the site of injury is thought to be due to its accumulation after inhibition of axoplasmic flow (Shigematsu and McGeer 1992). Increased APP immunoreactivity has been demonstrated in white matter of rats after transient and permanent MCA occlusion and thromboembolic stroke (Stephenson et al. 1992; Yam et al. 1997; Dietrich et al. 1998; Imai et al. 2001; Gresle et al. 2006). One of the problems in detecting axonal injury by APP is that the immunostaining is restricted mainly to the margins of the ischemic lesion, and hence is largely absent from the ischemic core (Yam et al. 1997; Gresle et al. 2006). Another immunostaining marker used to detect axonal injury is SMI-32. The SMI-32 antibody reacts with dephosphorylated

neurofilament H within the neuronal and axonal cytoskeleton. Neurofilament proteins are highly phosphorylated under physiological conditions and axonal injury causes a decrease in phosphorylated neurofilament and an increase in dephosphorylated neurofilament (Trapp et al. 1998). Therefore, the SMI-32 antibody can be used to detect axonal injury under many conditions (Gresle et al. 2006; Yoshioka et al. 2011b). In contrast to APP immunostaining, SMI-32 immunostaining can visualize axons within the ischemic core as changes in the state of phosphorylation. Myelin impairment has been evaluated using immunostaining with myelin basic protein (MBP). In histologically normal tissue, MBP immunoreactivity was detected in myelinated fiber tracts. Changes in MBP staining after cerebral ischemia differ among ischemia models and conditions. Marked reduction in MBP levels within ischemic tissue was detected 1–2 weeks after MCA occlusion (Irving et al. 2001), while in endothelin-1-injection ischemia models MBP staining was reduced 1–7 days after injection (Souza-Rodrigues et al. 2008; Sozmen et al. 2009; Moxon-Emre and Schlichter 2010).

White matter injury has also been reported in transient global cerebral ischemia models. Some authors reported axonal injury after global ischemia in the stratum radiatum of the CA1 subregion, the cerebral subcortical region, and the corpus callosum in rodent models (Pluta et al. 2006; Kubo et al. 2009; Walker and Rosenberg 2010). Oligodendrocytes are also vulnerable to global ischemia. The number of CC-1-positive mature oligodendrocytes in the corpus callosum significantly decreased 3 days after transient global ischemia (Walker and Rosenberg 2010). Petito et al. (1998) reported that oligodendrocytes were more vulnerable than neurons in the cerebral cortex and thalamus in a rat transient global cerebral ischemia model. We reported severe injury in oligodendrocytes and axons in the striatum after prolonged transient global cerebral ischemia (Yoshioka et al. 2011b). The cell processes of oligodendrocytes depicted with receptor-interacting protein (RIP) immunostaining in the striatum began to change 1–3 days after transient global ischemia, and disappeared 7 days after ischemia. Intense expression of SMI-32 was observed in the fiber fascicles of the internal capsule of the striatum 3 days after ischemia. At the same time point, MBP staining of the fiber fascicles became coarse, and vacuolation of the fascicles was observed (Fig. 20.2) (Yoshioka et al. 2011b).

Participation of oxidative stress in white matter injury after acute ischemia and the effectiveness of antioxidants have been investigated using these rodent models and immunostaining markers. 4-HNE staining was observed in axons within core and peri-lesion areas after transient MCA occlusion in rats (Imai et al. 2001). Pretreatment with α-phenyl-*n*-*tert*-butyl-nitrone reduced by 55 % the number of tau-positive injured oligodendrocytes in the subcortical white matter of the ischemic hemisphere compared with untreated animals after permanent MCA occlusion (Irving et al. 1997). Ebselen, which has potent antioxidant effects by acting as glutathione peroxidase and phospholipid glutathione peroxidase mimics, reduced oxidative stress in white matter and consequently lessened axon and oligodendrocyte injury in a rat MCA occlusion model (Imai et al. 2001). Administration of Edaravone 60 min after transient global cerebral ischemia reduced axonal damage in

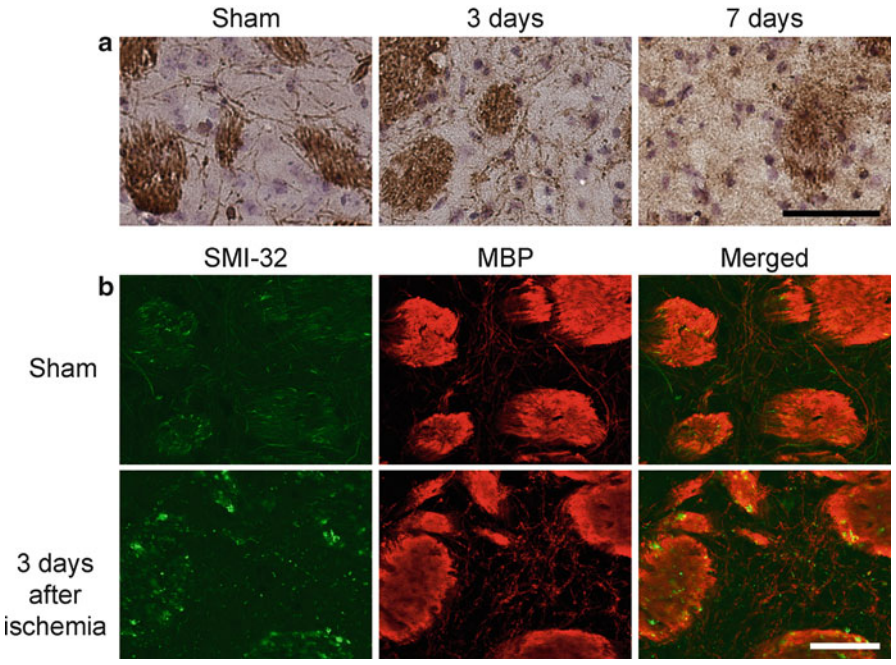

Fig. 20.2 White matter injury in the striatum after transient global cerebral ischemia. (**a**) Representative photomicrographs of RIP staining. RIP-positive processes were thin and smooth in the sham animals. Three days after bilateral common carotid artery occlusion, they became tangled and intermittent. Seven days after bilateral common carotid artery occlusion, RIP-positive processes disappeared, though staining of cell bodies was observed. Nuclei were counterstained with hematoxylin. Scale bar: 50 μm. (**b**) Representative photomicrographs of SMI-32 (*green*) and MBP (*red*) staining of the striatum. Three days after ischemia, intense expression of SMI-32 was observed in the fiber fascicles of the internal capsule of the striatum. With MBP staining, coarse change and vacuolation of the fiber fascicles were also observed. Scale bar: 50 μm

the hippocampus CA1 subregion and the corpus callosum in rats (Kubo et al. 2009). These studies support a pivotal role for oxidative stress in acute ischemic white matter injury.

20.3.2.2 Chronic Hypoperfusion

White matter lesions are often observed in patients with ischemic cerebrovascular diseases (such as Binswanger disease). They are most likely caused by chronic cerebral ischemia and are believed to be responsible for cognitive impairment (Pantoni and Garcia 1997). Neuropathological changes in these lesions are characterized by diffuse demyelination, the loss of axons, and gliosis (Shibata et al. 2004). Many processes, including inflammation and apoptosis of oligodendrocytes, contribute to white matter lesions, and oxidative stress is an important factor among them.

Nonhuman primates, dogs, cats, and rodents have been used to investigate pathological processes of white matter lesions. Nonhuman primates probably represent the best model because their vascular architecture and gyrencephalic brain with extensive white matter more closely resemble those of humans. Nevertheless, most experiments are performed in rodents because of lower cost and higher acceptability from ethical committees (Ginsberg and Busto 1989). Permanent occlusion of both common carotid arteries has been frequently used in rats (Wakita et al. 1994; Ihara et al. 2001; Ueno et al. 2002), while permanent narrowing of both common carotid arteries has been used in gerbils and mice, in which posterior communicating arteries are undeveloped (Hattori et al. 1992; Kudo et al. 1993; Kurumatani et al. 1998; Shibata et al. 2004). Several studies have identified the optic tract and the corpus callosum as predominant locations of vulnerable white matter lesions in the rat brain, while the internal capsule and the caudoputamen seem to be preserved (Wakita et al. 1994; Takizawa et al. 2003; Farkas et al. 2004). Hattori et al. (1992) reported that after more than 8-weeks' duration of brain hypoperfusion in gerbils, there were two types of white matter lesions: one similar to that found in gray matter, and the other observed only in white matter, including in the internal capsule, corpus callosum, ventral hippocampal commissure, and the fiber bundle of the caudoputamen. In mice, white matter lesions were most intense in the median of the corpus callosum, moderate in the paramedian of the corpus callosum, caudoputamen, and internal capsule, and slight in the anterior commissure and the optic tract (Shibata et al. 2004).

The temporal profile of white matter lesions is also different among species. In rats, white matter lesions in the optic nerve and the optic tract were observed 3–7 days after surgery, and those in the corpus callosum, anterior commissure, internal capsule, and the fiber bundle of the caudoputamen were not detected until 14 days (Wakita et al. 1994). In gerbil brains, the lesions specific to white matter were not detected before 8 weeks, and significantly increased in number and size by 12 weeks after surgery (Hattori et al. 1992), while white matter lesions in a mouse model were not detected until 14 days after surgery (Shibata et al. 2004). The different time courses of white matter lesions among these species may be attributed to the degree of change in cerebral blood flow and the vulnerability of white matter.

Antioxidants and free radical scavengers are effective against white matter injury after chronic hypoperfusion in rodent models. Edaravone suppressed accumulation of 4-HNE and 8-hydroxy-deoxyguanosine (an oxidative stress marker) and loss of oligodendrocytes in the corpus callosum after ligation of the bilateral common carotid artery (Ueno et al. 2009). Quercetin, a flavonoid known to scavenge free radicals, reduced vacuolar changes in the optic tract after ligation of the bilateral common carotid artery (Takizawa et al. 2003). Angiotensin-converting enzyme inhibitor and angiotensin II type 1 receptor blocker suppress superoxide production in experimental acute cerebral ischemia models (Ravati et al. 1999; Iwai et al. 2004; Sugawara et al. 2005; Wakai et al. 2011). These reagents are also reported to have protective effects against white matter injury after chronic hypoperfusion due to scavenging free radicals. Angiotensin-converting enzyme inhibitor treatment significantly suppressed the level of MDA and the oxidized glutathione–total

glutathione ratio and reduced white matter lesions in the optic tract, anterior commissure, corpus callosum, internal capsule, and caudoputamen after chronic hypoperfusion (Kim et al. 2008). Telmisartan, an angiotensin II type 1 receptor blocker with peroxisome proliferator-activated receptor-γ-modulating activity, reduced the degree of oxidative stress in vascular endothelial cells in the corpus callosum and attenuated oligodendrocyte loss and demyelinating change in the corpus callosum (Washida et al. 2010). Direct renin inhibition via aliskiren ameliorated NOX activity in the brain and a hypoperfusion-induced increase in cerebral nitrotyrosine levels, and protected the corpus callosum against chronic hypoperfusion (Dong et al. 2011). In addition to these reagents related to the renin–angiotensin system, cilostazol, a potent inhibitor of type III phosphodiesterase, markedly suppressed accumulation of 4-HNE and loss of oligodendrocytes (Watanabe et al. 2006). This evidence supports the participation of oxidative stress in the development of white matter lesions after chronic hypoperfusion and suggests that oxidative stress could be a target of treatment.

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References

Arvin KL, Han BH, Du Y, S-Z L, Paul SM, Holtzman DM (2002) Minocycline markedly protects the neonatal brain against hypoxic-ischemic injury. Ann Neurol 52:54–61

Back SA, Rivkees SA (2004) Emerging concepts in periventricular white matter injury. Semin Perinatol 28:405–414

Back SA, Volpe JJ (1997) Cellular and molecular pathogenesis of periventricular white matter injury. Ment Retard Dev Disabil Res Rev 3:96–107

Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM (2002) Selective vulnerability of late oligodendrocyte progenitors to hypoxia–ischemia. J Neurosci 22:455–463

Baud O, Haynes RF, Wang H, Folkerth RD, Li J, Volpe JJ, Rosenberg PA (2004) Developmental up-regulation of MnSOD in rat oligodendrocytes confers protection against oxidative injury. Eur J Neurosci 20:29–40

Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 87:1620–1624

Bedard K, Krause K-H (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 87:245–313

Bernardo A, Greco A, Levi G, Minghetti L (2003) Differential lipid peroxidation, Mn superoxide, and bcl-2 expression contribute to the maturation-dependent vulnerability of oligodendrocytes to oxidative stress. J Neuropathol Exp Neurol 62:509–519

Bogousslavsky J, Regli F (1992) Centrum ovale infarcts: subcortical infarction in the superficial territory of the middle cerebral artery. Neurology 42:1992–1998

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. general properties and effect of hyperbaric oxygen. Biochem J 134:707–716

Cai Z, Lin S, Fan L-W, Pang Y, Rhodes PG (2006) Minocycline alleviates hypoxic–ischemic injury to developing oligodendrocytes in the neonatal rat brain. Neuroscience 137:425–435

Chan PH (1996) Role of oxidants in ischemic brain damage. Stroke 27:1124–1129
Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. J Cereb Blood Flow Metab 21:2–14
Cheepsunthorn P, Palmer C, Connor JR (1998) Cellular distribution of ferritin subunits in postnatal rat brain. J Comp Neurol 400:73–86
Cheepsunthorn P, Palmer C, Menzies S, Roberts RL, Connor JR (2001) Hypoxic/ischemic insult alters ferritin expression and myelination in neonatal rat brains. J Comp Neurol 431:382–396
Chen H, Song YS, Chan PH (2009) Inhibition of NADPH oxidase is neuroprotective after ischemia–reperfusion. J Cereb Blood Flow Metab 29:1262–1272
Chen H, Yoshioka H, Kim GS, Jung JE, Okami N, Sakata H, Maier CM, Narasimhan P, Goeders CE, Chan PH (2011) Oxidative stress in ischemic brain damage: mechanisms of cell death and potential molecular targets for neuroprotection. Antioxid Redox Signal 14:1505–1517
Connor JR, Menzies SL (1996) Relationship of iron to oligodendrocytes and myelination. Glia 17:83–93
Craig A, Luo NL, Beardsley DJ, Wingate-Pearse N, Walker DW, Hohimer AR, Back SA (2003) Quantitative analysis of perinatal rodent oligodendrocyte lineage progression and its correlation with human. Exp Neurol 181:231–240
Deguchi K, Mizuguchi M, Takashima S (1996) Immunohistochemical expression of tumor necrosis factor α in neonatal leukomalacia. Pediatr Neurol 14:13–16
Dewar D, Dawson D (1995) Tau protein is altered by focal cerebral ischaemia in the rat: an immunohistochemical and immunoblotting study. Brain Res 684:70–78
Dewar D, Yam P, McCulloch J (1999) Drug development for stroke: importance of protecting cerebral white matter. Eur J Pharmacol 375:41–50
Dewar D, Underhill SM, Goldberg MP (2003) Oligodendrocytes and ischemic brain injury. J Cereb Blood Flow Metab 23:263–274
Dietrich WD, Kraydieh S, Prado R, Stagliano NE (1998) White matter alterations following thromboembolic stroke: a β-amyloid precursor protein immunocytochemical study in rats. Acta Neuropathol 95:524–531
Dong Y-F, Kataoka K, Toyama K, Sueta D, Koibuchi N, Yamamoto E, Yata K, Tomimoto H, Ogawa H, Kim-Mitsuyama S (2011) Attenuation of brain damage and cognitive impairment by direct renin inhibition in mice with chronic cerebral hypoperfusion. Hypertension 58: 635–642
Dröge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95
Farkas E, Donka G, de Vos RAI, Mihály A, Bari F, Luiten PGM (2004) Experimental cerebral hypoperfusion induces white matter injury and microglial activation in the rat brain. Acta Neuropathol 108:57–64
Fern R, Möller T (2000) Rapid ischemic cell death in immature oligodendrocytes: a fatal glutamate release feedback loop. J Neurosci 20:34–42
Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370
Folkerth RD, Haynes RL, Borenstein NS, Belliveau RA, Trachtenberg F, Rosenberg PA, Volpe JJ, Kinney HC (2004a) Developmental lag in superoxide dismutases relative to other antioxidant enzymes in premyelinated human telencephalic white matter. J Neuropathol Exp Neurol 63:990–999
Folkerth RD, Keefe RJ, Haynes RL, Trachtenberg FL, Volpe JJ, Kinney HC (2004b) Interferon-γ expression in periventricular leukomalacia in the human brain. Brain Pathol 14:265–274
Gerstner B, Lee J, DeSilva TM, Jensen FE, Volpe JJ, Rosenberg PA (2009) 17β-Estradiol protects against hypoxic/ischemic white matter damage in the neonatal rat brain. J Neurosci Res 87: 2078–2086
Ginsberg MD, Busto R (1989) Rodent models of cerebral ischemia. Stroke 20:1627–1642
Gresle MM, Jarrott B, Jones NM, Callaway JK (2006) Injury to axons and oligodendrocytes following endothelin-1-induced middle cerebral artery occlusion in conscious rats. Brain Res 1110:13–22

Halliwell B (1989) Oxidants and the central nervous system: some fundamental questions. Is oxidant damage relevant to Parkinson's disease, Alzheimer's disease, traumatic injury or stroke? Acta Neurol Scand Suppl 126:23–33

Harrison PM, Arosio P (1996) The ferritins: molecular properties, iron storage function and cellular regulation. Biochim Biophys Acta 1275:161–203

Hattori H, Takeda M, Kudo T, Nishimura T, Hashimoto S (1992) Cumulative white matter changes in the gerbil brain under chronic cerebral hypoperfusion. Acta Neuropathol 84:437–442

Haynes RL, Folkerth RD, Keefe RJ, Sung I, Swzeda LI, Rosenberg PA, Volpe JJ, Kinney HC (2003) Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J Neuropathol Exp Neurol 62:441–450

Haynes RL, Baud O, Li J, Kinney HC, Volpe JJ, Folkerth RD (2005) Oxidative and nitrative injury in periventricular leukomalacia: a review. Brain Pathol 15:225–233

Husain J, Juurlink BHJ (1995) Oligodendroglial precursor cell susceptibility to hypoxia is related to poor ability to cope with reactive oxygen species. Brain Res 698:86–94

Ihara M, Tomimoto H, Kinoshita M, Oh J, Noda M, Wakita H, Akiguchi I, Shibasaki H (2001) Chronic cerebral hypoperfusion induces MMP-2 but not MMP-9 expression in the microglia and vascular endothelium of white matter. J Cereb Blood Flow Metab 21:828–834

Imai H, Masayasu H, Dewar D, Graham DI, Macrae IM (2001) Ebselen protects both gray and white matter in a rodent model of focal cerebral ischemia. Stroke 32:2149–2154

Infanger DW, Sharma RV, Davisson RL (2006) NADPH oxidases of the brain: distribution, regulation, and function. Antioxid Redox Signal 8:1583–1596

Irving EA, Yatsushiro K, McCulloch J, Dewar D (1997) Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. J Cereb Blood Flow Metab 17:612–622

Irving EA, Bentley DL, Parsons AA (2001) Assessment of white matter injury following prolonged focal cerebral ischaemia in the rat. Acta Neuropathol 102:627–635

Iwai M, Liu H-W, Chen R, Ide A, Okamoto S, Hata R, Sakanaka M, Shiuchi T, Horiuchi M (2004) Possible inhibition of focal cerebral ischemia by angiotensin II type 2 receptor stimulation. Circulation 110:843–848

Juurlink BHJ, Thorburne SK, Hertz L (1998) Peroxide-scavenging deficit underlies oligodendrocyte susceptibility to oxidative stress. Glia 22:371–378

Kadhim H, Tabarki B, Verellen G, De Prez C, Rona A-M, Sébire G (2001) Inflammatory cytokines in the pathogenesis of periventricular leukomalacia. Neurology 56:1278–1284

Kadhim H, Tabarki B, De Prez C, Rona A-M, Sébire G (2002) Interleukin-2 in the pathogenesis of perinatal white matter damage. Neurology 58:1125–1128

Kim YS, Kim SU (1991) Oligodendroglial cell death induced by oxygen radicals and its protection by catalase. J Neurosci Res 29:100–106

Kim MJ, Shin K-S, Chung Y-B, Jung KW, Cha CI, Shin DH (2005) Immunohistochemical study of $p47^{Phox}$ and $gp91^{Phox}$ distributions in rat brain. Brain Res 1040:178–186

Kim J-S, Yun I, Choi YB, Lee K-S, Kim Y-I (2008) Ramipril protects from free radical induced white matter damage in chronic hypoperfusion in the rat. J Clin Neurosci 15:174–178

Kim GS, Jung JE, Niizuma K, Chan PH (2009) CK2 is a novel negative regulator of NADPH oxidase and a neuroprotectant in mice after cerebral ischemia. J Neurosci 29:14779–14789

Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH (1991) Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. Proc Natl Acad Sci U S A 88:11158–11162

Kubo K, Nakao S, Jomura S, Sakamoto S, Miyamoto E, Xu Y, Tomimoto H, Inada T, Shingu K (2009) Edaravone, a free radical scavenger, mitigates both gray and white matter damages after global cerebral ischemia in rats. Brain Res 1279:139–146

Kudo T, Takeda M, Tanimukai S, Nishimura T (1993) Neuropathologic changes in the gerbil brain after chronic hypoperfusion. Stroke 24:259–264

Kurumatani T, Kudo T, Ikura Y, Takeda M (1998) White matter changes in the gerbil brain under chronic cerebral hypoperfusion. Stroke 29:1058–1062

Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA (2005) Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. Proc Natl Acad Sci U S A 102:9936–9941

Lin S, Rhodes PG, Lei M, Zhang F, Cai Z (2004) α-Phenyl-*n-tert*-butyl-nitrone attenuates hypoxic–ischemic white matter injury in the neonatal rat brain. Brain Res 1007:132–141

Lo EH, Dalkara T, Moskowitz MA (2003) Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 4:399–415

Lyons SA, Kettenmann H (1998) Oligodendrocytes and microglia are selectively vulnerable to combined hypoxia and hypoglycemia injury *in vitro*. J Cereb Blood Flow Metab 18:521–530

McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1–19

Mitrovic B, Ignarro LJ, Vinters HV, Akers M-A, Schmid I, Uittenbogaart C, Merrill JE (1995) Nitric oxide induces necrotic but not apoptotic cell death in oligodendrocytes. Neuroscience 65:531–539

Mitrovic B, Parkinson J, Merrill JE (1996) An *in vitro* model of oligodendrocyte destruction by nitric oxide and its relevance to multiple sclerosis. Methods 10:501–513

Moxon-Emre I, Schlichter LC (2010) Evolution of inflammation and white matter injury in a model of transient focal ischemia. J Neuropathol Exp Neurol 69:1–15

Niizuma K, Yoshioka H, Chen H, Kim GS, Jung JE, Katsu M, Okami N, Chan PH (2010) Mitochondrial and apoptotic neuronal death signaling pathways in cerebral ischemia. Biochim Biophys Acta 1802:92–99

Noble PG, Antel JP, Yong VW (1994) Astrocytes and catalase prevent the toxicity of catecholamines to oligodendrocytes. Brain Res 633:83–90

Pantoni L, Garcia JH (1997) Pathogenesis of leukoaraiosis. Stroke 28:652–659

Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. Stroke 27:1641–1646

Petito CK, Olarte J-P, Roberts B, Nowak TS Jr, Pulsinelli WA (1998) Selective glial vulnerability following transient global ischemia in rat brain. J Neuropathol Exp Neurol 57:231–238

Pluta R, Ułamek M, Januszewski S (2006) Micro-blood-brain barrier openings and cytotoxic fragments of amyloid precursor protein accumulation in white matter after ischemic brain injury in long-lived rats. Acta Neurochir Suppl 96:267–271

Ravati A, Junker V, Kouklei M, Ahlemeyer B, Culmsee C, Krieglstein J (1999) Enalapril and moexipril protect from free radical-induced neuronal damage in vitro and reduce ischemic brain injury in mice and rats. Eur J Pharmacol 373:21–33

Shen Y, Liu X-B, Pleasure DE, Deng W (2012) Axon–glia synapses are highly vulnerable to white matter injury in the developing brain. J Neurosci Res 90:105–121

Shibata M, Ohtani R, Ihara M, Tomimoto H (2004) White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. Stroke 35:2598–2603

Shigematsu K, McGeer PL (1992) Accumulation of amyloid precursor protein in neurons after intraventricular injection of colchicine. Am J Pathol 140:787–794

Souza-Rodrigues RD, Costa AMR, Lima RR, Dos Santos CD, Picanço-Diniz CW, Gomes-Leal W (2008) Inflammatory response and white matter damage after microinjections of endothelin-1 into the rat striatum. Brain Res 1200:78–88

Sozmen EG, Kolekar A, Havton LA, Carmichael ST (2009) A white matter stroke model in the mouse: axonal damage, progenitor responses and MRI correlates. J Neurosci Methods 180: 261–272

Stephenson DT, Rash K, Clemens JA (1992) Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res 593:128–135

Sugawara T, Kinouchi H, Oda M, Shoji H, Omae T, Mizoi K (2005) Candesartan reduces superoxide production after global cerebral ischemia. Neuroreport 16:325–328

Takizawa S, Fukuyama N, Hirabayashi H, Kohara S, Kazahari S, Shinohara Y, Nakazawa H (2003) Quercetin, a natural flavonoid, attenuates vacuolar formation in the optic tract in rat chronic cerebral hypoperfusion model. Brain Res 980:156–160

Tejada-Simon MV, Serrano F, Villasana LE, Kanterewicz BI, Wu G-Y, Quinn MT, Klann E (2005) Synaptic localization of a functional NADPH oxidase in the mouse hippocampus. Mol Cell Neurosci 29:97–106

Thorburne SK, Juurlink BHJ (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem 67:1014–1022

Trapp BD, Stys PK (2009) Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol 8:280–291

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285

Ueno M, Tomimoto H, Akiguchi I, Wakita H, Sakamoto H (2002) Blood–brain barrier disruption in white matter lesions in a rat model of chronic cerebral hypoperfusion. J Cereb Blood Flow Metab 22:97–104

Ueno Y, Zhang N, Miyamoto N, Tanaka R, Hattori N, Urabe T (2009) Edaravone attenuates white matter lesions through endothelial protection in a rat chronic hypoperfusion model. Neuroscience 162:317–327

Wakai T, Yoshioka H, Yagi T, Kato T, Kinouchi H (2011) Effects of valsartan on neuroprotection and neurogenesis after ischemia. Neuroreport 22:385–390

Wakita H, Tomimoto H, Akiguchi I, Kimura J (1994) Glial activation and white matter changes in the rat brain induced by chronic cerebral hypoperfusion: an immunohistochemical study. Acta Neuropathol 87:484–492

Walder CE, Green SP, Darbonne WC, Mathias J, Rae J, Dinauer MC, Curnutte JT, Thomas GR (1997) Ischemic stroke injury is reduced in mice lacking a functional NADPH oxidase. Stroke 28:2252–2258

Walker EJ, Rosenberg GA (2010) Divergent role for MMP-2 in myelin breakdown and oligodendrocyte death following transient global ischemia. J Neurosci Res 88:764–773

Washida K, Ihara M, Nishio K, Fujita Y, Maki T, Yamada M, Takahashi J, Wu X, Kihara T, Ito H, Tomimoto H, Takahashi R (2010) Nonhypotensive dose of telmisartan attenuates cognitive impairment partially due to peroxisome proliferator-activated receptor-γ activation in mice with chronic cerebral hypoperfusion. Stroke 41:1798–1806

Watanabe T, Zhang N, Liu M, Tanaka R, Mizuno Y, Urabe T (2006) Cilostazol protects against brain white matter damage and cognitive impairment in a rat model of chronic cerebral hypoperfusion. Stroke 37:1539–1545

Xing C, Arai K, Lo EH, Hommel M (2012) Pathophysiologic cascades in ischemic stroke. Int J Stroke 7:378–385

Xu J, He L, Ahmed SH, Chen S-W, Goldberg MP, Beckman JS, Hsu CY (2000) Oxygen-glucose deprivation induces inducible nitric oxide synthase and nitrotyrosine expression in cerebral endothelial cells. Stroke 31:1744–1751

Xu L, Fagan SC, Waller JL, Edwards D, Borlongan CV, Zheng J, Hill WD, Feuerstein G, Hess DC (2004) Low dose intravenous minocycline is neuroprotective after middle cerebral artery occlusion-reperfusion in rats. BMC Neurol 4:7

Yam PS, Takasago T, Dewar D, Graham DI, McCulloch J (1997) Amyloid precursor protein accumulates in white matter at the margin of a focal ischaemic lesion. Brain Res 760:150–157

Yoon BH, Romero R, Kim CJ, Koo JN, Choe G, Syn HC, Chi JG (1997) High expression of tumor necrosis factor-α and interleukin-6 in periventricular leukomalacia. Am J Obstet Gynecol 177:406–411

Yoshioka H, Niizuma K, Katsu M, Okami N, Sakata H, Kim GS, Narasimhan P, Chan PH (2011a) NADPH oxidase mediates striatal neuronal injury after transient global cerebral ischemia. J Cereb Blood Flow Metab 31:868–880

Yoshioka H, Niizuma K, Katsu M, Sakata H, Okami N, Chan PH (2011b) Consistent injury to medium spiny neurons and white matter in the mouse striatum after prolonged transient global cerebral ischemia. J Neurotrauma 28:649–660

Yrjänheikki J, Keinänen R, Pellikka M, Hökfelt T, Koistinaho J (1998) Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. Proc Natl Acad Sci U S A 95:15769–15774