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REVIEW ARTICLE

Molecular mechanisms of acrolein-mediated myelin destruction in CNS trauma and disease

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Abstract

Myelin is a critical component of the nervous system facilitating efficient propagation of electrical signals and thus communication between the central and peripheral nervous systems and the organ systems that they innervate throughout the body. In instances of neurotrauma and neurodegenerative disease, injury to myelin is a prominent pathological feature responsible for conduction deficits, and leaves axons vulnerable to damage from noxious compounds. Although the pathological mechanisms underlying myelin loss have yet to be fully characterized, oxidative stress (OS) appears to play a prominent role. Specifically, acrolein, a neurotoxic aldehyde that is both a product and an instigator of OS, has been observed in studies to elicit demyelination through calcium-independent and -dependent mechanisms and also by affecting glutamate uptake and promoting excitotoxicity. Furthermore, pharmacological scavenging of acrolein has demonstrated a neuroprotective effect in animal disease models, by conserving myelin's structural integrity and alleviating functional deficits. This evidence indicates that acrolein may be a key culprit of myelin damage while acrolein scavenging could potentially be a promising therapeutic approach for patients suffering from nervous system trauma and disease.

Keywords: demyelination, spinal cord injury, multiple sclerosis, neurotrauma, hydralazine

Abbreviations: 4-AP, 4-aminopyridine; 4-AP-3-MeOH, 4-aminopyridine-3-methanol; CAP, compound action potential; CARS, Coherent Anti-Stokes Raman Scattering microscopy; Caspr, contactin-associated protein; CNS, central nervous system; GLT-1 (EAAT₂), glial glutamate transporter-1; iGluRs, ionotropic glutamate receptors; IP, intraperitoneal injection; EAE, experimental autoimmune encephalomyelitis; EGTA, ethylene glycol tetraacetic acid; GSH, glutathione; HNE, 4-hydroxynonenal; LPO, lipid peroxidation; MBP, myelin basic protein; MS, multiple sclerosis; NF155, neurofascin 155; NF186, neurofascin 186; OS, oxidative stress; ROS, reactive oxygen species; SCI, spinal cord injury

Introduction

Myelin damage is a significant pathogenic feature common to both trauma and disease that continues to pose a challenge in the treatment of neuropathologies. To establish novel and effective therapeutics, a strong understanding of the molecular mechanisms governing myelin damage is warranted. This review will focus on evidence implicating acrolein as a causative agent of myelin damage, and as a compound with great potential to be targeted therapeutically for the mitigation of myelin damage and the consequent functional deficits.

Pathological significance of myelin damage in CNS trauma and disease

The structural integrity of myelin is essential for normal function in the central nervous system (CNS), and its destruction is a key characteristic observed in many neuropathologies, including multiple sclerosis (MS) and spinal cord injury (SCI) [1–5]. The CNS myelin sheath

originates from oligodendrocytes and is composed of many compact layers tightly wrapped around axons, establishing a physical connection between myelin and axons at the paranodal region [1]. It has long been established that myelin insulates the internodal, juxtaparanodal, and paranodal regions, while leaving the nodes of Ranvier exposed. Given that this structure is vital for maintaining neuronal function, morphological studies have demonstrated significant demyelination in animal models of both SCI [4,5] and MS [6–8], implicating myelin injury as a common feature of CNS trauma and disease. For example, compression and stretch SCI have shown immediate myelin damage such as elongation of the nodes of Ranvier and separation of the myelin and axolemma [9–11]. Such demyelination has been shown to progress with graded lengthening of the nodes two and five hours post stretch SCI in rats [11]. Furthermore, strikingly similar damage was also observed upon morphological analysis of excised spinal cords of mice with experimental autoimmune encephalomyelitis (EAE), a well-established model of MS in which nodal lengthening and myelin retraction were also observed (unpublished observation, Shi). Thus the

presence of structural myelin damage is an important component of CNS trauma and disease.

The functional capabilities of most axons in the CNS are dependent on the structural integrity of the myelin sheath. In non-pathological conditions, axonal transmission of electrical depolarizing pulses is facilitated through the high expression of voltage-gated Na^+ channels present in the nodes of Ranvier, enabling saltatory conduction, in which pulses jump from one node to another [1]. Myelin enhances axonal conduction by both inhibiting ionic exchange across the membrane and increasing transverse resistance at the internodal region [12]. Accordingly, compromise of the structural integrity of myelin elicits significant conduction loss in spinal cord tissue, partly due to the exposure and the consequent activation of voltage-gated K^+ channels typically residing in the juxtaparanodal region [1,10,11,13–15]. *Ex vivo* studies of spinal cord tissue have demonstrated that myelin damage and the subsequent exposure of K^+ channels impaired signal propagation, indicated by a reduction of compound action potential (CAP) amplitude [10,11,14]. In fact, diminished CAP amplitudes were detected at the precise moment of physical damage, indicating that a number of axons were not capable of signal transduction, likely due to immediate myelin injury [9,10]. Interestingly, alleviation of functional deficits and partial recovery of CAP amplitude were observed following the application of K^+ channel blockers such as 4-aminopyridine (4-AP) and 4-aminopyridine-3-methanol (4-AP-3-MeOH), validating the role of K^+ channel dysfunction in conduction loss [9,11,14–16]. Reinstated function of injured axons following the application of potassium channel blockers such as 4-AP has been observed in various models such as rat spinal nerve roots, *ex vivo* rat sciatic nerves [17,18], and in cats with conduction and motor deficit due to chronic SCI [19–21]. While both morphological and electrophysiological evidence have demonstrated functional consequences in diseases such as MS and SCI, up until recently, knowledge regarding the mechanisms mediating myelin damage was limited. New evidence has emerged indicating that a variety of mechanisms could contribute to the demyelination characteristic of CNS pathology.

Mechanisms of myelin damage

Demyelination is generally attributed to damage by both physical and chemical processes. In the case of trauma, primary injury mediated by physical forces triggers subsequent pathological molecular mechanisms, deemed secondary injury [22,23]. Separation of these two components was postulated decades ago, and has been supported by many subsequent studies [13,22,23]. Recently, this phenomenon was demonstrated in an *ex vivo* acute SCI model where a mechanical stretch injury resulted in immediate myelin damage, indicated by lengthening of the nodal region and retraction of myelin from the paranodal region following the physical insult [11]. The detachment and degradation of myelin prompted immediate exposure of

the underlying juxtaparanodal K^+ channels and simultaneous conduction failure. Interestingly, computational modeling studies have revealed that the paranodal region is subjected to a significant amount of stress under an external load such as compression, supporting the observation that physical force can cause myelin damage, especially in the paranodal region, eliciting K^+ channel dysregulation and conduction failure [10,24]. The role of juxtaparanodal K^+ channel exposure in mediating conduction loss is further supported by the observation that application of the K^+ channel blocker 4-AP restored conduction in demyelinated axons, but was ineffective in myelin-intact axons [9,16,25,26]. Taken together, physical trauma can clearly produce myelin damage which could lead to functional loss.

It has been repeatedly shown that myelin damage and conduction loss directly resulting from physical trauma can be exacerbated even after the mechanical forces are removed, an effect attributed to myelin damage mediated by the pathophysiological molecular mechanisms of secondary injury [4,11,27]. For example, using a well-controlled *ex vivo* spinal cord white matter preparation, Sun and colleagues have shown that immediate myelin damage resulting from physical trauma can further deteriorate through chemical reactions without concomitant physical loading [11]. Interestingly, it was also noted that while myelin damage resulting from primary injury does not depend on extracellular calcium, some components of secondary injury do [11,28], further demonstrating the existence of two independent mechanisms.

It is worth noting that while myelin damage mediated by molecular signaling is clearly secondary to the physical impact in neurotrauma, chemical injury predominates in the case of neurodegenerative diseases where primary physical trauma is largely absent. The etiology of chemically-mediated injury in neurodegenerative disease is mostly unknown. It is generally believed that such pathology could be attributed to several known biochemical processes such as autoimmune responses, particularly in MS, where myelin-reactive T-lymphocytes perceive myelin as foreign to the body and mount an immune response, employing endogenous defense mechanisms to mediate its destruction [8,29]. Although distinct processes initiate the molecular mechanisms underlying demyelination in trauma and neurodegenerative disease, downstream chemically-mediated myelin damage appears to be similar in both neurotrauma and chronic disease [10,13,14,30]. Such scientific convergence facilitates the cross talk of these mechanisms and greatly enhances understanding of myelin damage in various pathological conditions as well as the development of effective treatments.

Based on decades of research in both the field of trauma and degenerative diseases, it is generally believed that biochemically induced myelin damage could result from multiple mechanisms including inflammation, oxidative stress, ischemia, and glutamate toxicity [31]. Many studies clearly indicate that biochemical mechanisms alone are capable of eliciting myelin damage similar to that elicited by physical force, although, over a prolonged

time course. In particular, oxidative stress has emerged as a crucial factor in instigating myelin damage, and a key factor of oxidative stress, acrolein, has been shown to cause significant myelin damage in the absence of physical trauma [6,30,31]. Due to the fact that acrolein appears to be a novel mechanism of myelin damage, this review will focus on discussing evidence attributing acrolein as a key factor of myelin damage and also as a novel and effective therapeutic target to deter myelin damage and functional loss.

Acrolein

Acrolein (2-propenal) is produced endogenously through various mechanisms such as intracellular enzymatic oxidation of polyamine metabolites [32–36], and more importantly, lipid peroxidation (LPO) [32,37,38]. As a highly electrophilic α , β -unsaturated aldehyde, acrolein is extremely reactive with nucleophilic DNA, the sulfhydryl groups of cysteine, histidine, lysine, and arginine residues in proteins as well as phospholipids, and can also generate free radicals [32,36,39]. Acrolein interacts with proteins via the Michael addition reaction and is rapidly incorporated into proteins, producing carbonyl derivatives [32,40,41]. In fact, oxidative stress is associated with the generation of many unsaturated aldehyde electrophiles such as 4-hydroxynonenal (HNE), 4-oxy-2-nonenal (ONE), malondialdehyde (MDA), 4-hydroxy-2E-hexenal (HHE), and crotonaldehyde. However, of the aforementioned LPO aldehyde byproducts, acrolein is at least 100 times more reactive and is produced at 40 times the concentration [32,36,42]. In addition to its high reactivity, acrolein is relatively stable in aqueous conditions and has a half-life that is several orders of magnitude longer than of better known reactive oxygen species (ROS) such as hydroxyl radicals that decay within nanoseconds [42–45], suggesting that acrolein could incite long-lasting damage. Given that ROS scavengers did not prove to be clinically successful in mitigating neurological injuries [8,31,46,47], acrolein is further promoted as a key factor in perpetuating oxidative stress and as a novel therapeutic target to mitigate such injury.

A unique characteristic of acrolein is its ability to exist both as a product and as a catalyst of LPO, indicative of a key role in perpetuating oxidative stress [6,48–51]. After its production via LPO, it can react with xanthine oxidase to be converted back to superoxide, which can then react to promote LPO and consequently produce more acrolein [52–54]. The significance of this finding is only enhanced when considered along with other studies that demonstrate the diffusive nature of acrolein. Hamann et al. incubated injured spinal cords and healthy segments, endogenously producing acrolein at an elevated concentration and rate to demonstrate superoxide production and acrolein-mediated damage in both injured and uninjured segments [55]. Natural physiological antioxidants, in particular glutathione (GSH), typically reduce this damage and prevent the elevation and diffusion of ROS and acrolein. As the most

powerful endogenous antioxidant, GSH continuously scavenges acrolein in normal and disease states through the 1, 4-addition reaction, forming a stable product that is non-toxic. However, GSH can be rapidly depleted during extreme oxidative stress [48,50,56]. Studies have shown that upon GSH depletion, pharmacological scavenging of acrolein in MS can suffice as a method for mitigating demyelination and the subsequent functional deficits [57]. Together, these studies demonstrate the key role of acrolein in enhancing oxidative stress, and thus it is likely responsible in part for perpetuating and propagating oxidative stress.

Acrolein-mediated myelin damage

In addition to its critical role in perpetuating oxidative stress, acrolein has also been implicated as a factor capable of directly exacerbating myelin damage. Oxidative stress in the CNS is especially detrimental to myelin, given that its main constituents are proteins and lipids—biopolymers vulnerable to progressively elevated acrolein levels due to the high potential for reactivity, rendering acrolein highly toxic to nervous tissue [53,54,58–61]. The presence of acrolein has been implicated in cases of enhanced oxidative stress, particularly where elevated tissue and systemic levels of acrolein have been shown in both SCI [55,56,61–64] and MS [57]. In fact, acrolein can increase up to 300% at 24 h post-SCI and remain elevated for at least 2 weeks in a clinically relevant rat spinal cord contusion injury model [63]. Given that demyelination is a hallmark of both MS and SCI, this evidence points toward acrolein-mediated myelin damage as a key factor underlying symptoms of CNS trauma and disease. Furthermore, removal of acrolein by application of the scavenger hydralazine in EAE mice demonstrated a marked reduction in myelin damage and a parallel improvement of motor function [57]. In light of the aforementioned evidence, it seems likely that acrolein in part mediates demyelination.

While elevated acrolein levels and concomitant demyelination are observed under conditions of high oxidative stress, the direct role of acrolein-mediated myelin damage was only addressed in recent *ex vivo* studies. Since it is extremely difficult to evaluate the effect of endogenous acrolein on myelin *in vivo*, studies using *ex vivo* spinal cords were advantageous in providing the relevant physiological tissue that could be monitored in a well-controlled environment. Perhaps the most powerful and direct study demonstrating acrolein-mediated myelin damage was the application of acrolein to isolated spinal cord tissue, which resulted in demyelination, nodal lengthening, and exposure of K^+ channels, visualized through the simultaneous use of label-free Coherent Anti-Stokes Raman Scattering (CARS) microscopy and immunohistochemistry, respectively [30]. The use of CARS is particularly unique, given that it is a label-free method for visualization of myelin and thus reduces the chance for further molecular perturbation [65]. Moreover,

acrolein-mediated structural damage caused an immediate reduction in electrophysiological recordings of the CAP amplitude [30,59,64]. While application of K^+ channels could restore the amplitude to some extent, acrolein scavenging with hydralazine significantly mitigated the progressive demyelination and conduction loss, demonstrating the key role of acrolein in mediating myelin damage. Thus, the combination of morphological and electrophysiological evidence clearly supports acrolein-mediated myelin damage in CNS trauma and disease by way of the mechanisms outlined in Figure 1, which will be further delineated in the following section.

Molecular mechanisms of acrolein-mediated myelin damage

Acrolein can induce direct damage to the myelin sheath through splitting or decompaction in a Ca^{2+} -independent manner. A physical connection among multiple layers of myelin lamellae is ensured by myelin basic protein (MBP) [66,67] (Figure 1). Contact between axons and myelin is

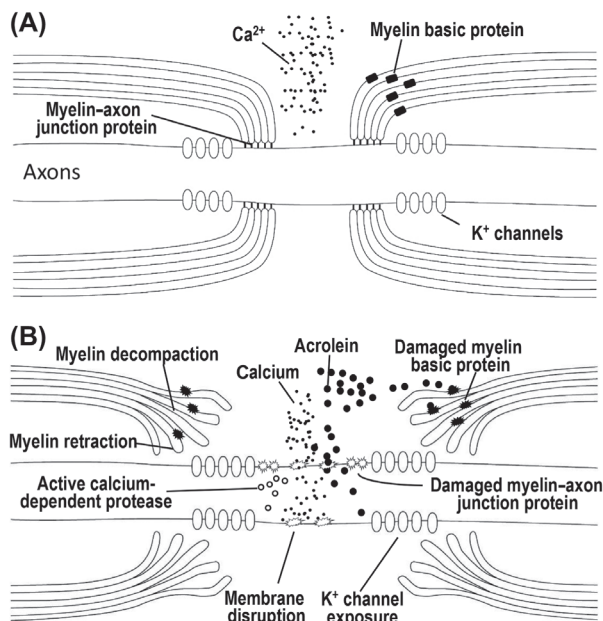


Figure 1. Diagram representing the steps in acrolein-mediated myelin damage. For healthy nerves (A), proteins such as Caspr, contactin, Nfasc, and spectrin comprise axo-glial septate junctions that secure the myelin to the axon at the periphery of the nodes of Ranvier. These act to confine voltage-gated fast K^+ channels to the internodal region. MBP acts to hold the multilayer of myelin together, forming a dense and compact structure of myelin. Intact axonal membranes at the nodal region maintain an ionic balance with low concentrations of intracellular Ca^{2+} , keeping intracellular proteases inactive. Exposure to acrolein results in damage to myelin, MBP, the myelin-axon junction, and the axonal membranes (B). Influx of calcium activates proteases, which may cause further damage to the paranodal axo-glial junction, results in the detachment of myelin from axon and the retraction of myelin. Damage to myelin and MBP leads to myelin decompaction. Ultimately, myelin decompaction and retraction cause exposure of K^+ channels and prevent salutatory conduction, consequently resulting in axonal conduction block and the disruption of neuronal function.

established through various proteins that form the axo-glial complex, including contactin-associated protein (Caspr) [68], neurofascin 155 (NF 155) [69], contactin [70], protein 4.1 [71,72], spectrin, and actin [73,74]. Given the existence of amino acid residues that are vulnerable to acrolein attack in MBP [75–77], acrolein likely reacts readily with the protein to form protein adducts, resulting in the disruption of protein structure and function. Such damage likely culminates in decompaction or splitting of the myelin lamellae. In fact, direct treatment of spinal cord tracts with acrolein resulted in degraded MBP

and concomitant myelin splitting in the paranodal region, visualized by CARS and immunohistochemistry [30]. Damage to MBP mediated by acrolein was shown to be Ca^{2+} -independent, given that incubation with the Ca^{2+} chelator–ethylene glycol tetraacetic acid (EGTA), did not alleviate paranodal myelin splitting [30]. These data are consistent with the hypothesis that acrolein attacks MBP directly, likely forming acrolein-MBP adducts, which does not require calcium.

The existing evidence suggests that myelin damage can also be initiated through acrolein-mediated enzymatic damage by Ca^{2+} -dependent mechanisms. In contrast with myelin splitting, acrolein-mediated retraction of myelin from the paranodal region only occurs in the presence of extracellular Ca^{2+} , since application of EGTA blocked acrolein-mediated myelin retraction [30]. Additionally, calcium is a major coenzyme of proteases such as calcium-activated neutral proteinase (calpain) [78]. Given that calpain has direct access to interact with and damage proteins critical for the function of the axo-glial complex, specifically spectrin and protein 4.1, activation of calpain could lead to the disruption of these structures and the consequent instability of the axo-glial junction, disassociation of myelin from axon, and subsequently, the retraction of myelin at the paranodal region [78,79]. This was demonstrated when acrolein application for 12 h significantly altered the structure of the axo-glial complex as seen by Caspr dissociation from the paranodal region [30]. Axonal neurofascin 186 (NF186) and myelinic NF155 were also shown to separate into three noticeable components, indicative of NF186 remaining in the nodal region, while NF155 receded with the retracting myelin, further demonstrating the disassociation and destruction of paranodal myelin structure [30]. Furthermore, abnormal distribution of Caspr is also observed in cases of chronic MS and associated with myelin damage [80]. Together, this data indicates that acrolein-mediated damage directly affects the myelin sheath and the paranodal protein complex in both a Ca^{2+} -independent (direct) and Ca^{2+} -dependent (indirect) manner, leading to myelin splitting and retraction.

Glutamate excitotoxicity is a recognized mechanism of CNS myelin damage [81–83]. It is known that glutamate can encourage calcium influx through the activation of NMDA receptors [84], where calcium activates calpain, which could lead to myelin damage [78,85,86]. Recent evidence points toward the critical role of acrolein in

mediating demyelination through inhibition of glutamate uptake transporters and enhancement of excitotoxic damage. Elevated levels of glutamate have been observed in the CSF of MS patients where chronic oxidative stress exists [87]. The significance of this finding is given by studies demonstrating that direct application of 1.0 and 0.1 mM glutamate to *ex vivo* spinal cord tissue resulted in splitting and retraction of myelin from the paranodal region, as well as disruption of the axo-glial complex [83]. This demyelination was also observed with exposure and redistribution of K⁺ channels and a consistent decrease in conduction, which could be recovered following application of 100 μ M 4-AP [83]. These characteristics parallel what is observed when acrolein is applied [30]. The link between acrolein and glutamate-mediated demyelination is further highlighted by the demonstrated ability of HNE, an aldehyde produced through LPO similar to acrolein but less reactive, to directly bind to the glutamate uptake transporter GLT-1 (EAAT2), to inhibit its activity and elevate extracellular glutamate levels [32,88–90]. Given the presence of cysteine residues within the active region of GLT-1 and acrolein's high reactivity to cysteine [91], it is likely that acrolein will also bind to this region and inhibit uptake activity. This is further strengthened by the fact that blockage of iGluRs, overactivated by excessive extracellular glutamate, can reduce demyelination, axonal damage, and motor deficits in MS [92]. Therefore, it is likely that acrolein-mediated inhibition of glutamate transporters and the subsequent increase of extracellular glutamate and excitotoxicity may also play key roles in causing acrolein-induced demyelination in both SCI and MS, where glutamate toxicity is a well-established pathological mechanism.

Mitigating acrolein-mediated myelin damage and improving functional recovery

Given its significant detrimental role in promoting myelin damage, neuroprotective strategies targeting acrolein removal have been investigated. Recent studies have demonstrated that the FDA approved antihypertensive hydralazine can scavenge and neutralize both acrolein and acrolein-protein adducts [48,55,64,93–96]. Binding of acrolein to hydralazine is initiated through a Michael addition reaction between the carbonyl and hydrazine functional groups, respectively [97]. Hydralazine application has demonstrated significant benefit by exhibiting therapeutic concentrations in the CNS only 2 h after application [63]. Specifically, 20 μ mol/L of hydralazine was detected in spinal cord tissue 2 h following an intraperitoneal injection (IP), which is comparable with a previous established therapeutic concentration [98]. Furthermore, low doses of hydralazine (1–5 mg/kg), comparable to a safe concentration used to treat pediatric patients (7.5 mg/kg), are still capable of significantly scavenging acrolein in rats and mice [57,62,63]. As no significant adverse side effects were observed with application of lower dosages [56,57], it is demonstrated that hydralazine has great potential for

neuroprotection given its nature to actively scavenge acrolein in the CNS at low concentrations.

Hydralazine's therapeutic benefit has been shown *in vitro* and *in vivo* in SCI and MS. *Ex vivo* SCI studies demonstrated that acrolein-mediated neuronal damage was alleviated in the presence of hydralazine [48,55,64]. Approximately 50 to 70 percent of acrolein levels in the spinal cord are sequestered following application of hydralazine [63]. *In vivo* studies of rat contusive SCI showed that neutralizing acrolein following hydralazine exposure resulted in significant reduction of tissue damage and motor and sensory deficits [62,63]. Similar effects are also observed in a murine model of MS [57]. EAE mice exhibiting elevated levels of acrolein and compromised motor function were treated with hydralazine, which inhibited further myelin damage and behavioral deficits and reversed the progressive symptoms of EAE [57]. Morphological and functional improvements elicited by anti-acrolein treatment further validate the causal role of acrolein in myelin damage. Therefore, there is great potential for hydralazine treatment in cases of MS and SCI where chronic oxidative stress and acrolein are present.

While the blood pressure-lowering properties of hydralazine may contraindicate its use as a carbonyl scavenger in some disease contexts, especially in acute stages when adequate blood perfusion of vital organs is critical, in the context of SCI these pharmacological properties may be desirable. Chronic SCI patients often experience acute hypertension due to autonomic dysreflexia which could lead to life-threatening consequences [99–101], and the systemic vasodilatory effects of hydralazine might be beneficial. Indeed, it has been shown that SCI patients receiving antihypertensive therapies were less likely to have elevated blood pressure and a consequent attack of autonomic dysreflexia [100]. Therefore, in addition to neutralizing acrolein, hydralazine may also offer a therapeutic benefit by curtailing post-SCI hypertension, effectively reducing the risk of autonomic dysreflexia in SCI patients.

Conclusion

Acrolein is likely a critical component of myelin damage in CNS trauma and disease. Elevated tissue and systemic levels have been determined in both SCI and MS. Given the composition of the myelin sheath, it is likely that acrolein can easily attack the lipid membrane and proteins critical for maintaining the axo-myelin junction and axolemma space. Furthermore, acrolein can lead to heightened calpain activation and excitotoxic damage. The mechanisms by which acrolein can damage myelin can be mitigated through acrolein scavenging strategies. *Ex vivo* and *in vivo* hydralazine application has exhibited a neuroprotective effect, reducing myelin damage in spinal cord tissue and improving conduction, indicative of its potential as an effective therapeutic intervention. Given that, like acrolein, many unsaturated aldehyde

electrophiles have been linked to various pathological conditions and likely share similar mechanisms of cellular destruction with acrolein, the knowledge gained in this line of research can facilitate the understanding of other various unsaturated aldehyde electrophiles. In addition, some known acrolein scavengers are also capable of scavenging other unsaturated aldehydes such as HNE and MDA, further strengthening the broader application of such knowledge in combating these unsaturated aldehyde toxicants [98]. Among many possible future studies that could advance our understanding of unsaturated aldehyde toxicants in the pathological processes of neuronal trauma and disease, the confirmation and characterization of protein damage by various unsaturated aldehyde electrophiles has great potential to further advance this line of work. This will require the use of a more chemically definitive method or more contemporary approaches, such as the biotin-tagging approach, to quantify the augmented protein adduction by LPO products—unsaturated aldehyde electrophiles—in relevant SCI and MS models [102]. Acquisition of additional data characterizing interactions between the aldehydes discussed and a variety of proteins will not only facilitate the utility of acrolein-protein adducts as biomarkers correlated with pathological changes, but also as an evaluation index of the effectiveness of anti-acrolein or anti-aldehyde treatment.

Declaration of interest

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