Modeling subcortical ischemic white matter injury in rodents: unmet need for a breakthrough in translational research

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Abstract

Subcortical ischemic white matter injury (SIWMI), pathological correlate of white matter hyperintensities or leukoaraiosis on magnetic resonance imaging, is a common cause of cognitive decline in elderly. Despite its high prevalence, it remains unknown how various components of the white matter degenerate in response to chronic ischemia. This incomplete knowledge is in part due to a lack of adequate animal model. The current review introduces various SIWMI animal models and aims to scrutinize their advantages and disadvantages primarily in regard to the pathological manifestations of white matter components. The SIWMI animal models are categorized into 1) chemically induced SIWMI models, 2) vascular occlusive SIWMI models, and 3) SIWMI models with comorbid vascular risk factors. Chemically induced models display consistent lesions in predetermined areas of the white matter, but the abrupt evolution of lesions does not appropriately reflect the progressive pathological processes in human white matter hyperintensities. Vascular occlusive SIWMI models often do not exhibit white matter lesions that are sufficiently unequivocal to be quantified. When combined with comorbid vascular risk factors (specifically hypertension), however, they can produce progressive and definitive white matter lesions including diffuse rarefaction, demyelination, loss of oligodendrocytes, and glial activation, which are by far the closest to those found in human white matter hyperintensities lesions. However, considerable surgical mortality and unpredictable natural deaths during a follow-up period would necessitate further refinements in these models. In the meantime, *in vitro* SIWMI models that recapitulate myelinated white matter track may be utilized to study molecular mechanisms of the ischemic white matter injury. Appropriate *in vivo* and *in vitro* SIWMI models will contribute in a complementary manner to making a breakthrough in developing effective treatment to prevent progression of white matter hyperintensities.

Key Words: animal model; axonal degeneration; demyelination; hypertension; ischemia; oligodendrocytes; subcortical ischemic white matter injury; vascular cognitive impairment; white matter hyperintensities

Introduction

With the advent and wide use of MRI technology, it has been increasingly appreciated that subcortical ischemic white matter stroke poses substantial risks in elderly heath. Initially termed as "leukoaraiosis," meaning rarefaction of the white matter on CT or MRI (Hachinski et al., 1987), the white matter ischemic lesions are sensitively detected as hyperintense signals on T2/FLAIR sequence MR images and thus frequently dubbed as white matter hyperintensities (WMHs) in the clinical realm. A large volume of clinical studies have shown that the WMHs are associated with worse outcomes in stroke and dementia, and an increase in mortality (Debette and Markus, 2010; Sabayan et al., 2015; Ryu et al., 2017; Alber et al., 2019). More importantly, the extent of WMHs progresses over time (Pantoni et al., 2005), providing a wide window of therapeutic interventions to prevent the WMH-associated neurological dysfunction and potentially worsening of Alzheimer's dementia that could be attributed to vascular factors.

Unfortunately, however, there is no currently available therapeutic modality except anti-platelet agents and modification of vascular risk factors, which are common for secondary prevention of the ischemic stroke of all kinds. To make situation even worse, there is no ongoing clinical trial targeting this important clinical entity based on the National Institutes of Health clinical trial database. One of the most significant reasons for the lack of clinical translation is the absence of adequate and standardized animal models, particularly using rodent species. The advantages of using rodent species include their small size facilitating easier access and maintenance and the availability of genetic resources and toolbox allowing more powerful experimental manipulation than other larger laboratory animals such as sheep, dog or pig. However, relatively small amount of white matter in the rodent brain makes modeling human WMHs in these species

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particularly challenging.

Human autopsy studies revealed that the pathologic correlates of the WMHs on MR images are comprised of axonal degeneration, demyelination or parlor of compact myelin, loss of glial cells, particularly oligodendrocytes, and impaired axon-glial junction (Munoz et al., 1993; Hinman et al., 2015). To understand how chronic ischemia leads to these diffuse white matter pathologies, it would be highly valuable to study with a rodent model in which these ischemic white matter lesions are produced consistently and quantifiably with a time course relevant for the human WMHs. As we will discuss in this review, currently available animal models are more or less short of the ideal concepts for modeling the white matter pathology.

In this review, we will discuss various animal models that have been reported to create ischemic injuries in the subcortical white matter. The discussions will be primarily focused on whether a specific animal model would be suitable to represent human white matter pathologies in rodent species, rather than reviewing specific results of individual studies. Here, subcortical ischemic white matter injury (SIWMI) in rodent models is defined as a pathologic process in the subcortical white matter leading to structural abnormalities in the components of the white matter, comprising axons, myelin sheaths, glial cells, and axon-glial junctions, primarily due to an ischemic insult. We prefer the term "ischemic injury" to "stroke" since the scope of this review will be confined to the structural pathologies irrespective of accompanying neurological dysfunctions. The most representative neurological dysfunction of SIWMI would be functional impairment in a wide range of cognitive domains, clinically known as subcorticalvascular cognitive impairment (SVCI) (Dichgans and Leys, 2017), which can be caused by not only diffuse white matter lesions such as leukoaraiosis or WMHs on MR images but also multiple focal lacunar infarctions in the subcortical white matter (**Figure 1**). In order to have SIWMI models exhibiting neurologic dysfunction mimicking human SVCI, it would be ideal to create ischemic injury to the subcortical white matter. Indeed, subcortical white matter corresponding to the cingulum was frequently targeted for injection of a vasoconstriction reagent to make ischemic lesion models (Sozmen et al., 2009; Rosenzweig and Carmichael, 2013). When SIWMI was produced as a result of chronic cerebral hypoperfusion, most studies examined the corpus callosum beneath the cingulum as potential pathological substrates for impaired cognitive dysfunction (Choi et al., 2015, 2016). It is not infrequent to observe motor dysfunction in patients with ischemic lesions in the subcortical white matter (Baezner et al., 2008; Kreisel et al., 2013). Therefore, the internal capsule would be targeted to mimic prominent motor dysfunction in human SIWMI.

It is also worth noting that we do not intend to cover animal models of vascular dementia or vascular cognitive impairment, where cognitive dysfunction is developed by ischemic insults not only in the white matter but also in any parts of the brain, thus not necessarily accompanied by the structural lesions in the white matter. Excellent reviews on the animal models of vascular dementia are available elsewhere (Jiwa et al., 2010; Gooch and Wilcock, 2016). We categorize the animal models of SIWMI into 1) chemically induced SIWMI models, 2) vascular occlusive SIWMI models, and 3) SIWMI models with underlying vascular risk factors. Since there is currently no optimal animal model for SIWMI, we will also introduce *in vitro* models of SIWMI as an alternative approach.

Search Strategy and Selection Criteria

The studies conducted on white matter stroke in animal models or *in vitro* models up to February 2020 were identified from PubMed, Web of Science, SCOPUS and Science direct. Search terms included: (white matter or white matter stroke) AND (animal model or *in vivo* or *in vitro* model) AND (axonal degeneration) AND (demyelination or oligodendrocyte) AND (hypertension or vascular risk factor or diabetes mellitus or metabolic syndrome) AND (white matter hyperintensities or leukoaraiosis). The search limited papers written in English. If only the abstract of the study was available, it was removed. The searched and identified titles and abstracts were initially reviewed and discarded if they were not relevant to the study.

Chemically Induced Subcortical Ischemic White Matter Injury Models

In this model, vasoconstriction reagent endothelin-1 (ET-1) is stereotaxically injected into either the subcortical white matter (cingulum) or the internal capsule in rats or mice. Although there was one study reporting the failure to create focal infarctions in mice using ET-1 (Horie et al., 2008), subsequent studies successfully produced focal white matter lesions using ET-1 in mice (Sozmen et al., 2009; Choi et al., 2014, 2017). Injection into the internal capsule has advantage of making accurate targeting easier than the cingulum injection, because the internal capsule is the thickest WM in rodent brain. Animals with the internal capsule lesion do not exhibit significant cognitive deficits but develop quantifiable motor impairment such as hemiparesis (Choi et al., 2017). Since ET-1 can produce direct effects on neurons and glial cells through endothelin receptors and affect remyelination process (Hammond et al., 2014), N^5-(1-Iminoethyl)-L-ornithine dihydrochloride, a chemical inhibitor of the endothelial nitric oxide synthetase, has been introduced as an alternative vasoconstrictive agent to cause chemically induced SIWMI model (Rosenzweig and Carmichael, 2013). The most beneficial feature of this model is to make highly reproducible lesions in predetermined locations within the white matter. This advantage allows quantitative comparison of the lesion volume as a readout of genetic manipulation or pharmacological treatment.

The biggest problem that makes this model not ideal for modeling human ischemic white matter injury is the rapid temporal progression of lesion development. The white matter lesions become identifiable as early as 3 hours after chemical injection and reach a maximal extent within 7 days (Nunez et al., 2016). This acute evolution is in contrast to slow progression of WMHs on human MR imaging over a range of 2 to 6 years (Schmidt et al., 2007). With this protracted evolution, it is highly likely that modest and chronic reduction of cerebral blood flow causes the degeneration of axons, glial cells, compact myelin sheaths, and axon-glia junctions in a slowly progressive manner. Injection of vasoconstrictive chemicals would elicit acute and drastic shortage of blood supply leading to acute and severe demise of the various white matter constituents. In this regard, chemically induced SIWMI models would be relevant more to lacunar infarction than to diffuse WMHs on MR imaging in human patients (**Figure 1**). WMHs is quite different from lacunes in that WMHs are diffuse or multifocal incomplete infarction caused by chronic ischemic injury (Pantoni and Garcia, 1997). On the contrary, lacunes are the results of focal acute complete infarction involving obliteration of small brain penetrating arterioles (Caplan, 2015)

Vascular Occlusive Subcortical Ischemic White Matter Injury Models

Surgical occlusion or stenosis of major blood vessels in the anterior circulation leads to a decrease in the blood flow to the forebrain, yet spared vertebrobasilar arteries provide collateral blood supply preventing overt cortical infarction. Resultant modest reduction of blood flow engenders critical shortage of the blood supply predominantly in the subcortical white matter (e.g., corpus callosum and cingulum) which is far from both major pial arteries and penetrating arteries and thus susceptible to global ischemia (i.e., border-zone or watershed ischemia). By far the most commonly utilized model in this category is the bilateral common carotid artery occlusion (BCCAO) in rats (Jiwa et al., 2010). To avoid high mortality from

BCCAO in mice, the bilateral carotid stenosis (BCAS) model was developed using metal micro-coils (Shibata et al., 2004). These models consistently exhibit cognitive dysfunction, making them suitable for modeling vascular cognitive impairment. More than several studies employing BCCAO in rats or BCAS in mice reported diffuse white matter lesions such as rarefaction, decreased myelin density, and increased microglial activation. Unfortunately, quantitative measurement of the white matter pathologies, especially in regard to white matter rarefaction or decreased myelination, was lacking in the majority of those studies. For example, white matter rarefaction in the BCAS model is described on the basis of the reported representative images (Shibata et al., 2004, 2007), but it is unclear how exactly the rarefaction is defined or how severe the observed rarefaction is as compared to control animals. Furthermore, there are some studies, including our own, that reported absence of overt axonal and myelin abnormality in BCCAO or BCAS model (Reimer et al., 2011; Choi et al., 2015). Intriguingly, the above two studies demonstrated disruption of axon-glial integrity in BCAS or BCCAO, raising a question whether the axon-glial junction is more vulnerable to ischemia than axon, myelin sheath, or oligodendrocytes (OLs) that produce compact myelin. Several recent papers with either BCAS or BCCAO models showed more quantitative evidence of diffuse white matter lesions using various immunohistochemical markers and/or diffusion tensor MR images (Choi et al., 2016; Koizumi et al., 2018; Wang et al., 2019). However, the reported extent of myelin loss is highly variable among these studies even though the same methods were adopted to generate the models. Furthermore, there are no convincing data of quantitative comparison of OL number or degeneration that could explain a diffuse decrease in the myelin density in those studies. Therefore, we speculate that more consistent pathological findings need to be reproduced to reach a consensus on the adequacy of the vascular occlusive SIWMI models in modeling human ischemic white matter injuries.

Variations in vascular occlusion methods have been reported. To reduce blood supply more gradually, the ameroid constrictor ring was applied to rats or mice (Kitamura et al., 2012; Hattori et al., 2014). This gradual narrowing resulted in smaller and less severe white matter damage compared to BCCAO rat models (Kitamura et al., 2012). In contrast, applying the ameroid constrictor in mice caused higher mortality and more severe pathology not only in the white matter but also gray matter than that in BCAS model (Hattori et al., 2014). Combination of the common carotid artery stenosis on one side and the ameroid constrictor on the other side in mice resulted in multiple subcortical infarctions on the ameroid constrictor side and in diffuse white matter pathology on the BCAS side, which was still lacking quantitative measurements (Hattori et al., 2015).

Subcortical Ischemic White Matter Injury Models with Comorbid Vascular Risk Factors

WMHs in human patients develop due to underlying cerebral small vessel diseases (Pantoni, 2010). In this sense, vascular occlusive SIMWI models ignore underlying vascular pathology leading to progressive stenosis of occlusion of the small vessels. Therefore, integration of underlying vascular risk factors in SIWMI models would be more appropriate to represent the pathological processes in humans.

Hypertension is the most significant modifiable risk factor for the cerebral small artery diseases. Inbred rat strain of hypertension, spontaneously hypertensive rats (SHRs), which do not spontaneously develop stroke, were subjected to BCCAO (Masumura et al., 2001; Choi et al., 2015). Although there were differences in the basal white matter lesion in normotensive rats, both studies reported no significant influence of hypertension on the white matter pathology following BCCAO. Interestingly, our own study revealed disruption of the blood-brain barrier only in hypertensive rats (Choi et al., 2015), although the mechanism behind the interaction between hypertension and chronic ischemic in developing BBB disruption remains to be determined. In contrast to SHRs, stroke prone SHRs (SHRSPs) typically develop spontaneous stroke several months after birth, but in the absence of cerebral hypoperfusion, they do not exhibit ischemic white matter lesions before cortical strokes develop (Brittain et al., 2013). When SHRSPs were subjected to unilateral carotid occlusion in combination with stroke permissive diet and high salt water, they exhibited unequivocal rarefaction of the white matter accompanied by a decrease in myelin content and an increase in OL death (Jalal et al., 2012). In addition, the loss of myelin and OL death were appropriately quantified. These pathological changes were supported by premortem MR imaging that showed diffuse white matter hyperintensities resembling WMHs in humans. The white matter lesions in this model were replicated in the subsequent studies by the same group (Jalal et al., 2015; Yang et al., 2018). Although the white matter pathologies reported in this model are quite obvious and indisputable, major drawback as an SIWMI model is the co-occurrence of the cortical infarction at the side of carotid occlusion. Another potential disadvantage of this model is the natural death of model animals within 4 weeks after the carotid occlusion with dietary modification. In our own experience, high mortality even within 2 weeks made it difficult to secure sufficient number of model animals with the white matter pathologies (unpublished observation).

As compared to SHR or SHRSP models, renovascular hypertension (RVH) can be experimentally induced by simply clipping renal arteries in wild type rats, thus avoiding potential genetic influence on brain pathology. Although the finding that RVH rats are prone to developing spontaneous stroke was reported in Zeng et al. (1998), it seems that RVH stroke models have received less attention than SHRSP models. Reported white matter lesions, measured by MRI and histology, are unequivocal and straightforward sufficient to be quantified (Fan et al., 2015). The white matter pathologies included the rarefaction of axonal fibers, decreased myelin, and formation of vacuoles, which are predominantly observed in the corpus callosum (Fan et al., 2015; Lin et al., 2017). Involvement of the cerebral cortex and other gray matter was not explicitly mentioned in the above studies, but one study reported quite frequent cortical lesions, both hemorrhagic and ischemic, on MRI (Ménard et al., 2017). Therefore, it remains to be determined whether RVH animals produce predominant white matter lesions or cerebral lesions both in gray and white matters. The disadvantage of this model seems to be the unpredictability of the interval between the renal artery clipping and the development of white matter lesions. Not all animals develop SIWMI at the final time point of the experiment, whereas some animals exhibit early and severe lesion leading to spontaneous death before the determined end point. Combining sequential occlusion of bilateral carotid arteries with RVH improved stability of this model and resulted in more extensive white matter lesions (Lin et al., 2017). Although the mortality rate did not increase by the combination of the vascular occlusion, considerable mortality related to RVH itself would be a challenge to determine earlier time points for mechanistic studies. Further refinements will be needed to make this model more appropriate for modeling human SIWMI in rats.

Various comorbid risk factors other than hypertension have been considered in generating animal models of vascular cognitive impairment, but very few studies examined whether the white matter injuries are accompanied in these models (Jiwa et al., 2010). Apolipoprotein E knockout mice, a model of atherosclerosis, were subjected to vascular occlusion using BCAS and exhibited a decrease in myelin density in the corpus callosum without signal changes on MR images (Lee et al., 2017). A recent study reported that a sequential occlusion of the two carotid arteries resulted in atrophy of the white matter in a rat model metabolic syndrome (Livingston et al., 2020). Since detailed analyses on the white matter lesions were

lacking in these studies, further studies will be needed to see if these comorbid vascular risk factors would be needed to develop prominent white matter lesions when combined with cerebral hypoperfusion.

In Vitro Modeling of Ischemic White Matter Injury

The above arguments and discussions on various *in vivo* SIWMI models highlight difficulties and challenges in appropriate modeling of human ischemic white matter lesions in rodents. In addition, the majority of the *in vivo* models, particularly for vascular occlusive SIWMI models and SIWMI models with comorbid vascular risk factors, takes longer than at least a couple of months to generate and evaluate the white matter ischemic lesions, which makes these *in vivo* models even more challenging for mechanistic studies to elucidate molecular mechanisms behind degeneration of the white matter components composed of axons, OLs, myelin, and axon-glia junctions. In this regard, an *in vitro* modeling that can recapitulate the myelinated white matter tracks with all the white matter components would have its own value as an alternative for *in vivo* SIWMI models.

A series of studies using the *in vitro* rat optic nerve preparation have provided important mechanistic insights into the acute phase of central white matter injury (Stys, 1998). This simple preparation works for recording electrophysiological changes in axons following ischemia but would not be a suitable model for evaluation of pathological changes in various white matter components. Cultured slice preparation would allow interaction of multiple white matter components in the same environment thus more closely resembling *in vivo* situation (Doussau et al., 2017). In acute coronal brain slices, oxygen-glucose deprivation for 30 minutes resulted in axonal degeneration in the corpus callosum accompanied by widespread OL death (Tekkok and Goldberg, 2001; McCarran and Goldberg, 2007). The *in vitro* system in these studies facilitated elucidation of AMPA/Kainate receptors mediating the oxygen-glucose deprivation-induced axonal injury and OL death. The functionality of acute slice preparation, however, can be preserved for only several hours. Therefore, it is impossible to implement chronic and prolonged ischemic insult, which is more relevant for human white matter ischemia, in the acute brain slices. In addition, inevitable degeneration in the white matter in acute slice preparation even in control conditions might be a source of confusion in the evaluation of oxygen-glucose deprivation-induced axonal degeneration and OL death.

To circumvent the problems inherent in the acute slice preparation, we recently established organotypic cerebellar slice cultures that can be subjected to chronic hypoxic insult (Cui et al., 2020). We initially attempted to obtain cultured coronal cerebral slices containing the corpus callosum. In our hands, however, it was impossible to secure survival of the corpus callosum longer than a few of days, probably due to severance of callosal axons from originating commissural neurons during slicing. In contrast, sagittal cerebellar slices were successfully cultured on a cell culture insert with the majority of Purkinje neurons and their axons maintained. Importantly, when slices were obtained from P12 rat pups, the white matter tract consisting of the Purkinje axons was densely myelinated, recreating the complex environment consisting of axons, myelin sheaths, OLs, and axon-glial junctions in an *in vitro* system. The cultured cerebellar slice system allowed us to subject the white matter tract to milder and prolonged hypoxic insult, which is more relevant to human white matter ischemia. Axons in the subcortical white matter developed progressive degeneration in response to the 48-hour duration hypoxic insult (Cui et al., 2020). The degenerating axons showed focal constrictions and fragmentations with frequent swelling (**Figure 2**), a granular morphology that is frequently observed in various neurodegenerative diseases (Wang et al., 2012). This

Clinical syndrome of human SIWMI	Radiologic or pathologic manifestation of human SIWMI	Conceptual animal model	Operational animal model	*In vitro* white matter injury model
• Subcortical vascular cognitive impairment	• Leukoaraiosis or WMHs	• Progressive and diffuse ischemic injury in the white matter	• Vascular occlusive SIWMI models with or without comorbid vascular risk factors	• Acute anoxic insult to optic nerve preparation • OGD in acute slice preparation • Chronic hypoxic insult in organotypic cerebellar slice cultures
	• Lacunar stroke	• Rapid and focal ischemic injury in the white matter	• Chemically induced SIWMI models	

Figure 1 | Subcortical ischemic white matter injury: from human manifestations to animal models and *in vitro* modeling.
OGD: Oxygen-glucose deprivation; SIWMI: subcortical ischemic white matter injury; WMHs: white matter hyperintensities.

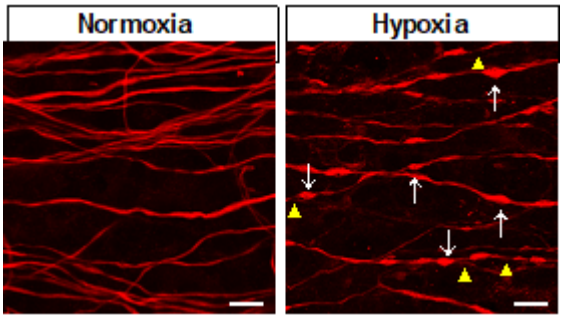

Figure 2 | Representative images of the cerebellar cultured slices subjected to normoxic (left) or hypoxic (right) condition for 48 hours.
White arrows indicate axonal swellings and yellow arrowheads mark axonal segments with focal constrictions. Scale bars: 10 μm. Reprint from Cui et al. (2020).

in vitro model of ischemic axon degeneration enabled us to test multiple pharmacological reagents to delineate molecular mechanisms mediating the ischemic axonal degeneration, fulfilling the primary motivation of an *in vitro* modeling. A weakness of this model is that pathological changes related to myelin sheaths and OLs were not replicated. Myelin sheaths remained surrounding degenerating axons even with severe swellings and fragmentations as the number of OLs did not decrease in hypoxic condition (Cui et al., 2020). While the reason for the remarkable resilience of myelin and OLs could not be answered in that study, the differential susceptibility to ischemic insult may suggest an important implication in the pathogenesis of diffuse ischemic white matter injury in humans.

Conclusions and Future Perspectives

Human radiologic or pathological manifestations of SIWMI, which causes a syndrome of SVCI, could be classified into WMHs (or leukoaraiosis) and lacunar infarction based on the nature of ischemic insult (**Figure 1**). Conceptual animal models to mimic WMHs necessitates gradually progressive diffuse ischemic injury to the white matter. On the other hands, rapid focal ischemic insult in the white matter is necessary for the animal model of lacunar infarction. Various rodent SIWMI models have been developed based on this conceptual speculation. Briefly, as the local injection of vasoconstrictive chemicals into the white matter results in focal acute ischemic injury, chemically induced SIWMI model is used to make lacunar infarction model. On the other hand, occlusion or stenosis of major arteries such as common carotid artery with or without comorbid vascular risk factors could lead to a chronic and diffuse decrease in blood supply in the subcortical white matter, the most susceptible region to the hypoperfusion (**Figure 1**). Unfortunately, these operational animal models are considered to be still incomplete in fulfilling the conceptual ideation of the SIWMI models.

In this review, we introduced various operational SIWMI animal models and discussed their advantages and disadvantages

primarily in regard to the pathological manifestations of white matter components including axons, myelin sheaths, glial cells, and axon-glial junctions. Most frequently appearing in the literature are vascular occlusive SIWMI models. Although these models consistently exhibit cognitive impairment following vascular occlusion, the adequacy of the vascular occlusive SIWMI models in modeling bona fide white matter injuries remains to be determined until more consistent data on the myelin pathology by BCCAO in rats or BCAS in mice will be available. Chemically induced models display unequivocal lesions with demyelination, OL loss, and axonal degeneration in predetermined locations. However, the abrupt nature of lesion development renders these models appropriate for lacunar infarctions rather than diffuse white matter injury that usually evolve in a slowly progressive manner. The SIWMI models with comorbid vascular risk factors (specifically hypertension), when combined with vascular occlusion, seem to produce progressive and definitive white matter lesions that were demonstrated by pathologic analysis and MR images. The white matter pathologies involve diffuse rarefaction, demyelination, OL loss, and glial activation, which are by far the closest to those found in human WMH lesions. However, considerable surgical mortality and unpredictable natural death during a follow-up period would give rise to a problem of securing sufficient number of animals at specified time points. Thus, further refinements will be needed to furnish these models with stability and consistency, which are important qualities in animal models in general.

In the meantime, combining *in vivo* SIWMI animal models and *in vitro* modeling of white matter injury would be a feasible alternative to make a progress in translational research of the white matter ischemic injury (**Figure 1**). In particular, *in vitro* slice models that recapitulate myelinated white matter track may be utilized to study molecular mechanisms of SIWMI. In addition to the axonal degeneration model we have developed, it could be conceivable to devise a different *in vitro* model where demyelination and OL death are predominant. Finely adjusting ischemic or hypoxic severity *in vitro* would make it possible to address the issue of differential sensitivity to ischemia among various components of the white matter such as axons, myelin sheaths, glial cells, and axon-glial junctions.

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