HIGHLIGHTS

Hydrogen sulfide controls peripheral nerve degeneration and regeneration: a novel therapeutic strategy for peripheral demyelinating disorders or nerve degenerative diseases

After peripheral nerve injury, the process of Wallerian degeneration is initiated in the distal stump of injured nerves. Wallerian degeneration in peripheral nerves involves axonal degeneration and degradation of the myelin sheath in Schwann cells. This provides the necessary conditions for axonal regeneration and remyelination. After nerve injury, macrophages are also recruited to the distal nerve stump and, together with Schwann cells, play a role in the clearance of myelin debris. Thus, a series of processes help to promote peripheral nerve regeneration, which includes axonal regeneration and remyelination. This is in contrast to injuries within the adult central nervous system, in which successful regeneration encounters several significant barriers: myelin-associated inhibition (Neuman et al., 2002), diminished axonal growth capacity (Ruff et al., 2008) and glial scarring (Yiu and He, 2006). Because the successful regeneration of injured peripheral nerves relies on a harmonious degenerating process, it is essential to identify a molecular mechanism that regulates axonal degeneration or myelin fragmentation during Wallerian degeneration to foster the conditions allowing efficient peripheral nerve regeneration. We have recently shown that hydrogen sulfide (H_2S) is important for axonal degradation and demyelination. We focus here on the effects of H_2S on axonal degradation and on understanding the underlying mechanisms of H_2S-associated demyelination, dedifferentiation and proliferation in Schwann cells during Wallerian degeneration. In addition, we discuss a novel strategy for nerve regeneration in the injured perip heral nerve or peripheral neuropathy.

The synthesis and regulation of H_2S in the nervous system: H_2S, the most recently described gas signaling molecule, performs a variety of physiological functions (Kimura, 2013). H_2S is produced from pyridoxal-5′-phospate (PLP)-dependent enzymes [cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE)] and 3-mercaptopyruvate sulfurtransferase (MST), along with cysteine aminotransferase (CAT). These enzymes play physiological roles in a variety of human tissues in the body. In the central nervous system (CNS), the synthesis of H_2S is regulated by CBS activity, and the imbalance in H_2S production is linked to several CNS diseases including Alzheimer's disease (Beard and Bearden, 2011). We found that the peripheral nervous system (PNS) shows a very different pattern of enzymatic activity for H_2S production. In the PNS, CSE and MST/CAT, but not CBS, are expressed in the normal nerves. There is reason to believe that H_2S may play a significant role in the degeneration of peripheral nerves following injury, based on comparisons with nitric oxide (NO) and carbon monoxide (CO). Like H_2S, NO and CO are gas transmitters used in a variety of signaling pathways. After nerve injury, inducible NO synthase (iNOS) is up-regulated in the distal stump of peripheral nerves, and iNOS knockout mice exhibit delayed demyelination during Wallerian degeneration (Levy et al., 2001; Campuzano et al., 2008). Previous studies suggest that NO is linked to delayed Wallerian degeneration after peripheral nerve injury and also point to the possibility that the other gasotransmitters CO or H_2S may be related to nerve degeneration and regeneration. Of the three aforementioned gasotransmitters, the physiological functions of H_2S are similar to those of NO. In other words, H_2S dynamics are likely similar to NO dynamics during Wallerian degeneration in peripheral nerves. We have gathered enough evidence to support the hypothesis of a relationship between H_2S dynamics and peripheral nerve degeneration/regeneration. After peripheral nerve injury, CSE is up-regulated, and its up-regulation occurs in Schwann cells, but not in axons, in mouse tissue *in vivo*. CSE activity is distributed diffusely in the cytoplasm of Schwann cells after nerve injury, whereas in uninjured Schwann cells, the localization of CSE has yet to be identified. These *in vivo* findings indicate a relationship between H_2S and Wallerian degeneration, especially that mediated by CSE activity.

H_2S dynamics during Wallerian degeneration: Demyelination, which results in the degradation of the myelin sheath, is one of the pathological phenotypes observed during Wallerian degeneration. During demyelination, the myelin sheath is fragmented and the myelin debris is engulfed and removed by Schwann cells and macrophages. The successful removal of myelin debris does not interrupt axonal regeneration. In our laboratory, we employed N-ethylmaleimide (NEM, inhibitor of all cysteine peptidases) to inhibit H_2S production in Schwann cells during Wallerian degeneration. Through the blockage of all cysteine peptidases, the prevented increase in H_2S production in Schwann cells during Wallerian degeneration regulates myelin ovoid fragmentation and influences axonal degradation (**Figure 1**). We propose that during Wallerian degeneration, activated H_2S production in Schwann cells breaks down myelin sheaths mechanically, leading to myelin ovoid fragmentation. Because the activation of H_2S production does not occur in the peripheral axons, the effect of the inhibitor on H_2S production is restricted to Schwann cells. This implies that mechanical forces related to H_2S production in myelin fragmentation during Wallerian degeneration may be sufficient for axonal degradation. However, we cannot exclude the possibility that H_2S-mediated extracellular signaling affects axonal degradation directly. In addition, the inhibition of H_2S production in the injured peripheral nerve blocks the recruitment of macrophages (unpublished observation). Although Wallerian degeneration is a condition that results when the peripheral nerve is injured, a related process is relevant to many neurodegenerative diseases and it is termed 'Wallerian-like degeneration' (Coleman and Freeman, 2010). Thus, effective control of H_2S production in the injured peripheral nerve is important for myelin sheath dynamics or

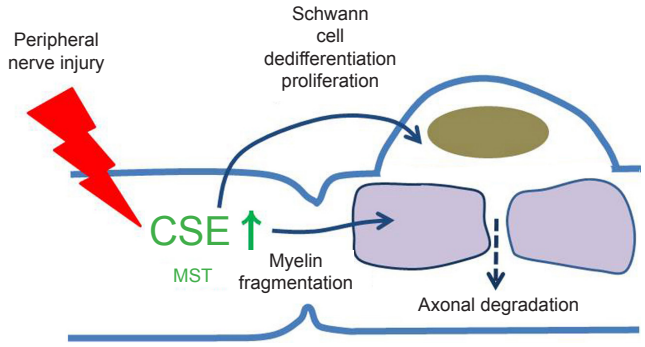

Figure 1 Hydrogen sulfide (H_2S) is essential for Wallerian degeneration.
H_2S functions as a physiological gas transmitter in both normal and pathophysiological cellular events. H_2S is produced from cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase/cysteine aminotransferase in normal peripheral nerves. Injured static nerves *in vivo* up-regulate CSE in Schwann cells during Wallerian degeneration, influencing Schwann cell dedifferentiation/proliferation and demyelination. However, CSE was not up-regulated in peripheral axons.

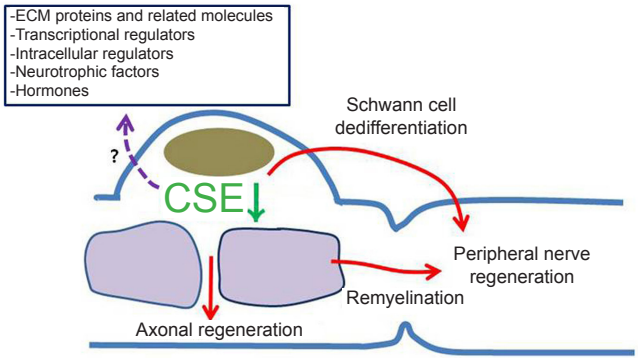

Figure 2 Hydrogen sulfide (H_2S) is a key modulator for peripheral nerve regeneration.
A model of H_2S dynamics in Schwann cells during peripheral nerve regeneration. Schwann cells may be regulated by H_2S production, potentially an important aspect of successful regeneration. Schwann cell dedifferentiation/proliferation and remyelination are key processes potentially involving Schwann cell responses to improve the regenerative environment.

myelin debris clearance. Furthermore, the effective regulation of H_2S production through the inhibition of CSE expression may contribute to a novel therapeutic strategy for demyelinating diseases, such as Guillain-Barré syndrome and Charcot-Marie Tooth Type 1 disease.

During Wallerian degeneration after peripheral nerve injury, Schwann cell dedifferentiation, which refers to the denervated state of Schwann cell (Jessen and Mirsky, 2008) and proliferation (Siironen et al., 1994) are essential for axonal regenerative processes and subsequent successful nerve regeneration. In our laboratory, we have shown that the inhibition of H_2S production in sciatic explants suppressed Schwann cell dedifferentiation and proliferation during Wallerian degeneration (**Figure 1**). The expression of several Schwann cell dedifferentiation or immaturity markers, LAMP1, $p75^{NTR}$, c-Jun and p-ERK1/2, was inhibited by the H_2S inhibitor in sciatic nerve explants *in vitro*. This indicates that H_2S signaling broadly affects the processes of Schwann cell dedifferentiation through lysosomal protein degradation, neurotropin receptors, the MAPK pathway, and transcriptional regulation. In addition, expression of the proliferation marker ki67 was inhibited by the H_2S inhibitor *in vitro*. Intriguingly, the activity of krox20, a marker for differentiation, myelination or maturity, was maintained after treatment with H_2S inhibitors during Wallerian degeneration. Transcriptional regulation through several antagonistic interactions between transcriptional factors, such as c-Jun and Krox20, affects the denervated state (Jessen and Mirsky, 2008). We suggest the possibility that H_2S regulates the delay or progression of Schwann cell differentiation, myelination or maturity through Krox20 and c-jun transcriptional regulation.

Unsolved questions in peripheral nerve regeneration: One of the remaining issues regarding the role of H_2S is the possible link with peripheral nerve regeneration. For successful regeneration, the important issue centers on the responses of Schwann cells to H_2S production. Because peripheral axons do not show altered CSE-associated H_2S production during Wallerian degeneration, H_2S-associated repair of peripheral axons is likely dependent on Schwann cell dynamics, but not axons, during the regeneration process. Thus, the key processes pertaining to Schwann cell responses are Schwann cell dedifferentiation, proliferation and remyelination. H_2S broadly affects various events occurring in Schwann cells during Wallerian degeneration. First, H_2S production is involved in Schwann cell dedifferentiation. After nerve injury, myelinated Schwann cells dedifferentiate into immature Schwann cells resembling undeveloped cells (Jessen and Mirsky, 2008). The inhibition of H_2S production results in the down-regulation of several Schwann cell dedifferentiation markers such as LAMP1, p75NTR, c-Jun and p-ERK1/2. Therefore, at the endpoint of Wallerian degeneration, the efficient decrease of H_2S production in Schwann cells may contribute to improved Schwann cell guidance for growth of regenerating axons or for remyelination through the suppression of the dedifferentiation-related molecules (**Figure 2**). Second, H_2S production is involved in Schwann cell proliferation. After peripheral nerve injury, the immature Schwann cells proliferate in the presence of the endoneurium (Siironen et al., 1994). The proliferated Schwann cells guide an axonal sprout from the injured site to reach the target organ. Thus, the effective regulation of H_2S production during Wallerian degeneration may enhance Schwann cell proliferation (**Figure 2**). Third, H_2S dynamics may be involved in Schwann cell remyelination. Even when the growing axon terminals correctly reach the ending organ, if Schwann cell remyelination is not performed efficiently, nerve regeneration is incomplete. Because H_2S production affects transcriptional regulation through krox20 and c-jun, effective transcriptional regulation through the inhibition of H_2S production may influence the improvement in Schwann cell remyelination ability (**Figure 2**). Thus, we believe that H_2S is a key modulator for peripheral nerve regeneration. In

addition, many questions still remain regarding the role of H_2S in peripheral nerve regeneration. It is important to assess the relationship between H_2S production and various factors that have been implicated previously in the regulation of Schwann cell responses during peripheral regeneration. For example, it is necessary to evaluate the relationship between H_2S production and remyelination-associated ECM proteins and related molecules (laminins, dystroglycan, L-periaxin, tPA/plasminogen and fibrin; Chen et al., 2007). Neurotrophic factors may be relevant, and receptors such as BDNF, FGF-2, and TGF-β, along with p75NTR, may be affected by H_2S production. Future research should also address intracellular regulators (PI3-kinase/Akt signaling, cyclin D1 and ski; Chen et al., 2007), several hormones (progesterone and thyroid hormones; Chen et al., 2007) or transcriptional regulators (Oct-6, Sox-10, Brn2, NF-κB, Notch, Sox-2, Pax-3 and Id2; Jessen and Mirsky, 2008) (**Figure 2**). Further studies along these lines would provide important insight into peripheral nerve regeneration and contribute to the development of a novel therapeutic strategy for peripheral demyelinating diseases or nerve degenerative diseases.

This work was supported by Dong-A University research fund. The authors have no conflicts of interest to disclose.

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Accepted: *2014-11-08*
doi:*10.4103/1673-5374.147940* ***http****://www.nrronline.org/*
Jung J, Jeong NY. Hydrogen sulfide controls peripheral nerve degeneration and regeneration: a novel therapeutic strategy for peripheral demyelinating disorders or nerve degenerative diseases. Neural Regen Res. 2014;9(24):2119-2121.

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