

Considerations for the optimization of induced white matter injury preclinical models

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White matter (WM) injury in relation to acute neurologic conditions, especially stroke, has remained obscure until recently. Current advances in imaging technologies in the field of stroke have confirmed that WM injury plays an important role in the prognosis of stroke and suggest that WM protection is essential for functional recovery and post-stroke rehabilitation. However, due to the lack of a reproducible animal model of WM injury, the pathophysiology and mechanisms of this injury are not well studied. Moreover, producing selective WM injury in animals, especially in rodents, has proven to be challenging. Problems associated with inducing selective WM ischemic injury in the rodent derive from differences in the architecture of the brain, most particularly, the ratio of WM to gray matter in rodents compared to humans, the agents used to induce the injury, and the location of the injury. Aging, gender differences, and comorbidities further add to this complexity. This review provides a brief account of the techniques commonly used to induce general WM injury in animal models (stroke and non-stroke related) and highlights relevance, optimization issues, and translational potentials associated with this particular form of injury.

Keywords: corpus callosum, lysophosphatidylcholine, mouse, NOS inhibitor, posterior limb internal capsule, stroke, vasoconstriction

Introduction

The human brain comprises both gray matter and white matter (WM), with the latter constituting roughly 60% of the total volume. Gray matter consists of neuronal cell bodies, their dendrites and axons, glial cells, and blood vessels (1). On the other hand, WM consists of myelinated and unmyelinated axons that connect various gray matter areas of the brain and support communication between neurons, as well as convey information among the network of efferent and afferent axonal

Abbreviations: CBF, cerebral blood flow; CC, corpus callosum; EB, ethidium bromide; ET-1, endothelin 1; eNOS, endothelial NOS; L-NIO, L-N⁵-(1-iminoethyl)ornithine; LPC, lysophosphatidylcholine; LPS, lipopolysaccharide; PLIC, posterior limb internal capsule; WM, white matter.

fibers. Disruption of these conduction pathways may cause motor and sensory dysfunction, neurobehavioral syndromes, and cognitive impairment (2–4). In clinical settings, WM injury can occur at any time in the life span, such as with the development of periventricular leukomalacia due to hypoxic ischemic injury in infants, cardiac arrest and stroke in adults, and vascular dementia in the elderly (5–8). WM injury is the major cause of paresis in all types of stroke (9). Most obviously this is true for lacunar infarcts, which comprise about 25% of all strokes, and for lower extremity paresis in large vessel distribution strokes (except in the rare circumstance that the anterior cerebral artery territory is involved). However, because anterior circulation large vessel strokes are almost always due to clots embolizing or propagating to the carotid T-junction or the proximal middle cerebral artery, and because infarcts in both locations cause ischemia in the posterior periventricular WM, through which corticospinal and corticobulbar pathways pass, ischemic WM injury also accounts for most upper extremity paresis in large vessel distribution strokes. Furthermore, the site of periventricular WM lesions that cause paresis is also the site of crossing callosal fibers. Damage to these may contribute to apraxia after left brain stroke and may interfere with language recovery after stroke.

Thus, the extent to which WM injuries contribute to neurological impairment after stroke and the frequency with which WM damage contributes to other neurologic disorders highlights the need for therapeutic intervention strategies aimed at ameliorating WM damage or promoting WM recovery, as well as the need to dissect the molecular mechanisms involved in the pathophysiology of this injury. This review focuses essentially on techniques reported to induce WM injury. For other topics such as WM injury induced by traumatic brain injury, the pathophysiology of WM injury, WM hypersensitivity, and genetics variants leading to stroke and WM injury, which are beyond the scope of this paper, see reviews (10–17) and original research articles (18–20).

Broad Classifications of Animal Models with WM Injuries

Attempts have recently been made to develop animal models with WM injury; however, all of these WM injury models have certain limitations. A majority of experimental studies have been designed using rodents, despite their having only about 14% of the relative WM volume of humans (1). Although disease models using primates or other higher order mammals are more readily translated to the human brain, small rodents are privileged for preclinical testing because they are cost effective and easy to acquire, handle, and monitor from a physiological standpoint. Furthermore, murine models provide researchers more flexibility in characterizing disease mechanisms because of the availability of genetically modified (transgenic or knockout) animals. The variety of methods used to induce WM injury in rodents reflects not only the complexity of the disease, but also the need for different paradigms to identify mechanisms that cause WM damage. Such considerations provide the foundation for the development of preclinical novel therapeutic approaches. In general, WM lesion models can be classified into two main categories, as discussed below.

Focal WM Injury

Focal WM injuries are produced at a specific locus in a WM region of the brain. Periventricular WM (PVWM) and posterior limb internal capsule (PLIC) are the most commonly targeted areas to produce injury in WM injury models (**Figure 1**). PVWM injuries are produced by targeting the corpus callosum (CC). The PLIC, which contains corticospinal tracts from the motor cortex, can be targeted to induce motor deficits that are important in determining the efficacy of a therapeutic approach when dealing with functional outcomes (see **Table 1**).

Global WM Injury

Global WM injuries are reflected by diffuse axonal injury occurring in the entire brain as a result of hypoxic/ischemic brain injury, heightened inflammatory states resulting from autoimmune diseases or infection, and/or exposure to demyelinating agents. It is well recognized that the perinatal WM is susceptible to hypoxic/ischemic injury, especially in the preterm period (47). A well-established model of perinatal hypoxic/ischemic injury is unilateral ligation of one common carotid artery (CCA) in combination with hypoxia in the neonatal rodent. It will result in a decrease in cerebral blood flow (CBF) in the middle cerebral artery territory, which corresponds to clinical birth asphyxia (48). Diffused WM injury may also occur in global hypoperfusion, which commonly occurs in cardiac arrest patients. In this context, Shibata and colleagues developed an adult rodent model of chronic cerebral hypoperfusion that provided further insight into mechanisms of WM injury pathogenesis (35).

Ischemic Stroke-Related Focal WM Injury Models

Determining the damage to specific regions of motor pathways can help predict potential functional outcomes. An animal model of a focal WM infarct has been developed using stereotaxic injections of selective drugs in rodents. Stereotactic delivery of selective vasoconstrictors or demyelinating agents induces the local degeneration of WM in a distinct region of the brain by ischemia or the loss of myelin. Subcortical WM, such as the PVWM and the PLIC, contains descending axonal fibers originating in the motor cortex and ascending fibers conveying sensory information to the somatosensory cortex. Therefore, the loss of these connections, as observed in stroke in general and in WM injury in particular, results in a significant clinical phenotype reflected by substantial sensorimotor deficits. Considering the importance of these networks and connections, it is important to develop a WM injury model by targeting PVWM or PLIC.

Target Brain Regions to Induced Focal WM Injury

Paraventricular White Matter

PVWM refers to the WM adjacent to the bodies of the lateral ventricles. It includes ascending and descending fibers in continuation with the internal capsule (the corona radiata), callosal fibers, and, more laterally, long anterior–posterior fasciculi.

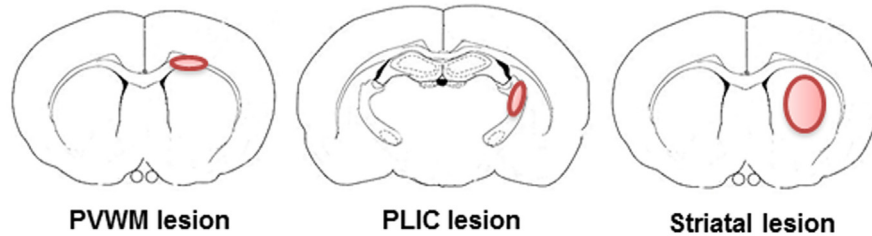


FIGURE 1 | Representative illustrations of targets to induce focal white matter injury in rodents. In rodents, only a small percentage of the brain is WM and WM is mostly located in the periventricular white matter, posterior limb internal capsule, and striatum. Therefore, all of the rodent models of WM injury employ one or more of these areas.

Periventricular leukomalacia in infants is caused by a decrease of blood or oxygen flow during the perinatal period and is characterized by necrosis, astrogliosis, microglial infiltration, and depletion of premyelinating oligodendrocytes in the PVWM (49, 50). Periventricular leukomalacia most often occurs in premature infants. It is associated with neurodevelopmental impairments such as cerebral palsy, with or without mental retardation, learning disability, or epilepsy (48, 51). White matter injuries in PVWM are associated with cognitive and emotional decline (52, 53). Executive, and to a lesser extent, episodic memory formation and declarative memory retrieval are particularly susceptible to deep WM injury because of the particular dependency of these functions on the integrity of long WM pathways (54).

In an effort to develop relevant animal models, several studies have successfully induced infarction and/or demyelination by targeting the PVWM (21, 29–31). Unfortunately, in contrast to what has been reported in the human studies, these animal lesion models have not been associated with significant functional deficits (Table 1), and further refinement is warranted.

Posterior Limb Internal Capsule

In humans, the PLIC contains corticospinal fibers and sensory projections from the thalamus deriving from both lemniscal and anterolateral systems. The anterior limb of the internal capsule includes frontopontine and thalamocortical projections, which modulate higher cognitive functioning (55). The PLIC is vascularized by the anterior choroidal branches of the internal carotid artery and lenticulostriate branches of the middle cerebral artery. Damage to corticospinal motor fibers in the PLIC results in sensorimotor deficits in patients. In human stroke, damage to sensorimotor fibers passing through the PLIC is by far the most important contributor to hemiplegia or hemiparesis, although this damage most often occurs more rostrally, in the PVWM or the centrum semiovale (9). Paresis is characteristically worst in the arm but often involves the face and lower extremity. Dysarthria, dysphagia, and sensory symptoms may also be present in these patients (8). With stereotaxic injection of the vasoconstricting agent endothelin 1 (ET-1) in the PLIC, Frost et al. (24) and Lecrux et al. (25) induced infarction in the PLIC that was associated with functional deficits (24, 25). Thus, selective targeting of the PLIC by vasoconstricting or demyelinating agents can be used to induce a reproducible WM injury model with anatomical and functional deficits.

Vasoconstricting Agents Used to Induce Focal WM Injury

Vasoconstrictors have been reported to selectively induce focal WM injuries. Potent vasoconstrictors are potential tools that need to be fully exploited to further evaluate the mechanisms associated with ischemic WM injury and potential therapeutic treatments. The following is a list of some of the vasoconstrictors that are commonly being used to develop WM injuries in animals.

Endothelin 1

Endothelin 1, a 21-amino acid peptide that is produced by vascular endothelial cells, is a potent vasoconstrictor (56–58). It participates in the development of atherosclerosis, hypertension, and vasospasm following subarachnoid hemorrhage (59). ET-1 coexists with other isoforms, ET-2 and ET-3, which are synthesized by a variety of cells, including glial cells and neurons (60–63). Within the central nervous system, ET-1 is distributed widely within the brain and spinal cord (60, 64–66), but it cannot cross the blood–brain barrier (67, 68). Two classes of ET receptors, ET_A and ET_B, exist in many tissues. The ET_A receptor has a high affinity for ET-1 and ET-2, but a low affinity for ET-3, whereas the ET_B receptor has equal affinity for all three isoforms (69).

At present, the cellular mechanism of ET secretion in neurons and glial cells is poorly understood because the content of ET in neurons and glia is far less than what is found in endothelial cells. ET is thought to bind to its receptors and mobilize intracellular calcium via phospholipase C activation and/or through calcium channels (70). Application of ET-1 in the subcortical WM (such as the PVWM or PLIC) will reduce blood flow, disrupt myelin, and cause axonal injury (12, 71). When ET-1 is administered via intraparenchymal injection or applied topically to cerebral blood vessels, it elicits a sustained vasoconstriction and leads to a monophasic reduction in microvascular blood flow (72–75). Furthermore, the hypoxia of endothelial cells surrounding cerebral vasculature after infarction will trigger *de novo* synthesis of ET-1 and contribute further to vasospasm-induced brain ischemia (76). The application of ET-1 has been shown to effectively induce stroke in rats (75, 77, 78). However, additional precaution should be taken while considering this model because application of ET-1 topically or in brain parenchyma can lead to non-specific injury to the WM and gray matter. Indeed, when ET-1 or other vasoconstriction agents are applied in brain parenchyma, such as striatum, it affects the cerebral fibers network; however, other striatal circuitries are also affected non-selectively. A striatal-induced stroke/lesion is likely

TABLE 1 | Preclinical models of white matter brain injury.

Focal white matter injury	Animal	Lesion location	Morphological changes	Functional deficits, reference
ENDOTHELIN 1 (ET-1)				
	Mouse	CC/PVWM	Gliosis, axonal degeneration, myelin loss	No report (21)
	Mouse	Subcortical WM, striatum	None	No report (22)
	Rat	CC, striatum	Gliosis, demyelination	No report (23)
	Rat	PLIC	Demyelination	Yes (24)
	Rat	PLIC	Lesion	Yes (25)
	Rat	PLIC	Axonal degeneration, myelin loss	Yes (26)
AchA OCCLUSION				
	Rat	IC, lateral and medial hypothalamus	Infarct	Yes (27)
	Pig	IC	Infarct	Yes (28)
PHOSPHATIDYLCHOLINE				
LPC	Rat	CC/PVWM	Demyelination	No report (29, 30)
	Mouse	CC/PVWM	Demyelination	No report (31)
NOS INHIBITOR				
L-NAME + ET1	Mouse	Subcortical WM, striatum	Infarct	Yes (22)
L-NIO	Mouse	Subcortical WM, CC	Infarct, demyelination	No report (32)
OTHER				
Myelin debris	Rat	CC/PVWM	Gliosis, demyelination	No report (33)
Global white matter injury				
CHRONIC HYPOPERFUSION				
Bilateral CCA ligation	Rat	CC, IC, caudate-putamen	Hippocampal cell death	Cognitive impairment (34)
	Mouse	Optic tract, CC, IC	Demyelination, gliosis	No report (35)
	Mouse	Hippocampus	Neuronal death	Memory loss (36)
	Gerbil	Hippocampus, Cortex	Gliosis, neuronal death	Yes (37)
	Gerbil	Whole brain	Ventricular dilation, atrophy, gliosis, demyelination, NF degeneration	No report (38, 39)
Asymmetric CCA ligation	Mouse	Subcortical WM	WM rarefaction, gliosis, axonal damage	Motor and cognitive deficits (40)
HYPOXIC-ISCHEMIC INJURY				
Unilateral ligation + hypoxia	Rat	Cortex, striatum	Cell death, atrophy	Yes (41)
	Rat	Hippocampus	Demyelination	Memory loss (42)
	Mouse	CC, hippocampus, Thalamus	Demyelination, necrosis	No report (43)
Hypoxia only	Rat	CC, PVWM	NOS, gliosis, axonal degeneration	No report (7)
LPS				
	Rat	CC	Demyelination (delayed)	Acute motor deficits (44)
	Sheep	PVWM, cortex	Inflammation, gliosis	Cardiovascular changes (45)
	Sheep	IC, EC	Demyelination (delayed)	No report (46)

AchA, anterior choroidal arteries; CCA, common carotid artery; CC, corpus callosum; EC, external capsule; IC, internal capsule; L-NAME, L-N^G-nitroarginine methyl ester; L-NIO, L-N^G-(1-iminoethyl)ornithine; LPC, lysophosphatidylcholine; NF, neurofilament; NOS, nitric oxide synthase; PLIC, posterior limb internal capsule; PVWM, paraventricular white matter; WM, white matter.

a combination of a white and gray matter lesion. The bundles of penetrating corticopetal fibers in the rodent striatum are indeed injured or destroyed in such a model. Therefore, in an effort to develop a focal WM infarction model that can mimic the lasting neurologic deficits, which is a clinical feature of WM stroke, a recent report in rodents shows that ET-1 injection into the internal capsule results in focal infarction and severe axonal and myelin loss. Such a rodent model also exhibit significant functional deficit at 1 month post-injection (26). However, when ET-1 is applied to cortical tissue in mouse brain, it has produced inconsistent results (22, 79), suggesting dissimilarities between mice and rats in response to ET-1 because of differences in expression of ET-1 receptor isoforms (80). On the other hand, a combination of ET-1 and L-N^G-nitroarginine methyl ester [L-NAME, a nitric oxide synthase (NOS) inhibitor] increased the probability of producing an infarct in mice; however, the lesion was still relatively small and strain dependent (22).

Nitric Oxide Synthase Inhibitors

Nitric oxide synthase plays important roles in physiological and pathological events, including blood pressure homeostasis, neurotransmission, and immune function in the central nervous system (81). Endothelial NOS (eNOS) is found in the endothelium of cerebral vessels and also in a subset of neurons. Nitric oxide (NO) generated by eNOS is crucial for vascular function and hemostasis. By contrast, NO produced by the neuronal and inducible isoforms of NOS can be neurotoxic (82, 83). Furthermore, overproduction of NO may also have pathological consequences (84). Nevertheless, NO plays a critical role in neuroprotection against ischemic stroke through various processes, including ischemic pre- and post-conditioning (85–87). Consequently, some of these NOS inhibitors are being used to produce focal ischemic brain damage.

L-NAME is an arginine analog commonly used as a potent inhibitor of NOS. It has been shown to induce a rapid rise in blood

pressure and a decrease in cardiac output (88, 89). It has been reported that a striatal injection of 100 mM L-NAME alone in the rat produces minor injury, whereas when it is co-administered with quinolinic acid (an NMDA receptor agonist), it augments striatal injury (90). Similarly, co-administration of L-NAME with ET-1 augments infarct volume. However, it has also been reported that the final outcomes following such combinational treatments are strain dependent and produce a relatively small lesion size (22). By contrast, L-NAME has also been shown to have neuroprotective effects in rodent models of cerebral ischemia. It has reduced the effect of ischemic brain injury and prevented blood–brain barrier disruption in animal models (91–93).

L-N⁵-(1-iminoethyl)ornithine (L-NIO, a selective eNOS inhibitor) is approximately five times more potent as an eNOS inhibitor than any other arginine analog, including L-NAME (94). The inhibitory effects of L-NIO are rapid in onset and irreversible, in contrast to other arginine analogs (95). Carmichael and colleagues demonstrated that microinjection of L-NIO in either the cortex or CC induced focal cortical or callosal WM stroke (32). This microinjection resulted in axonal fiber loss and myelin damage within the CC.

Non-Ischemic Stroke-Related Focal WM Injury Models

Ethidium Bromide

Ethidium bromide (EB) has been used extensively to induce demyelination in animal models because of its cytotoxic effect on cells. Many reports have shown the effectiveness of EB-induced focal demyelination in various WM regions of the brain (96–99). The size of the injury is dependent on the volume and concentration of EB, and survival time points (100). Compared to other demyelinating agents, EB administration results in relatively delayed remyelination. Oligodendrocyte and astrocyte loss occurs within the epicenter of the lesion. Neurons and endothelial cells are less sensitive than glia, and axons remain unaffected (101–103).

Myelin-Enriched Brain Debris

This method was recently introduced by Clarner and colleagues (33). Myelin-enriched debris taken from the CC of 7-week-old C57BL/6 mice is homogenized with Precellys® zirconium oxide beads. After extraction of the protein contained within the debris using the Bradford method, 6500 µg/mL of protein in a total volume of 1 µL was stereotactically injected into the CC or the cortex of deeply anesthetized animals (33). This direct application of myelin-enriched brain debris induces profound myelin loss and inflammation.

Demyelinating Agents Used to Induce Focal WM Injury

Lysophosphatidylcholine

The partial hydrolysis of phosphatidylcholines generates lysophosphatidylcholine (LPC), which is also known as lysolecithin. This hydrolysis is generally the result of phospholipase A₂ enzymatic action on phospholipids, which generates free fatty acids such as arachidonic acids and lysophospholipids such as

LPC (104). LPC is believed to play an important role in atherosclerosis and inflammatory diseases by altering various functions in a number of cell types, including monocytes, macrophages, and T-cells (105). LPC is a potent demyelinating agent and has been used widely to study various aspects of demyelination and remyelination in the mature rodent central nervous system (106, 107) as well as *in vitro* organotypic cerebellar slice culture (108). LPC induces demyelination of the brain by the action of recruited macrophages and microglia, which phagocytose nearby myelin. Several reports have demonstrated that microinjection of LPC in the PVWM and WM regions of the spinal cord results in demyelination, an increase in proinflammatory genes, and the induction of gliosis (29–31, 109–111).

A significant limitation of LPC-induced injury, at least from a stroke and rehabilitation point of view, is the potential for reversibility of the injury. Lesions rapidly become repopulated with oligodendrocyte precursors, which initiate remyelination. In rodents, remyelination begins 7 to 14 days post-lesion, depending on the location of the lesion and the age of the animal. Most of the remyelination is essentially complete by 3–4 weeks (112).

Ischemic Stroke-Related Global WM Injury

Chronic Cerebral Hypoperfusion Model

Neonatal WM damage in the periventricular region is often identified as periventricular leukomalacia. Factors predisposing patients to periventricular leukomalacia include immature cerebrovascular development and reduction of CBF in association with hypoxic/ischemic insults such as birth trauma, asphyxia, respiratory failure, cardiopulmonary defects, and premature birth and/or low birth weight (49). The pathological processes involved in global WM injury are still unknown. However, the intrinsic vulnerability of oligodendrocyte precursors to hypoxic/ischemic injury is considered to be central to the pathogenesis of periventricular leukomalacia. These cells are susceptible to a variety of injurious stimuli, including free radical exposure, the lack of trophic factors, and excitotoxicity resulting from cerebral hypoperfusion, as well as secondary events consequent to microglial activation and astrogliosis (49). The increased expression of proinflammatory cytokines (such as TNF α , IL-1 β , and IL-6) in amniotic fluid has also been reported as an identifiable risk factor for the development of brain WM injury (113).

Since the development of the first rodent neonatal hypoxic/ischemic brain injury model, this model has been routinely used to better understand the neuropathology of stroke in neonates (51, 114, 115). The injury in neonatal rat pups at postnatal day 7 (P7) is induced through unilateral ligation of the CCA followed by creation of hypoxia (6–8% oxygen mixed with nitrogen) for a certain period of time (51, 114, 115). Brain damage, seen histologically, is generally confined to the cerebral hemisphere ipsilateral to the arterial occlusion. Depending on the duration of the hypoxia, injury may include the cerebral cortex, hippocampus, striatum, and thalamus. The duration of hypoxia determines the extent and severity of WM damage observed in subcortical and PVWM. The procedure was later adapted to the preterm equivalent rat at P2, which corresponds to the developmental

stage/phenotype present in a preterm infant. At this time, highly vulnerable pre-oligodendrocytes are present in high numbers. In these circumstances, damage occurs throughout cerebral gray and WM (116) and is associated with behavioral deficits (41).

Hypoperfusion models in adult animals involving bilateral CCA stenosis have been introduced by Hattori and co-workers (37) and by Shibata and colleagues (35). Microcoils are wrapped around both CCA below the carotid bifurcation in mice to reduce the lumen diameter. This method results in a chronic reduction of CBF in the brain and induction of WM injury after 14 days without any gray matter involvement. In this model, the most severe WM injury occurs in the medial portion of the CC adjacent to the lateral ventricle and in the PVWM; moderate lesions are found in fiber bundles of the caudate-putamen and internal capsule and less severe lesions develop in the anterior commissure and the optic tracts (35). Interestingly, Yatomi et al. (117) reported that ligation of both CCA in rats decreased CBF by up to 60% with gradual improvement in flow over time. However, CBF never returned to baseline despite a survival time of 28 days. Following chronic cerebral hypoperfusion, blood-brain barrier disruption, especially in the CC, along with glial activation, may contribute to the pathogenesis of WM injury (118). A recent asymmetrical hypoperfusion model by Ihara and colleagues reports the use of a microcoil on one CCA while an ameroid constrictor was applied on the other CCA. Ameroid constrictor absorbs water and swells, resulting in the constriction of the CCA while use of a microcoil acts as vascular stenosis. The use of these two different approaches in the same mouse led to a gradual decrease in CBF and by day 28, these mice developed functional deficits and WM degeneration (40).

Non-Ischemic Stroke-Related Global WM Injury

Hypomyelination Induced by Lipopolysaccharide in the Developing Brain

The pathogenesis of periventricular leukomalacia appears to be multifactorial. Two important factors are hypoxic/ischemic injury and maternal-fetal infection. It has been hypothesized that maternal infection during the preterm period can lead to fetal brain injury. Clinical, epidemiological, and neuropathological data support maternal-fetal infection as an important pathogenic factor in infants with periventricular leukomalacia. Maternal infection increases proinflammatory cytokines levels in amniotic fluid and fetal blood and is associated with WM disorders such as periventricular leukomalacia (113, 119). Animal models of periventricular leukomalacia-like WM injury can be mimicked by injecting lipopolysaccharide (LPS) into the intraperitoneal cavity of pregnant rodents (120, 121) or by intravenous injection in ovine fetuses (45, 46). LPS has been reported to cross the placental barrier and induce systemic inflammation (45, 122). Maternal injection of LPS at gestational day 18 or 19 (G18 or G19) increases proinflammatory cytokines such as TNF α , IL-1 α , and IL-6 and leads to generation of reactive oxygen species by inducing depletion of peroxisomes in premyelinating oligodendrocytes in the fetal brain (113, 120, 123). Apoptosis has also been reported to be a contributing mechanism of cell death in infants with WM

injury (124). Both *in vitro* and *in vivo* studies have shown that LPS causes significant toxicity to premyelinating oligodendrocytes, suggesting that these cells are more vulnerable to proinflammatory cytokines and/or oxidative stress than mature cells (125, 126). Furthermore, astrogliosis and microglial activation are hallmarks of neuroinflammation and are associated with the apoptosis of premyelinating oligodendrocytes that occurs in the wake of maternal LPS exposure (44).

Functional Deficits in the WM Injury Model

Preclinical research on stroke has predominantly focused on models that replicate the vascular events experienced by humans. However, because of the enormous anatomic differences between rodent and human brains, the neuropathological consequences of induced vascular events in rodents are substantially different from those in patients. Thus, traditional rodent models may not be optimal for the study of prognosis or neurorehabilitative intervention. More targeted approaches are needed. Because paresis in human stroke is substantially attributable to WM ischemic injury, the development of ischemic stroke models in rodents that predominantly involve WM is essential if we are to make progress in rehabilitation of paresis after stroke. If, on the other hand, the rehabilitation focus in patients is behavioral deficits wrought of gray matter injury (e.g., aphasia, apraxia, impairment in visuospatial function, hemispatial neglect, and deficits in emotional function), then the traditional middle cerebral occlusion model in rodents, which produces predominantly gray matter injury, may be optimal. Furthermore, because of the degree of "cerebralization" of function in the human brain, it is simply not possible to recapitulate the functional consequences of human hemispheric stroke in a rodent model. For example, most patients with middle cerebral artery stroke permanently lose all or nearly all upper extremity function. Rodents with the analogous vascular lesion demonstrate modest and transient impairment. The central experimental challenge in rodent models is to develop functional measures sufficiently sensitive to reveal differences between treatment and control groups weeks or months following the injury.

To study rehabilitation therapies, suitable animal models of WM injury to produce functional deficits in defined brain circuits and functional improvement following therapeutic intervention are needed. For example, in human stroke, the type and extent of behavioral deficits are dependent on different parts of the somatosensory cortex related to the lesion locus. In global WM injury, in which most of the injury will encompass many functional circuits in the brain, morphological changes as well as functional deficits will become evident (**Table 1**). However, in focal WM injury models, results are inconsistent and depend on the techniques, drugs/chemicals used, and the location of lesions. The application of ET-1-induced middle cerebral artery occlusion is a standard ischemic model that also induces WM damage. This method is relatively less invasive, and important parameters related to reperfusion can be easily tested (127, 128). Similarly, occlusion of the anterior choroidal arteries (without compromising the anterior or middle cerebral artery origins) exclusively produced infarction in the striatum, including the internal capsule in rats (27) and pigs (28). Acute damage to the

PLIC can be a useful predictor of motor outcomes and prognosis for stroke patients (8). ET-1 has been successfully used to induce PLIC damage and produce a functional deficit (24, 25). However, injury in the CC or PVWM in animal models does not result in behavioral deficits (29–31), in contrast to patients with paraventricular leukomalacia, who do exhibit significant behavioral deficits. One of the important reasons for this discrepancy could be the substantial differences in the composition of the gray and WM in humans and rodents. The PLIC injuries identified in current studies are more likely to demonstrate functional deficits than the PVWM injuries; therefore, PLIC injuries represent a viable model for measuring motor deficits.

Mechanisms of WM Injury

Innovative histological techniques have provided the foundation for current cellular and molecular studies on the role of proinflammatory cytokines, gliosis, reactive oxygen species, glutamate toxicity-induced oligodendroglial cell death, and demyelination in WM. Since the early 1970s, Leviton and Gilles have systematically studied PVWM injury and subsequent neurological disability (e.g., cerebral palsy and cognitive deficits) in premature infants (5, 129–132). The spectrum of chronic PVWM injury includes focal cystic necrotic lesions and diffuse demyelination. The demyelination process will then provoke the proliferation and migration of microglia into the lesion, and astrocytes around the lesion (133, 134).

The pathways (intrinsic and extrinsic) that regulate the response of the oligodendrocyte lineage during initial and ensuing phases of injury (including myelination failure) remain unclear. Further neuropathological studies are needed to provide guidance for developing animal models of WM injury. There is no definitive explanation of the mechanism of the WM damage caused by injury to oligodendrocytes produced by such acute neural insults, but based on some preliminary studies, it is believed that at least four major factors can contribute: (1) hypoxic/ischemic injury, (2) vasoconstriction (that may be induced by agents such as ET1, NOS inhibitors, etc.), (3) direct demyelination (that may be induced by agents such as LPC, EB, etc.), and (4) inflammatory processes (maternal-fetal infection induced by agents such as LPS) (Figure 2).

Hypoxic/ischemic conditions and vasoconstrictor agents reduce CBF to the target area of the brain and induce WM injury. WM of the mammalian central nervous system suffers irreversible injury when subjected to anoxia/ischemia (19, 135, 136). Furthermore, WM in older adult mammals is more susceptible to ischemic injury than that in younger adults (137). Ischemia reduces adenosine triphosphate (ATP) and precipitates glutamate release due to reverse Na-dependent transport (136, 138), resulting in the overactivation of AMPA/Kainate receptors. This excitotoxic condition then causes the accumulation of Ca^{2+} in cytoplasm and triggers oligodendrocyte death and axonal disruption (7, 135, 136, 139, 140).

The role of inflammation and excitotoxicity following WM injury has been suggested in numerous reports. Evidence suggests that several proinflammatory cytokines, including $IFN\gamma$, $TNF\alpha$, $IL-1\beta$, $IL-2$, and $IL-6$, are elevated in periventricular leukomalacia

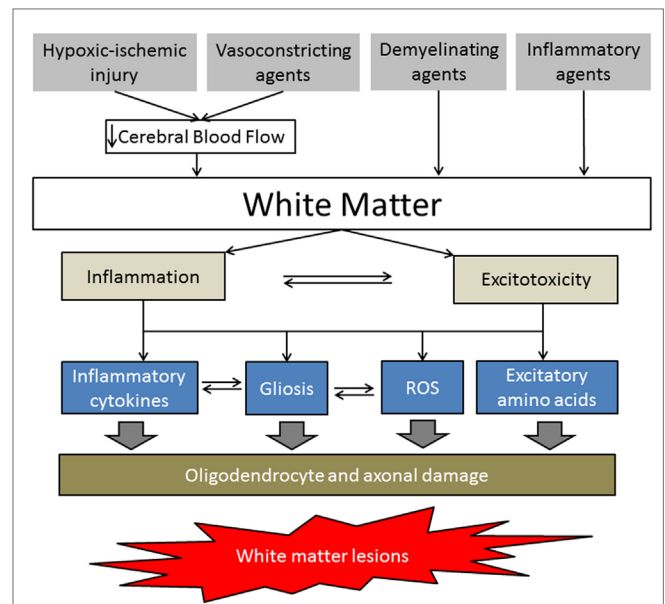


FIGURE 2 | Simplified schematic of white matter injury pathogenesis.

The hypoxic-ischemic injury and vasoconstricting agents decrease cerebral blood flow, resulting in energy depletion and blood–brain barrier disruption in the WM. Demyelinating and inflammatory agents directly affect WM by increasing proinflammatory cytokines, gliosis, reactive oxygen species, and/or excitatory amino acids, leading to oligodendrocyte precursor and axonal damage.

(113, 141–143) and may play pivotal roles in perinatal WM injury (49, 144, 145). Microglial activation and astrogliosis have also been reported in prenatal periventricular leukomalacia (71, 146, 147) and in an animal model of this disorder (114). Moreover, it has also been shown that glial cells express glutamate receptors and are thus susceptible to excitotoxicity following hypoxic/ischemic insult or treatment with a demyelinating agent (140, 148, 149).

There is ample evidence suggesting that oxidative stress correlates with the inflammatory response to ischemia-induced WM injury. Cerebral WM is susceptible to oxidative stress that particularly targets the immature stages of oligodendrocyte development. In addition, following hypoxic/ischemic injury, oxygen-free radicals contribute to chronic cerebral hypoperfusion-induced WM injuries via direct cytotoxic damage involving lipid peroxidation and by induction of inflammatory processes (14, 16, 140, 149–151).

Various Techniques Employed to Investigate WM Injury Outcomes in WM Preclinical Models

Because preclinical and clinical investigations have pointed toward the significant role of WM in various physiologic and pathophysiologic conditions, these studies have primarily utilized novel imaging and neuropathologic tools to better understand the mechanisms of WM injury. In the following sub-sections, some of these studies have been summarized to familiarize the reader with these techniques; however, for greater in-depth

understanding of these techniques, please refer to some of these articles (12, 14, 151–154).

Imaging

Imaging techniques have improved substantially and have paved the way for the analysis of WM functions. Diffusion tensor imaging (DTI) has been used to determine WM degeneration in a mouse model of hypoperfusion (155). Similarly, in a model of neonatal hypoxic/ischemic brain injury, WM microstructure integrity was assessed by MRI (156). Traumatic brain injury also leads to microstructural disruption in WM and these changes can be assessed by DTI (157, 158). Delayed WM injury can lead to significant functional deficit; therefore, early detection of such impairments is highly recommended. In a model of hypoxic/ischemic brain damage, delayed WM injury is detected by longitudinal diffusion tensor and manganese-enhanced MRI (154). In a mouse model of WM injury, ET-1 was injected in the subcortical WM and the axonal damage was studied by performing MRI (21). The use of such imaging techniques has improved the diagnostics of WM injury and provided the greater details of structural changes post-ischemic and other neurologic conditions.

Neuropathology

In preclinical models, several neuropathologic techniques have been used to test various parameters to determine WM injury. In a model of TBI, axonal disruption in WM was assessed by transmission electron microscopy (TEM) or immunostaining with various specific markers (e.g., NF200), or by performing Luxul Fast Blue staining (158). In a recently described model of hypoperfusion leading to WM injury and dementia, the severity of the WM lesions was determined by performing immunohistochemistry for the markers of astrocytes (anti-GFAP antibody), microglia (anti-Iba1 antibody), oligodendrocyte (anti-GST- π antibody), and damaged axons (anti-neurofilament H non-phosphorylated (SMI32) antibody) (40). The damage in WM resulted from hypoperfusion leading to vascular cognitive impairment and was quantified by performing staining for myelin basic protein (anti-MBP) (159). Several other immunohistochemical markers have been targeted to understand some of the mechanisms associated with WM injury (160–163). These well-established immunohistochemical protocols provide greater detail into the role of WM in acute neurologic conditions and help in better understanding the mechanisms associated with WM injuries.

Optimization of Induced WM Injury

As discussed above, several models of WM injury induced by vasoconstricting agents, demyelinating agents, or vascular manipulations have been reported. The extent of injury is dependent on many factors, including the drugs or agents administered, brain areas targeted, gender, age, and survival time point post-injection. Consequently, most of these studies report anatomical outcomes and only a few studies correlate anatomical outcomes with functional changes (Table 1). Use of agents such as ET-1, LPC, or L-NIO, in addition to other compounds/strategies and their related injection sites, provide a plethora of information that has improved our understanding toward the pathophysiology of

WM injury during stroke. However, WM injury models that can be truly referred as a WM injury model of ischemic stroke need further refining. Therefore, some considerations, as discussed below, should be taken into account for optimizing an induced WM injury model relevant with stroke.

First, it is important to choose a brain region that is mostly affected during ischemic stroke. One such area is PLIC or IC. This brain region is an important area to control motor functions and is substantially affected soon after the onset of stroke (164–167). Therefore, targeting this WM brain area will most likely provide a better and consistent WM injury model. The second aspect to consider is to have a better choice of WM injury-inducing chemicals (such as ET-1, L-NIO, or LPC). The advantage with ET-1 and L-NIO, in terms of stroke research, is that these vasoconstricting agents result in irreversible focal infarction, whereas the demyelinating agent LPC induces diffused reversible WM injury. A limitation of ET-1 is that it also has receptor-mediated effects on OPC and astrocytes. Therefore, use of ET-1 as an agent to induce ischemic stroke in WM may confound the interpretation of neural repair tissue outcomes because these outcomes could be due to the direct ischemic effect of ET-1, or due to the effect of ET-1 on astrocytes and OPCs. Third, the dose of these agents at one single location or multiple locations of the same WM region should also be considered for a sustained WM injury model. Because the effectiveness of ET-1 in mice is still questionable, a combination of ET-1 and L-NIO can also be considered as an option for the stroke-related WM injury model. These are some of the basic considerations to optimize chemical-induced WM injury models. Some considerations for other WM injury models that are beyond the scope of this review can be found in these articles (11–16, 19, 20).

Conclusion

Given the advances in various animal models of WM injury developed to understand the mechanisms of disease, a focus on disease management continues to increase. The crucial need to understand the cellular and molecular events that accompany the progressive phases of WM injury and demyelination in patients has led to the use of animal models. However, there has only been moderate success in translation using the proposed WM injury models, especially for mimicking functional deficits that occur in patients. Although current animal models do not reproduce all the clinical features of WM disease, they provide valuable information regarding disease mechanisms and disease-related neurobiological pathways, and they assist in testing possible interventions. The fact that the human brain differs considerably from rodents must be appreciated: such differences account for variations in lesion volumes and behavioral outcomes. In finding novel therapies for WM injuries, the first and foremost requirement is an in-depth knowledge of the neuropathology associated with the disorder while taking into account the complexity of WM and its distribution in the brain. Such an effort on development of optimized WM preclinical models holds great promise in leading to a comprehensive understanding of the disease mechanisms and in finding novel therapies that can alleviate the suffering of a significant portion of the population that is encountering this devastating disease.

Author Contributions

AA, SD, and IS contributed to the design of the work, IS and AA participated in data acquisition, analysis, interpretation, and drafting the manuscript, and JF contributed to the data acquisition and analysis and drafting the manuscript. SN and SD contributed to the conception and design of the work and critically revised the manuscript. All authors agreed with final version of the manuscript, and agree to be accountable for all aspects of the work.

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