● **PERSPECTIVES**

Membrane resealing as a promising strategy for early treatment of neurotrauma

Traumatic injuries to the central nervous system (CNS), including traumatic brain injury (TBI) and spinal cord injury (SCI), often involve an immediate mechanical damage to plasma membrane that surrounds neuronal somata and axons. This initial disruption of plasma membrane following injuries has been convincingly demonstrated by increased membrane permeability to large molecules and dyes that are normally inaccessible to cellular plasma (Farkas et al., 2006; Cho and Borgens, 2012). Further evidence comes from experiments that showed ultra-structural changes of plasma membranes, axons, and organelles, and subsequent neuronal death and axotomy (Povlishock and Pettus, 1996; Whalen et al., 2008).

Disruption of plasma membrane is catastrophic to the injured neurons and axons. A leaky membrane makes it impossible for the neurons and axons to maintain critical differences in ions and small molecules, particularly calcium ion, between the extracellular and intracellular compartments. The influx of calcium ion down its electrochemical gradient leads to the destruction of membranes and cytosol and constitutes a key initial event in TBI and SCI. Progressive episodes of secondary injury subsequently increase reactive oxygen species and energy dysfunction, and trigger inflammation, leading to more severe behavioral dysfunction of the brain or spinal cord. For neuronal somata, loss of plasma membrane integrity after TBI initiates a series of events such as the failure of oxidative metabolism of the mitochondria, formation of reactive oxygen species, and the activation of apoptosis pathways. These events subsequently lead to cell degeneration and cell death (Farkas and Povlishock, 2007; Whalen et al., 2008). Neurons with plasmalemma damage, even though there are signs of spontaneous resealing of the membrane, may inevitably degenerate and die chronically (Whalen et al., 2008). For axons, disruption of the axolemma, such as in diffuse axonal injury following TBI and compressive axon injury following SCI, causes axon beading and intracellular calcium increase, which activates a number of intracellular pathways including local degradation of the cytoskeleton, failure of mitochondria function and energy deficit, and proteolytic reactions that involve calpain and caspase, eventually leading to axotomy (Buki and Povlishock, 2006; Kilinc et al., 2008). In SCI, the initial mechanical damage to axons is believed to play a significant role in producing functional deficits.

Given the critical role of membrane disruption in the pathophysiology of neurotrauma, resealing the damaged membrane of neuronal somata and axons appears to be an efficient strategy for repairing and rescuing the damaged neurons and axons, stopping secondary injury, and promoting functional recovery. Molecular resealing of damaged membrane may be accomplished with local application or intrave-

nous injection of hydrophilic polymers such as polyethylene glycol (PEG)(Cho and Borgens, 2012) or biocompatible surfactants such as poloxamers and poloxamines (*e.g.*, poloxamer 188). In *in vitro* and *in vivo* models of TBI and SCI, these polymers and surfactants have been shown to be effective in sealing cell membranes, quickly recovering the conductivity of axons, and promoting behavioral recovery (Cho and Borgens, 2012). PEG is able to fuse severed myelinated axons, repair severely injured spinal cords, and promote recovery of axonal conduction. The mechanism of this repair process may involve its ability to dehydrate cellular membranes quickly, thereby permitting membrane structural components to resolve and reassemble upon rehydration. The use of these molecules may provide an opportunity for rescuing the damaged neurons or the transected or compressed axons that often progress to cell death or axotomy.

Recent progress in using a membrane-resealing strategy for neurotrauma treatment is the development and testing of nanoparticles. Several specifically designed nano-particles are found not only to be effective in resealing disrupted membrane following traumatic CNS injuries, but also to possess unique properties such as good biocompatibility and tissue penetration, long blood retention time, and capability of carrying drugs and peptides (Cho and Borgens, 2012; Tyler et al., 2013). One type of these nanoparticles is the recently developed monomethoxy PEG-poly(D,L-lactic acid) (mPEG-PDLLA) micelles. The micelles contain a hydrophilic PEG shell and a hydrophobic poly(D, L-lactic acid) core (Shi et al., 2010), which may allow them to quickly fuse with plasma membrane for repairing damaged membrane as well as for delivering hydrophobic drugs. The micelles have been shown to not only rapidly restore axon conductivity and reduce calcium influx in axons of injured spinal cord at low concentrations, but also improve the recovery of locomotor function and reduced lesion volume after SCI (Shi et al., 2010). Our recent study indicated that intravenous injection of mPEG-PDLLA micelles, when given immediately or at 4 hours after TBI in mice, significantly improved the function of brain axons, as indicated by increases in peak amplitudes of compound action potentials generated by myelinated and unmyelinated cortical axons (Ping et al., 2014). Using fluorescent dye-labeled mPEG-PDLLA micelles, we further demonstrated high fluorescent staining in cortical grey and white matters underneath the impact cortical site, suggesting that the micelles can penetrate the compromise the blood brain barrier and reseal the injured axolemma (Ping et al., 2014). Other nanoparticles, such as PEG-decorated silica nanoparticles, have also been shown to be effective in sealing axonal membrane, improving axon conduction, and improving recovery of behavioral functions following SCI (Cho et al., 2010).

Although the current data support that surfactant molecules and membrane-sealing nanoparticles show promise in rescuing injured axons of the brain and spinal cord and improving their conduction function, the data on the effect of membrane sealing on saving damaged neurons are less encouraging. We found that treatment with the mPEG-PDLLA micelles following TBI did not result in significant decreases

in the number of membrane-disrupted cortical and hippocampal neurons (Ping et al., 2014). In another study, a membrane-resealing agent Kollidon VA64 was found to be effective in resealing the ruptured neuronal membrane in a controlled cortical impact model of TBI in mice, but it did not rescue the injured neurons from eventual death at 7 days after TBI (Mbye et al., 2012). This ineffectiveness of membrane resealing on neuronal survival may partially explain the modest therapeutic efficacy often observed on behavioral recovery in animal models of TBI *in vivo*. Indeed, most previous studies focused on the mechanisms and consequences of membrane disruption on neurons and the short-term effect of membrane-sealing treatment on them, the long-term effect of such treatment and the eventual fate of the membrane-ruptured neurons are not well understood. On the other hand, the ineffectiveness in rescuing membrane-disrupted neurons may also suggest that a membrane-sealing treatment may not be sufficient as a stand-alone therapy for early neuroprotection. Given the great complexity of pathological processes that are initiated following neurotrauma and membrane disruption, combining membrane-sealing treatment with other drugs that target other biological processes would be necessary for achieving a better therapeutic effect on neuroprotection as well as on axon repair. Furthermore, the beneficial effects of membrane-sealing molecules or nanoparticles on TBI or SCI may also involve mechanisms other than membrane repair. For example, the effect of Kollidon VA64 on TBI is found to be mainly mediated by reducing blood brain barrier damage, tissue loss, and brain edema, instead of rescuing the membrane-interrupted neurons (Mbye et al., 2012). Increased penetration of nanoparticles in the injured brain tissue may involve a repair effect of the nanoparticles on the damaged blood vessels or glial cells in the injured brain regions. Therefore, when considering the effect and mechanism of membrane-sealing molecules, it would be important to take into account various potential mechanisms other than a membrane-sealing effect.

Because repairing the plasma membrane is targeted to the initial event following neurotrauma, timing is likely critical for the success of this type of treatment. The sooner the broken membrane is repaired, the more likely the neurons may recover their structural integrity and prevent secondary injury. We found that the micelles were effective within 4 hours after TBI; this time period may be sufficient for clinical intervention in a larger percentage of patients. The time window for the treatment of SCI seems much longer. For example, PEG was found to be effective after at least 8 hours after SCI, although such efficacy is lost at 24 hours after SCI. Nevertheless, further study to define the effective time window will be critical for the eventual clinical application of these molecules.

Although the current data do not support the efficacy of membrane sealing on preventing injured brain neurons from eventual death, further study on the effects and mechanisms of membrane-sealing agents for SCI and TBI treatment is well justified by the critical roles of axon injury in the pathophysiology of both TBI and SCI, and by the *in vitro* and *in vivo* efficacy of membrane sealing on improving axon structure and function. Since PEG has been used safely in the clinic, and PEG-based micelles for neurotrauma treatment are nontoxic, PEG-based micelles would facilitate clinical translation, particularly as a combinatorial treatment and as a drug carrier for neurotrauma therapies that have limited CNS tissue penetration.

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