

● PERSPECTIVE

Potassium channel blockers restore axonal conduction in CNS trauma and diseases

Myelin damage in the central nervous system (CNS) plays an important role in motor and sensory dysfunction (Shi and Sun, 2011). This neuropathology is observed widely in neurotrauma such as spinal cord injuries (SCI) and is also a distinguishing feature of many neurological diseases such as multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) (Shi and Sun, 2011). Damage to myelin structure leads to severe consequences at both a macromolecular and systems levels, which remains as one of the most challenging medical problems for both basic researchers and clinicians (Shi and Sun, 2011). Consequently, few successful therapeutic strategies exist today that can reverse or even slow the progression of this CNS pathology. Understanding the mechanisms underlying myelin damage is crucial for designing new, more effective treatments to mitigate symptoms and bring hope to millions of victims.

The molecular architecture of myelin in the CNS has been relatively well-characterized. Composed primarily of lipids, myelin extends from oligodendrocytes to wrap itself tightly around neighboring axons, resulting in highly conserved distinct structural elements: the paranodal, juxtapanodal, and internodal regions (Poliak and Peles, 2003). In the paranodal region, myelin forms a physical link with axons through the axo-glial protein junction; a complex composed of multiple proteins expressed in both axons and myelin. Nodes of Ranvier, which interrupt the myelinated segments, reveal axolemmae lacking myelin and therefore allowing for direct exposure of the axonal membrane, which contains high density of voltage-gated sodium channels, to the extracellular environment. The presence of nodal regions is vital for axonal conduction of electrical signals in the form of action potentials (APs), which are generated through depolarization of the membrane and subsequent activation of voltage-gated sodium channels. Once an AP is triggered at one node, it will drive the positive charge towards the next node, leading to membrane depolarization and consequential generation and propagation of AP, a process known as saltatory conduction. Interestingly, although absent in the node of Ranvier, voltage-gated potassium channels exist in the axonal membrane ensheathed by myelin, the juxtapanodal region. As such, while the myelin sheath renders the neurons capable of the rapid and efficient conductance of action potentials, such unique cellular structure also makes the axons vulnerable to mechanical insults which can lead to impulse conduction block (Poliak and Peles, 2003; Shi and Sun, 2011).

In cases of neurotrauma, we and others have uncovered evidence that myelin damage could result from both primary physical trauma and secondary biochemical insults (Shi and Sun, 2011; Shi et al., 2011, 2015; Sun et al., 2012). In neurodegenerative diseases that lack significant physical trauma, such as MS, biochemical insults are the dominating causative factors of myelin damage (Shi et al., 2011, 2015). Recently, we have demonstrated these two distinct phases, primary and secondary myelin damage, in an *ex vivo* acute spinal cord injury model. Specifically, we showed that a mechanical stretch injury produced immediate myelin damage indicated by lengthening of the nodal region and the retraction of myelin from the paranodal region, a process primarily influenced by the rate and mag-

nitude of physical force (Sun et al., 2012). Interestingly, computational modeling revealed that the paranodal region is a site of stress concentration when axons are deformed via mechanical loading, supporting the observation that physical force alone can cause myelin damage, especially in the paranodal region (Shi and Sun, 2011; Babbs and Shi, 2013).

It is important to note that, in the event of CNS trauma, mechanically-induced myelin disruption is followed by chemically-induced myelin damage. Using an *ex vivo* spinal cord injury model we have shown that chemical factors not only exacerbate primary myelin damage, but also elicit myelin damage in the absence of physical forces (Shi et al., 2011; Sun et al., 2012). As one of the most well-established injurious biochemical mechanisms, oxidative stress is known to be especially detrimental to myelin integrity due to its diffusive nature and high lipid reactivity (Shi et al., 2015). We have shown that acrolein, both a product and a catalyst of lipid peroxidation and therefore a perpetuator of oxidative stress, can directly attack myelin (Shi et al., 2011, 2015). Elevated acrolein is observed in experimental SCI and MS animal models where myelin damage is a well-established pathology (Shi et al., 2015). Furthermore, application of acrolein at a pathologically relevant concentration to *ex vivo* spinal cord tissue resulted in myelin splitting and retraction, nodal lengthening, potassium channel exposure and relocation and loss of axonal conduction (Shi et al., 2011). Finally, lowering acrolein levels in experimental MS and SCI leads to a reduction of myelin damage and improved axonal conduction (Shi et al., 2015). These studies strongly suggest that acrolein is a key factor in myelin damage in both SCI and MS.

As one of the key proteins critical for myelin integrity, myelin basic protein (MBP) establishes a physical connection between the layers of myelin lamellae which ensures the dense, compact structure of myelin (Shi et al., 2015). We have provided clear evidence that MBP is damaged when myelin is exposed to acrolein in *ex vivo* experiments (Shi et al., 2011). Interestingly, acrolein-induced MBP damage and consequential myelin splitting and decompaction of the myelin lamellae is not dependent on Ca^{2+} . This finding suggests that the acrolein/MBP interaction is likely direct and not mediated through Ca^{2+} -dependent pathways (Shi et al., 2011). Conversely, we have provided compelling evidence that acrolein-mediated paranodal myelin retraction is mediated by Ca^{2+} -dependent activation of calpain, which in turn breaks down the axo-glial complex. One key piece of evidence we have obtained demonstrating the acrolein-mediated disintegration of the axo-glial junction is that contactin-associated protein (Caspr), a key component of the complex that originates from the axonal membrane but is closely associated with myelin, is separated from myelin following acrolein exposure (Shi et al., 2011). These findings further implicate the existence of acrolein-mediated myelin damage in both SCI and MS.

It is well established that exposure of potassium channels in the juxtapanodal region due to retraction of paranodal myelin is the key pathological mechanism underlying axonal conduction failure (Poliak and Peles, 2003; Shi and Sun, 2011; Shi et al., 2011). This notion is supported by the fact that potassium channel blockers, such as 4-aminopyridine (4-AP), could restore conduction in injured axons with myelin damage, a result also demonstrated through mathematical modeling (Jensen and Shi, 2003; Babbs and Shi, 2013). In light of this, one of the most successful strategies for restoring function in injured axons to date is the application of a potassium channel blocker. By reducing potassium efflux, such blockers increase paranodal membrane resistance and increase the probability of adequate membrane depolarization at the nodes of Ranvier and, ultimately, the generation of APs (Shi and Sun, 2011).

We and others have shown that 4-AP, a potassium channel blocker known to block fast (A-type) channels expressed in the juxtaparanodal region, could restore axonal function following spinal cord injury (Shi and Sun, 2011). Specifically, 4-AP could augment the amplitude of compound action potentials (CAPs) of a physically-injured cord segment in an *ex vivo* preparation (Jensen and Shi, 2003). The CAP was measured with double sucrose gap technique developed in our lab (Jensen and Shi, 2003). In addition, we have noted that 4-AP could also restore motor function in an *in vivo* study of rat contusive SCI (unpublished observation). These findings not only further support the role of potassium channels in axonal conduction failure stemming from myelin damage, but further suggest potassium channel blockade as an effective strategy to restore axonal conduction and neurological functions in CNS trauma and diseases.

As the most studied potassium channel blocker for reestablishing function following nerve injury, 4-AP has been investigated extensively as the leading compound for axonal functional restoration efforts following myelin damage in both SCI and MS (Shi and Sun, 2011). While the use of 4-AP was approved for MS patients following successful clinical trials, the compound unfortunately failed to demonstrate significant benefits for SCI victims in phase three clinical trials (Shi and Sun, 2011). The failure of 4-AP clinical trials in treating SCI patients was, among other factors, largely due to significant adverse side effects and a narrow range for safe clinical dosing (Sun et al., 2010; Shi and Sun, 2011). Therefore, while potassium channel blockade remains an important mechanism for restoring signal conduction due to myelin damage, better tolerated and more effective potassium channel blockers need to be developed to re-establish function in SCI patients. Motivated by such a need, we have investigated a variety of 4-AP derivatives as potential alternatives for blocking potassium channels (Sun et al., 2010). One in particular, 4-aminopyridine-3-methanol (4-AP-3-MeOH), showed similar efficacy, a 10-fold increased potency and overall less side effects in restoring electrical conduction in injured axons following mechanical insult (Sun et al., 2010). Therefore, while 4-AP seems like the logical choice to treat demyelination in the CNS, 4-AP-3-MeOH emerges as a viable alternative and perhaps an even stronger candidate for functional restoration in SCI patients.

While 4-AP-3-MeOH clearly shows strong efficacy in restoring axonal conduction when compared to 4-AP in mechanically injured spinal cords, such comparison has not been conducted in chemically-injured axons prior to the current study (Jensen and Shi, 2003; Sun et al., 2010). The current investigation was the first to examine the effect of 4-AP-3-MeOH in restoring spinal cord CAP amplitude following the exposure of acrolein which is known to cause myelin damage (Yan et al., 2016). In addition, both 4-AP and 4-AP-3-MeOH were tested side-by-side in the same study for the first time. This study confirmed that 4-AP-3-MeOH works to restore conduction in axons with acrolein-mediated myelin damage and also displayed greater efficacy and wider therapeutic range than 4-AP. Furthermore, 4-AP-3-MeOH restores axonal conduction without negatively affecting the property of either the absolute and relative refractory periods or repetitive firing. On the other hand, 4-AP treatment resulted in an increase of both the relative and absolute refractory periods and the reduction of axonal capabilities to follow train stimuli. This phenomenon has been observed in both mechanically and chemically-injured axons (Jensen and Shi, 2003; Sun et al., 2010; Yan et al., 2016). Taken together, the results from this and other studies confirm that acrolein-mediated myelin damage, both in absence of (MS) or following CNS trauma (SCI), leads to potassium channel exposure and

conduction block, which can then be reversed by potassium channel blockers, particularly 4-AP-3-MeOH.

It is clear that functional deficits as a result of physically induced myelin damage can be reversed through the application of potassium channel blockers such as 4-AP and 4-AP-3-MeOH (Jensen and Shi, 2003; Sun et al., 2010; Shi and Sun, 2011; Yan et al., 2016). While 4-AP has previously been shown to be effective for improving axonal conduction caused solely by the chemical injury such as acrolein, a key factor in oxidative stress, we now know that 4-AP-3-MeOH has similar capabilities with perhaps even greater efficacy and fewer side effects (Shi and Sun, 2011; Yan et al., 2016). Consistent with the current study, 4-AP-3-MeOH has been shown to improve axonal conduction in EAE mice, the leading animal model of MS and is known to be associated with acrolein pathology (Leung et al., 2011). In summary, it appears that 4-AP-3-MeOH is effective in restoring axonal conduction as a result of both mechanical and chemical insults in animal models. The significance of these findings could potentially be translated into the effective use of 4-AP-3-MeOH for the treatment of axonal damage in both trauma and disease and serves as a viable alternative for 4-AP.

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References

- Babbs CF, Shi R (2013) Subtle paranodal injury slows impulse conduction in a mathematical model of myelinated axons. *PLoS One* 8:e67767.
- Jensen JM, Shi R (2003) Effects of 4-aminopyridine on stretched mammalian spinal cord: the role of potassium channels in axonal conduction. *J Neurophysiol* 90:2334-2340.
- Leung G, Sun W, Brookes S, Smith D, Shi R (2011) Potassium channel blocker, 4-Aminopyridine-3-Methanol, restores axonal conduction in spinal cord of an animal model of multiple sclerosis. *Exp Neurol* 227:232-235.
- Poliak S, Peles E (2003) The local differentiation of myelinated axons at nodes of Ranvier. *Nat Rev Neurosci* 4:968-980.
- Shi R, Sun W (2011) Potassium channel blockers as an effective treatment to restore impulse conduction in injured axons. *Neurosci Bull* 27:36-44.
- Shi R, Page JC, Tully M (2015) Molecular mechanisms of acrolein-mediated myelin destruction in CNS trauma and disease. *Free Radic Res* 49:888-895.
- Shi Y, Sun W, McBride JJ, Cheng JX, Shi R (2011) Acrolein induces myelin damage in mammalian spinal cord. *J Neurochem* 117:554-564.
- Sun W, Fu Y, Shi Y, Cheng JX, Cao P, Shi R (2012) Paranodal myelin damage after acute stretch in Guinea pig spinal cord. *J Neurotrauma* 29:611-619.
- Sun W, Smith D, Fu Y, Cheng JX, Bryn S, Borgens R, Shi R (2010) Novel potassium channel blocker, 4-AP-3-MeOH, inhibits fast potassium channels and restores axonal conduction in injured guinea pig spinal cord white matter. *J Neurophysiol* 103:469-478.
- Yan R, Page JC, Shi R (2016) Acrolein-mediated conduction loss is partially restored by K⁺ channel blockers. *J Neurophysiol* 115:701-710.