Neurosci Bull February 1, 2011, 27(1): 36-44. http://www.neurosci.cn
DOI: 10.1007/s12264-011-1048-y

·Minireview·

Potassium channel blockers as an effective treatment to restore impulse conduction in injured axons

Riyi SHI, Wenjing SUN

Department of Basic Medical Sciences, School of Veterinary Medicine, Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana 47907, USA

Abstract: Most axons in the vertebral central nervous system are myelinated by oligodendrocytes. Myelin protects and insulates neuronal processes, enabling the fast, saltatory conduction unique to myelinated axons. Myelin disruption resulting from trauma and biochemical reaction is a common pathological event in spinal cord injury and chronic neurodegenerative diseases. Myelin damage-induced axonal conduction block is considered to be a significant contributor to the devastating neurological deficits resulting from trauma and illness. Potassium channels are believed to play an important role in axonal conduction failure in spinal cord injury and multiple sclerosis. Myelin damage has been shown to unmask potassium channels, creating aberrant potassium currents that inhibit conduction. Potassium channel blockade reduces this ionic leakage and improves conduction. The present review was mainly focused on the development of this technique of restoring axonal conduction and neurological function of demyelinated axons. The drug 4-aminopyridine has recently shown clinical success in treating multiple sclerosis symptoms. Further translational research has also identified several novel potassium channel blockers that may prove effective in restoring axonal conduction.

Keywords: axon; conduction; potassium channel; injury; demyelination; 4-aminopyridine

1 Introduction

The majority of the axons in vertebrate nervous systems are wrapped with an insulating material called myelin. It is this myelin sheath that enables rapid and efficient conductance of action potential by neurons[1,2]. However, the unique cellular structure that enables efficient saltatory conduction is vulnerable to mechanical insults which can lead to impulse conduction block. Specifically, myelin damage is associated with axonal conduction loss in trauma and chronic neurodegenerative diseases[3-10]. The mechanisms of myelin damage-induced axonal conduction loss have attracted much attention these years. Unveiling of this question can help overcome the deficits and restore axonal conduction and the overall neurological function in demyelinating diseases and trauma. The translational research has achieved great progresses in developing effective therapeutic interventions to restore function. This review mainly discussed the role of axonal potassium channels in conduction loss in spinal cord mechanical injury as well as in other conditions characterized by myelin damage such as multiple sclerosis. The preclinical development of potassium channel blockers as effective tools for axonal conduction restoration was specially focused on. Recent clinical development was also discussed.

Corresponding author: Ri-Yi SHI
Tel: +1-765-496-3018; Fax: +1-765-494-7605
E-mail: riyi@purdue.edu
Article ID: 1673-7067(2011)01-0036-09
Received date: 2010-12-21; Accepted date: 2011-01-06

2 Normal structure of myelinated axons in the central nervous system (CNS)

In the vertebrate CNS, most axons are myelinated by oligodendrocytes which enwrap the axons. These sheathe the axon in longitudinal segments that are interrupted by bare portions called nodes of Ranvier. By reducing the capacitance and increasing the transverse resistance, myelin significantly reduces current flow across axonal membranes, and therefore increases longitudinal conduction. This system enhances electrical impulse transmission, as depolarization jumps from node to node in a process called saltatory conduction. Because the action potentials are only generated at the node of Ranvier, conduction through this system is faster and much more efficient than conduction through unmyelinated axons[2,11,12].

Saltatory conduction relies on both myelin encasement and the asymmetric distribution of ionic channels[2,11,12]. Compared to the internodal region, the node of Ranvier has a significantly higher density of sodium channels, which enables the generation of action potential at the node. Specifically, when the node membrane is adequately depolarized by incoming current, voltage-dependent sodium channels will open, allowing sodium influx and initiating the action potential. Potassium channels reside at a high density beneath the myelin at the juxtaparanodel area, separated from the node by the paranodal region[2,11,12]. Although the functions of these potassium channels in normal healthy axons remain entirely unclear, these channels are thought to be involved in stabilizing the axonal membrane potential[13-16].

The paranodal region where myelin is physically connected to the axon, serves as a physical barrier to maintain the asymmetric distribution of sodium and potassium channels, which is essential for saltatory conduction[12,17,18]. Tight septate junctions connect the axonal and myelin membranes. This axo-glial structure is composed of several proteins including contactin associated protein (Caspr or paranodin) and F3/contactin from axonal side, and glial neurofasin 155[12]. The axo-glial junction is critical for maintaining the properly specialized domains characteristic of normal myelinated axons and insulating the juxtaparanodal potassium channels from the extracellular environment[12]. However, there is evidence that mechanical trauma to the spinal cord can produce significant stress in this axonal region[19]. The induced damage to the myelin barrier can produce significant functional consequences in injured axons[4,9,10,19-22].

3 Paranodal myelin damage: anatomical and functional evidence

Research over the last several decades has come to a general agreement that mechanical insult to the CNS results in myelin damage, especially in the paranodal region[10,19,22-26]. This is consistent with the mathematical modeling showing significant stress in this area under mechanical insults[19]. Such myelin damage may result in disruption of the axo-glial junction and cause retraction of myelin away from the node[10,19]. This would then induce exposure or unmasking of potassium channels that otherwise reside underneath the myelin and are physically separated from the extracellular environment[10,12,19]. The exposure of voltage-dependent potassium channels has been widely demonstrated, using various labeling and imaging techniques. For example, Fehlings and colleagues have observed abnormal distributions of potassium channels following mechanical injury[23,24]. However, these studies did not simultaneously image myelin, so the relationship between myelin and the underlying potassium channels remained unclear.

In a recent study, using a combination of traditional (coherent anti-stokes Raman scattering, CARS) and novel multimodal imaging techniques (two photon excitation fluorescence microscopy)[27], Shi and colleagues provided unequivocal anatomical evidence demonstrating the exposure of potassium channels following mechanical insult in guinea pig spinal cord[10,19]. By using CARS to image unlabelled myelin and observing immunofluorescence of stained potassium channels, they demonstrated for the first time that myelin recedes away from the node following trauma, and exposes potassium channels[10,19]. Besides, it is difficult or even impossible for other methods to obtain such an observation[23,24,28]. These findings are consistent with observations under similar situations and further confirm the previous hypothesis[4,9,20,23,24,29].

In addition to the overwhelming evidence of myelin dam-

age in chronic mechanical trauma[4,20,22-24,26,30], acute trauma can also affect myelin[9,10,19]. Several studies have revealed myelin displacement immediately following mechanical insults such as stretch and compression[9,10,19]. In a recent study, a finite element mathematical model suggested that mechanical compression would produce high stresses in the paranodal axonal-glial junction[19]. This is consistent with the reported observations that this region is vulnerable to mechanical injury[9,10]. Furthermore, it also confirms that mechanical deformation alone is sufficient to rupture the tight, septate junctions that join myelin to the axonal membrane. However, such acute myelin damage is likely to be exacerbated by the secondary biochemical cascades that are also known to damage myelin[31,32]. Therefore, the severe myelin damage observed in chronic nerve injury almost certainly results from both acute, mechanical trauma and the secondary biochemical deterioration.

Parallel to the anatomical findings, electrophysiological evidence from various sources also strongly supports that potassium channel activity plays an important role in axonal conduction loss in demyelinated axons. Bostock *et al.*[33,34] were the first to show that 4-aminopyridine (4-AP) application could enhance conduction in rat spinal nerve roots. Similar results were reported by Targ and Kocsis describing restoration of axonal conduction in demyelinated rat sciatic nerves *ex vivo*[35]. Blight[36] pioneered these treatments in the central nervous system by restoring nerve conduction in cats with chronic spinal cord injury using 4-AP. Blight and colleagues further expanded this line of research showing that 4-AP improved motor behavioral response in chronic spinal-injured cats[37]. These studies have paved the way for the extensive laboratory testing of 4-AP[4,9,20,38] and the development of 4-AP usage in clinical applications[39-43]. These studies have suggested 4-AP as a leading choice of potassium channel blocker to restore axonal conduction in demyelinated peripheral and central nervous system nerves.

4 Suggested mechanisms of 4-AP-mediated restoration of axonal conduction in spinal cord injury

Exposure of potassium channels in the juxtaparanodal region is believed to be the underlying mechanism of conduction block in demyelinated axons[9,10,19]. Under normal conditions, deplorization at the node of Ranvier will drive potassium ions down to the electrical gradient. As a result, potassium ions flow within the axon, through the internodal region as a major intracellular positive charge carrier, and produce depolarization adequate in degree and duration to trigger an action potential in the next adjacent node. However, when the potassium channels in the juxtaparanodal region are exposed to the extracellular space, the potassium ions moving through the axoplasm will exit through these channels before reaching the nodal region. If this potassium exodus is severe, nodal depolarization may fail to reach a level adequate for sufficient sodium influx that triggers an action potential, thus preventing signal conduction (Fig. 1).

The second proposed mechanism of axonal conduction failure in demyelinated axons may be related to the duration of the Na^+ action current[40]. Potassium leakage due to channel exposure may shorten the duration of an action potential, resulting in reduction of the total amount of current, thus lessening the safety factor for conduction across the demyelinated internode. The safety factor is indicated as the ratio of current generated by an action potential to the minimal amount of current that ensures conduction. Reducing the safety factor may increase the likelihood of conduction block. In summary, exposure of potassium channels as a result of myelin damage can shorten circuit normal conduction and prevent adequate depolarization of nodal membrane, leading to conduction failure[9,20,36].

The potential therapeutic roles of potassium channel blockers are therefore obstructing the channels and reducing potassium efflux. These will increase the probability of action potential genesis and the duration of depolarization, therefore, the magnitude of sodium influx. By this way, the likelihood for conduction across the demyelinated internode will be increased. All these factors cooperate to ensure the transmission of action potential through the area of myelin damage, thereby restoring axonal conductivity. In this sense, potassium channel blockers function almost as artificial myelin, insulating the axons and preventing depolarizing transmembrane currents (Fig. 1).

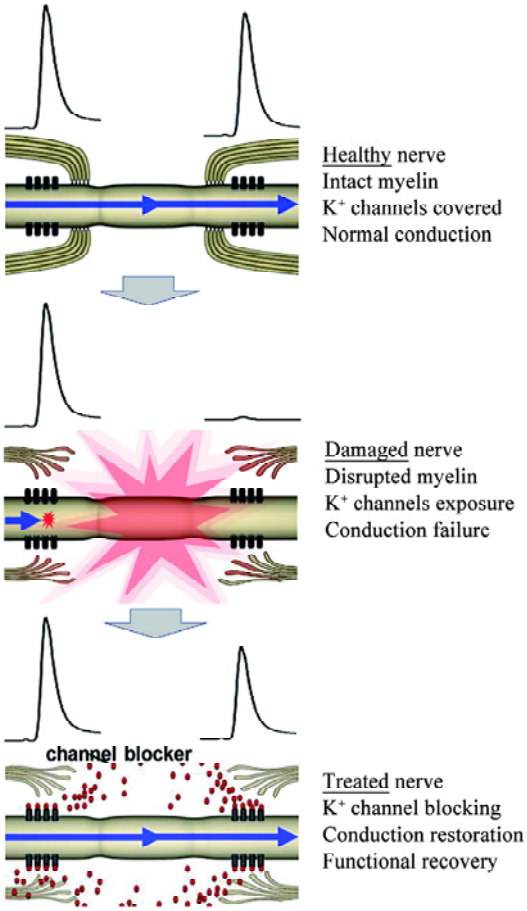

Fig. 1 A diagrammatic depiction of myelin damage in injured axons and the functional restoration by potassium channel blockade.

5 Pharmacology and dose-response of 4-AP-mediated conduction restoration

The sensitivity of vertebrate neuronal potassium channels to 4-AP has been explored in a wide array of physiological preparations[9,33-36,38], although only a handful of studies have examined a wide range of concentrations[4, 20]. Using a double sucrose-gap recording technique suitable for lengthy *ex vivo* examinations, Shi and colleagues have established the dose response curve for 4-AP treatment of both acute and chronic mechanical injury in isolated spinal cord white matter from guinea pig[20]. It is shown that concentration between 0.1-1 μmol/L is the threshold for 4-AP to produce significantly beneficial effects against both acute (hours) and chronic (weeks) mechanical injuries. In acute injury, the maximal effective concentration is 100 μmol/L while 10 μmol/L can maximize conduction restoration against chronic injuries. It has been estimated that the maximum safe serum concentration of 4-AP in human testing is around 0.1-1 μmol/L[42]. At this concentration, 4-AP exerts some beneficial neurological effects[41,42]. Therefore, the data from *ex vivo* testing support the argument that the clinically observed beneficial effects of 4-AP could be attributed to the enhanced axonal conduction across demyelinated internodes.

Another interesting finding from *ex vivo* experiments is that the effect of 4-AP is biphasic in restoring axonal conduction: 4-AP enhances conduction at micro-molar levels but suppresses it at concentrations above 1 mmol/L[4,20]. Negative effects on axonal conduction produced by 4-AP above 1 mmol/L were observed in acutely injured guinea pig spinal cord and were even noted in uninjured nerve fiber[4]. The concurrent depolarization shown by the sucrose-gap recordings may be due to the blockade of resting potassium conductance by 4-AP, and may partially explain the reduction in compound action potential amplitude. Depolarization of the axon would increase the inactivation of sodium channels in the nodal membrane. In this case, the decrease in amplitude of the compound action potential would result from a decreased amplitude of the action currents themselves, but would not necessarily reflect conduction failure. In fact, higher concentrations of 4-AP may improve conduction in injured axons while simultaneously decreasing the amplitude of the compound potential recorded from the uninjured part of the spinal cord strip. This may explain why 1 mmol/L 4-AP was found to improve conduction in some single-fiber recordings and increase the temperature of conduction block in one study[36], while similar concentrations reduced the compound action potential amplitude in another study[20].

6 Change of refractoriness in 4-AP-mediated axonal conduction restoration

Although 4-AP can induce a significant conduction recovery, there seems to be a concomitant increase of refractoriness in recovered spinal cords. This is evident in a study of Jensen *et al.*, showing that 4-AP significantly increased the lengths of both absolute and relative refractory periods

in acutely stretched guinea pig spinal cord[9]. In addition, the overall responsiveness to multiple stimuli was also decreased by 4-AP in the same samples[9]. Similar phenomena were noted by Targ and Kocsis in sciatic nerve[44]. The newly recruited spinal cord axons are likely to be responsible for this change. The primary cause of the enhanced refractoriness is not clear. It is possible that although some axons are able to conduct with blockade of voltage-dependent potassium channels, the safety factor for conduction remains significantly compromised by the damage to paranodal structures and the increased capacitive load provided by the internodal membrane[45]. It could also be attributed to the inhibited resting potassium conductance. The subsequent depolarization of axons may increase the inactivation of sodium channels in the nodal membrane and therefore reduces the responsiveness to dual and repetitive stimuli. Such mechanism may not prevent the genesis of the single action potential, but the condition would be exacerbated with multiple action potentials. Nevertheless, the exact cellular mechanism underlying the increase in refractoriness at the maximal effective level of 4-AP remains to be determined.

7 The effects of 4-AP in human testing

Due to its success in restoring axonal conduction in demyelinated axons in animal studies, 4-AP has been used in treating human demyelinating diseases, mainly spinal cord injury and multiple sclerosis. Although promising, 4-AP has not yet been approved for clinical use in spinal cord injury, a devastating disease with no established treatments despite years of investigation. It should be noted that 4-AP also fails to elicit statistically significant improvements when tested against a placebo in several clinical trials[46,47], including the 2 recently completed Phase III studies[47]. Nevertheless, two 4-AP trials have found statistically significant improvements in functional recovery[48, 49], suggesting that potassium channel blockade is still a viable mechanism for potentially restoring signal conduction in patients with spinal cord injury.

Similar to neurotrauma, multiple sclerosis has a long history of clinical testing of 4-AP[50-61]. Recently, a series of carefully designed clinical trials have provided significant improvement in walking ability of multiple sclerosis patients[43,62-64]. Since 4-AP has a half life of 3.5 h, frequent dosing is essential for maintaining effective serum concentrations[65]. To overcome this problem, a slow release formula has been developed, which significantly facilitates its clinical usage[66]. This leads to the final FDA approval of 4-AP for treating multiple sclerosis symptoms[43,62-64]. 4-AP is the first and thus far the only drug to improve ambulation in multiple sclerosis patients.

8 The limitations of 4-AP clinical use in spinal cord injury

While 4-AP has been demonstrated to be capable of improving sensory and motor functions in both animal and human spinal cord injuries, the overall therapeutic benefit of 4-AP remains modest[41,42]. One contributing factor is the discrepancy between the maximum tolerable blood level of 4-AP in human patients (0.5-1 μmol/L) and the most effective concentrations demonstrated *in vitro* (100 μmol/L in acute injury and 10 μmol/L in chronic injury)[4,20]. Concentrations beyond 1 μmol/L may increase the probability of side effects such as respiratory distress, anxiety, and epileptiform seizures[67-69]. Possible causes for the negative side effects associated with higher doses of 4-AP include increased synaptic transmission and additional blockade of potassium channel currents associated with the resting membrane potential[70]. Therefore, it seems that the narrow therapeutic range of 4-AP limits its clinical effectiveness.

9 4-AP derivatives as novel potassium channel blockers to restore axonal function

In order to overcome the limitations of 4-AP, efforts have been made to identify compounds whose structures are similar to, yet distinct from, 4-AP[10,71]. Such compounds may enhance conduction in the injured spinal cord as effectively as 4-AP, but with wider therapeutic ranges and fewer side effects[10,72]. Shi and colleagues have demonstrated that 4 newly identified analogs are capable of enhancing axonal conduction following *ex vivo* spinal cord trauma[10,72], including N-(4-pyridyl)-methyl carbamate, N-(4-pyridyl)-ethyl carbamate, N-(4-pyridyl)-tertbutyl (tBoc), and 4-aminopyridine-3-methanol (4-AP-3-MeOH). They have also

shown that these compounds are indeed capable of inhibiting 4-AP-sensitive fast potassium channels[10,73]. All these compounds can significantly enhance conduction in spinal cord white matter following mechanical injury[10, 72]. Similar to 4-AP, the derivatives do not preferentially enhance conduction based on axonal caliber[9,10,72]. Moreover, unlike 4-AP, the derivatives do not change the overall electrical responsiveness of axons to dual and multiple stimuli. Specifically, the relative and the absolute refractory periods remain the same following the application of the 4 analogs. In addition, the ability to follow multiple stimuli remained unchanged in analog-affected axons, compared with the healthy samples[10,72]. These data indicate that axons recruited by the derivatives conduct in a similar manner as healthy axons.

Dose-response studies have shown that the the first 3 compounds have similar thresholds in restoring impulse conduction as that of 4-AP (0.1-1 μmol/L)[74]. However, 4-AP-3-MeOH has a higher potency. The observed lowest effective concentration of 4-AP-3-MeOH to enhance conduction is between 0.01-0.1 μmol/L, a 10-fold increase of potency compared with the other 3 derivatives and 4-AP[10]. Since the side effects of 4-AP are dose-related[20], it is reasonable to speculate that a lower effective dosage may be accompanied by a reduced probability of inducing serious side effects. These results demonstrate that the novel constructs are promising alternatives of 4-AP for the purpose of reversing conduction deficits.

Besides enhancing action potential conduction in *ex vivo* preparations, 4-AP-3-MeOH and N-(4-pyridyl)-methyl carbamate can also inhibit "fast" or 4-AP–sensitive potassium current, as demonstrated by patch clamp recording techniques[10,73]. Interestingly, 4-AP-3-MeOH inhibits approximately 75% of 4-AP sensitive K^+ current, significantly more than that of methyl carbamate which inhibits only 30%[10,73]. As gauged by blocking the 4-AP-sensitive current, 4-AP-3-MeOH is a more effective blocker than methyl carbamate, which is more effective than ethyl carbamate and tBoc in restoring action potential[10,73]. This may in part contribute to the 10-fold increase in the potency of 4-AP-3-MeOH compared to methyl carbamate[10,72,74].

10 Conclusions

It is well established that the increase in potassium channel activity is an important mechanism of conduction failure in spinal cord injury and multiple sclerosis. Suppression of aberrant potassium current by potassium channel blockade is an effective way for restoring signal conduction in demyelinated axons. 4-AP treatment restores conduction and neurological function *in vitro*, in animal models, and in clinical trials. However, its clinical application is limited by the narrow therapeutic range and the severe side effects. Several new compounds have exhibited efficacy in restoring action potential conduction in injured axons. Of these potassium channel blockers, 4-AP-3-MeOH is more potent than 4-AP. Furthermore, compared with the 4-AP-rescued axons, the derivative-rescued axons conduct electric impulses in a manner more similar to that of healthy axons. Therefore, these new derivatives are potential alternatives to 4-AP in reversing conduction block in spinal cord injury and other demyelinating diseases. Currently, 4-AP is under study for its use in treating spinal cord injury, because blocking potassium channels remains a viable strategy in restoring axonal conduction and improving overall neurological function. Further studies on potassium blockade therapy for treating demyelinating diseases are warranted, as 4-AP has been approved to be capable of improving ambulatory ability in multiple sclerosis patients.

Acknowledgements: This work was supported by the Purdue Research Foundation (No. 61133) and National Institute of Health, USA (No.1R21 NS050174-01A1). We also thank Mr. Todd Rickett for his critical reading of this manuscript.

References:

[1] Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science. 4th ed. New York: McGraw-hill, 2000.

[2] Waxman SG, Kocsis JD, Stys PK. The Axon. New York Oxford: Oxford University Press, 1995.

[3] Blight AR. Delayed demyelination and macrophage invasion: a candidate for secondary cell damage in spinal cord injury. Central Nervous System Trauma 1985, 2: 299-315.

[4] Shi R, Blight AR. Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord. Neuroscience 1997, 77: 553-562.

[5] Nashmi R, Fehlings MG. Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord. Neuroscience 2001, 104: 235-251.

[6] Waxman SG. Membranes, myelin, and the pathophysiology of multiple sclerosis. N Engl J Med 1982, 306: 1529-1533.

[7] Waxman SG. Demyelinating diseases—new pathological insights, new therapeutic targets. N Engl J Med 1998, 338: 323-325.

[8] Waxman SG. Ion channels and neuronal dysfunction in multiple sclerosis. Arch Neurol 2002, 59: 1377-1380.

[9] Jensen JM, Shi R. Effects of 4-aminopyridine on stretched mammalian spinal cord: the role of potassium channels in axonal conduction. J Neurophysiol 2003, 90: 2334-2340.

[10] Sun W, Smith D, Fu Y, Cheng JX, Bryn S, Borgens R*, et al.* 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 2010, 103: 469-478.

[11] Waxman SG, Ritchie JM. Molecular dissection of the myelinated axon. Ann Neurol 1993, 33: 121-136.

[12] Poliak S, Peles E. The local differentiation of myelinated axons at nodes of Ranvier. Nat Rev Neurosci 2003, 4: 968-980.

[13] Vabnick I, Trimmer JS, Schwarz TL, Levinson SR, Risal D, Shrager P. Dynamic potassium channel distributions during axonal development prevent aberrant firing patterns. J Neurosci 1999, 19: 747-758.

[14] Zhou L, Zhang CL, Messing A, Chiu SY. Temperature-sensitive neuromuscular transmission in Kv1.1 null mice: role of potassium channels under the myelin sheath in young nerves. J Neurosci 1998, 18: 7200-7215.

[15] Chiu SY. Asymmetry currents in the mammalian myelinated nerve. J Physiol 1980, 309: 499-519.

[16] Chiu SY, Ritchie JM. On the physiological role of internodal potassium channels and the security of conduction in myelinated nerve fibres. Proc R Soc Lond B Biol Sci 1984, 220: 415-422.

[17] Peles E, Salzer JL. Molecular domains of myelinated axons. Curr Opin Neurobiol 2000, 10: 558-565.

[18] Salzer JL, Brophy PJ, Peles E. Molecular domains of myelinated axons in the peripheral nervous system. Glia 2008, 56: 1532-1540.

[19] Ouyang H, Sun W, Fu Y, Li J, Cheng JX, Nauman E*, et al.* Compression induces acute demyelination and potassium channel exposure in spinal cord. J Neurotrauma 2010, 27: 1109-1120.

[20] Shi R, Kelly TM, Blight AR. Conduction block in acute and chronic spinal cord injury: Different dose-response characteristics for reversal by 4-Aminopyridine. Exp Neurology 1997, 148: 495-501.

[21] Waxman SG. Demyelination in spinal cord injury and multiple sclerosis: what can we do to enhance functional recovery? J Neurotrauma 1992, 9: S105-117.

[22] Blight AR. Morphometric analysis of a model of spinal cord injury in guinea pigs, with behavioral evidence of delayed secondary pathology. J Neurol Sci 1991, 103: 156-171.

[23] Nashmi R, Jones OT, Fehlings MG. Abnormal axonal physiology is associated with altered expression and distribution of Kv1.1 and Kv1.2 K^+ channels after chronic spinal cord injury. Eur J Neurosci 2000, 12: 491-506.

[24] Karimi-Abdolrezaee S, Eftekharpour E, Fehlings MG. Temporal and spatial patterns of Kv1.1 and Kv1.2 protein and gene expression in spinal cord white matter after acute and chronic spinal cord injury in rats: implications for axonal pathophysiology after neurotrauma. Eur J Neurosci 2004, 19: 577-589.

[25] McDonald JW, Belegu V. Demyelination and remyelination after spinal cord injury. J Neurotrauma 2006, 23: 345-359.

[26] Totoiu MO, Keirstead HS. Spinal cord injury is accompanied by chronic progressive demyelination. J Comp Neurol 2005, 486: 373-383.

[27] Wang H, Fu Y, Zickmund P, Shi R, Cheng JX. Coherent anti-stokes Raman scattering imaging of axonal myelin in live spinal tissues. Biophys J 2005, 89: 581-591.

[28] Howell OW, Palser A, Polito A, Melrose S, Zonta B, Scheiermann C*, et al.* Disruption of neurofascin localization reveals early changes preceding demyelination and remyelination in multiple sclerosis. Brain 2006, 129: 3173-3185.

[29] Shi R, Pryor JD. Pathological changes of isolated spinal cord axons in response to mechanical stretch. Neuroscience 2002, 110: 765-777.

[30] Waxman SG. Demyelination in spinal cord injury. J Neurol Sci 1989, 91: 1-14.

[31] Fu Y, Sun W, Shi Y, Shi R, Cheng JX. Glutamate excitotoxicity inflicts paranodal myelin splitting and retraction. PLoS One 2009, 4: e6705.

[32] Fu Y, Wang H, Huff TB, Shi R, Cheng JX. Coherent anti-Stokes Raman scattering imaging of myelin degradation reveals a calcium-dependent pathway in lyso-PtdCho-induced demyelination. J Neurosci Res 2007, 85: 2870-2881.

[33] Bostock H, Sears TA, Sherratt RM. The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. J Physiol 1981, 313: 301-315.

[34] Bostock H, Sherratt RM, Sears TA. Overcoming conduction failure in demyelinated nerve fibres by prolonging action

potentials. Nature 1978, 274: 385-387.

[35] Targ EF, Kocsis JD. 4-Aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. Brain Res 1985, 328: 358-361.

[36] Blight AR. Effect of 4-aminopyridine on axonal conduction-block in chronic spinal cord injury. Brain Res Bull 1989, 22: 47-52.

[37] Blight AR, Gruner JA. Augmentation by 4-aminopyridine of vestibulospinal free fall responses in chronic spinal-injured cats. J Neurol Sci 1987, 82: 145-159.

[38] Kaji R, Sumner AJ. Effects of 4-aminopyridine in experimental CNS demyelination. Neurology 1988, 38: 1884-1887.

[39] Hayes KC, Blight AR, Potter PJ, Allatt RD, Hsieh J, Wolfe DL, *et al.* Preclinical trial of 4-aminopyridine in patients with chronic spinal cord injury. Paraplegia 1993, 31: 216-224.

[40] Hayes KC. The use of 4-aminopyridine (Fampridine) in demyelinating disorders. CNS Drug Rev 2004, 10: 295-316.

[41] Donovan WH, Halter JA, Graves DE, Blight AR, Calvillo O, McCann MT, *et al.* Intravenous infusion of 4-AP in chronic spinal cord injured subjects. Spinal Cord 2000, 38: 7-15.

[42] Halter JA, Blight AR, Donovan WH, Calvillo O. Intrathecal administration of 4-aminopyridine in chronic spinal injured patients. Spinal Cord 2000, 38: 728-732.

[43] Goodman AD, Brown TR, Krupp LB, Schapiro RT, Schwid SR, Cohen R, *et al.* Sustained-release oral fampridine in multiple sclerosis: a randomised, double-blind, controlled trial. Lancet 2009, 373: 732-738.

[44] Targ EF, Kocsis JD. Action potential characteristics of demyelinated rat sciatic nerve following application of 4-aminopyridine. Brain Res 1986, 363: 1-9.

[45] Blight AR. Computer simulation of action potentials and afterpotentials in mammalian myelinated axons: the case for a lower resistance myelin sheath. Neuroscience 1985, 15: 13-31.

[46] van der Bruggen MA, Huisman HB, Beckerman H, Bertelsmann FW, Polman CH, Lankhorst GJ. Randomized trial of 4-aminopyridine in patients with chronic incomplete spinal cord injury. J Neurol 2001, 248: 665-671.

[47] Acorda. Acorda Therapeutics Reports Results of Fampridine-SR Clinical Trials (Press Release). 2004.

[48] Potter PJ, Hayes KC, Segal JL, Hsieh JT, Brunnemann SR, Delaney GA, *et al.* Randomized double-blind crossover trial of fampridine-SR (sustained release 4-aminopyridine) in patients with incomplete spinal cord injury. J Neurotrauma 1998, 15: 837-849.

[49] Grijalva I, Guizar-Sahagun G, Castaneda-Hernandez G, Mino D, Maldonado-Julian H, Vidal-Cantu G, *et al.* Efficacy and safety of 4-aminopyridine in patients with long-term spinal cord injury: a randomized, double-blind, placebo-controlled trial. Pharmacotherapy 2003, 23: 823-834.

[50] Stefoski D, Davis FA, Fitzsimmons WE, Luskin SS, Rush J, Parkhurst GW. 4-Aminopyridine in multiple sclerosis: Prolonged administration. Neurology 1991, 41: 1344-1348.

[51] Stefoski D, Davis FA, Faut M, Schauf CL. 4-Aminopyridine improves clinical signs in multiple sclerosis. Ann Neurol 1987, 21: 71-77.

[52] Fujihara K, Miyoshi T. The effects of 4-aminopyridine on motor evoked potentials in multiple sclerosis. J Neurol Sci 1998, 159: 102-106.

[53] Davis FA, Stefoski D, Quandt FN. Mechanism of action of 4-aminopyridine in the symptomatic treatment of multiple sclerosis. Ann Neurol 1995, 37: 684-684.

[54] Polman CH, Bertelsmann FW, De Waal R, Van DH, Uitdehaag B, Van Loenen AC, *et al.* 4-Aminopyridine is superior to 3,4-diaminopyridine in the treatment of patients with multiple sclerosis. Arch Neurol 1994, 51: 1136-1139.

[55] Polman CH, Bertelsmann FW, Van Loenen AC, Koetsier JC. 4-Aminopyridine in the treatment of patients with multiple sclerosis. Arch Neurol 1994, 51: 292-296.

[56] van Diemen H, Polman CH, van Dongen M, Nauta J, Strijers R, Van Loenen AC, *et al.* 4-Aminopyridine induces functional improvement in multiple sclerosis patients: A neurophysiological study. J Neurol Sci 1993, 116: 220-226.

[57] Bever CT Jr., Young D, Anderson PA, Krumholz A, Conway K, Leslie J, *et al.* The effects of 4-aminopyridine in multiple sclerosis patients: controlled, crossover trial. Neurology 1994, 44: 1054-1059.

[58] van Diemen HAM, Polman CH, van Dongen TMMM, van Loenen AC, Nauta JJP, Taphoorn MJB, *et al.* The effect of 4-aminopyridine on clinical signs in multiple sclerosis: a randomized, placebo-controlled, double-blind, cross-over study. Ann Neurol 1992, 32: 123-130.

[59] Davis FA, Stefoski D, Rush J. Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. Ann Neurol 1990, 27: 186-192.

[60] Jones RE, Heron JR, Foster DH, Snelgar RS, Mason RJ. Effects of 4-aminopyridine in patients with multiple sclerosis. J Neurol Sci 1983, 60: 353-362.

[61] Judge SI, Bever CT Jr. Potassium channel blockers in multiple sclerosis: neuronal Kv channels and effects of symptomatic treatment. Pharmacol Ther 2006, 111: 224-259.

[62] Goodman AD, Brown TR, Cohen JA, Krupp LB, Schapiro R, Schwid SR, *et al.* Dose comparison trial of sustained-release fampridine in multiple sclerosis. Neurology 2008, 71: 1134-1141.

[63] Goodman AD, Brown TR, Edwards KR, Krupp LB, Schapiro RT, Cohen R, *et al*. A phase 3 trial of extended release oral

dalfampridine in multiple sclerosis. Ann Neurol 2010, 68: 494-502.

[64] Goodman AD, Cohen JA, Cross A, Vollmer T, Rizzo M, Cohen R, *et al.* Fampridine-SR in multiple sclerosis: a randomized, double-blind, placebo-controlled, dose-ranging study. Mult Scler 2007, 13: 357-368.

[65] Van Diemen HA, Polman CH, Koetsier JC, Van Loenen AC, Nauta JJ, Bertelsmann FW. 4-Aminopyridine in patients with multiple sclerosis: dosage and serum level related to efficacy and safety. Clin Neuropharmacol 1993, 16: 195-204.

[66] Agoston S, Salt PJ, Erdmann W, Hilkemeijer T, Bencini A, Langrehr D. Antagonism of ketamine-diazepam anaesthesia by 4-aminopyridine in human volunteers. Br J Anaesth 1980, 52: 367-371.

[67] Pena F, Tapia R. Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus *in vivo*: role of glutamate- and GABA-mediated neurotransmission and of ion channels. Neuroscience 2000, 101: 547-561.

[68] Pena F, Tapia R. Relationships among seizures, extracellular amino acid changes, and neurodegeneration induced by 4-aminopyridine in rat hippocampus: a microdialysis and electroencephalographic study. J Neurochem 1999, 72: 2006-2014.

[69] Stork CM, Hoffman RS. Characterization of 4-aminopyridine in overdose. Clin Toxicol 1994, 32: 583-587.

[70] Felts PA, Smith KJ. The use of potassium channel blocking agents in the therapy of demyelinating diseases. Ann Neurol 1994, 36: 454-454.

[71] Smith DT, Shi R, Borgens RB, McBride JM, Jackson K, Byrn SR. Development of novel 4-aminopyridine derivatives as potential treatments for neurological injury and disease. Eur J Med Chem 2005, 40: 908-917.

[72] McBride JM, Smith DT, Byrn SR, Borgens RB, Shi R. 4-Aminopyridine derivatives enhance impulse conduction in guinea-pig spinal cord following traumatic injury. Neuroscience 2007, 148: 44-52.

[73] Sun W, Smith D, Bryn S, Borgens R, Shi R. N-(4-pyridyl) methyl carbamate inhibits fast potassium currents in guinea pig dorsal root ganglion cells. J Neurol Sci 2009, 277: 114-118.

[74] McBride JM, Smith DT, Byrn SR, Borgens RB, Shi R. Dose responses of three 4-aminopyridine derivatives on axonal conduction in spinal cord trauma. Eur J Pharm Sci 2006, 27: 237-242.

钾通道阻滞剂可以有效修复受伤轴突的冲动传导

史日异，孙文婧

普渡大学韦尔登生物医学工程学院兽医学院基础医学系，西拉斐特，印地安那州 47907，美国

摘要：在脊椎中枢神经系统中，少突胶质细胞能形成轴突的髓鞘。髓鞘对轴突具有保护作用，使轴突具有电绝缘的特性，其独特的节段状结构使髓鞘化的神经轴突能快速、跳跃式地传导神经冲动。髓鞘损伤常见于脊髓损伤和一些慢性神经退行性疾病，由其引起的轴突传导阻滞被认为是引起损伤相关的神经并发症的主要原因。钾离子通道在发生于脊髓损伤和多发性硬化征的轴突传导阻滞中扮演重要角色。髓鞘损伤后会暴露钾离子通道，引起钾离子泄漏，从而阻断神经传导。将钾离子通道阻滞后，离子泄漏得到抑制，进而能促进神经传导。本综述主要详细介绍了修复轴突神经传导功能技术的研究进展和脱髓鞘轴突的神经功能。最近的研究表明，4-氨基吡啶能有效治疗多发性硬化征。此外，转化型研究也筛选出了一些能有效修复轴突神经传导的新型的钾离子通道阻滞剂。

关键词：轴突；传导；钾离子通道；损伤；脱髓鞘；4- 氨基吡啶