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Acrolein as a novel therapeutic target for motor and sensory deficits in spinal cord injury

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Abstract

In the hours to weeks following traumatic spinal cord injuries (SCI), biochemical processes are initiated that further damage the tissue within and surrounding the initial injury site: a process termed secondary injury. Acrolein, a highly reactive unsaturated aldehyde, has been shown to play a major role in the secondary injury by contributing significantly to both motor and sensory deficits. In particular, efforts have been made to elucidate the mechanisms of acrolein-mediated damage at the cellular level and the resulting paralysis and neuropathic pain. In this review, we will highlight the recent developments in the understanding of the mechanisms of acrolein in motor and sensory dysfunction in animal models of SCI. We will also discuss the therapeutic benefits of using acrolein scavengers to attenuate acrolein-mediated neuronal damage following SCI.

Key Words: *oxidative stress; spinal cord injury; 3-hydrxypropyl mercapturic acid; acrolein-lysine adduct; hydralazine*

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Introduction

Traumatic spinal cord injury (SCI) is one of the major causes of irreversible nerve injury, resulting in both motor and sensory dysfunction. One of the critical contributing factors of SCI pathology is the secondary cascade of events occurring in the hours, days, and weeks after initial physical impact. This process exacerbates injury at the initial lesion site of impact and also results in spreading of the damage to adjacent tissue throughout the spinal cord. Secondary injury includes ischemia/reperfusion injury, inflammation, oxidative stress, and glutamate excitotoxicity (Braughler and Hall, 1989; Juurlink and Paterson, 1998; Hall and Springer, 2004), which all contribute to the eventual tissue degeneration and functional loss.

As a hallmark of secondary injury, oxidative stress plays an important role in mediating functional loss after SCI and, furthermore, has been implicated in several neurodegenerative diseases (Hall and Braughler, 1993; Smith et al., 1999). Under the high level of oxidative stress, the endogenous system of antioxidants, such as glutathione, vitamin E, and ascorbic acid, may be overwhelmed and depleted (Hall, 1996) which results in further damage in tissue following SCI. Although there is strong evidence that oxidative stress plays a critical role in the pathogenesis after SCI, clinical trials of free radical scavenging have not produced any effective treatments to promote functional recovery after traumatic SCI (George et al., 1995; Suberviola et al., 2008). Therefore, further understanding of the mechanisms of oxidative stress and identification of novel, more effective therapeutic targets are highly warranted.

As both a product of and catalyst for lipid peroxidation, the highly reactive α, β-unsaturated aldehyde, acrolein, induces a vicious cycle of oxidative stress, dramatically amplifying its effects and perpetuating cellular damage (Hamann and Shi, 2009). Furthermore, acrolein remains active in the body much longer than the more commonly studied reactive oxidative species (Ghilarducci and Tjeerdema, 1995). We have demonstrated that acrolein plays a critical role in oxidative stress and likely plays an important role in neuronal trauma and degenerative disorders (Shi et al., 2002; Luo and Shi, 2004). In particular, we have demonstrated that acrolein concentrations are increased in animal models of SCI and likely play a role in motor paralysis and neuropathic pain after SCI (Luo et al., 2005; Due et al., 2014; Park et al., 2014). Therefore, acrolein may be a key factor in propelling oxidative stress, and subsequently disrupting motor function and sensory hypersensitivity after traumatic SCI. In this review, we will focus on the discussion of possible mechanisms of acrolein in motor and sensory dysfunction after traumatic SCI in a clinically relevant contusive SCI animal model. Furthermore, we will demonstrate that acrolein scavenging strategies could be an effective treatment for neurological deficits to SCI victims.

The origin of acrolein and mechanisms of acrolein-mediated damage

Acrolein can originate through endogenous pathways such as lipid peroxidation (LPO) or produced and ingested from ex-

ogenous sources. Environmental exposure of acrolein occurs through incomplete combustion of organics, manufacturing processes, and cigarette smoke (Ghilarducci and Tjeerdema, 1995; Kirkham et al., 2003). Further, since acrolein is both a product and initiator of LPO, it has been implicated in many damaging biochemical mechanisms in diseases such as diabetes, cancer, and rheumatoid arthritis (Uchida, 1999).

Acrolein has been shown to be the most reactive electrophile produced by LPO and is found in at least 40 times greater concentration than other much studied reactive oxidative species (ROS). It can react with glutathione (GSH), an endogenous antioxidant, 100–150 times faster than 4-hydroxynonenal (HNE). In addition, the half-life of acrolein is significantly longer than transient ROS (on the order of days compared to fractions of a second, respectively). Furthermore, acrolein conjugates, which form with proteins and GSH, have much longer half-lives than free acrolein and have been shown to be highly reactive (Adams and Klaidman, 1993; Burcham et al., 2004; Kaminskas et al., 2004a). As an unsaturated aldehyde, acrolein presents a threat to proteins, DNA, phospholipids, and other biomolecules. Within proteins, acrolein binds to cysteine, histidine, and lysine residues, forming carbonyl acrolein-protein oligomers. Acrolein can also react with the nucleophilic bases of DNA to form exocyclic adducts (Kehrer and Biswal, 2000). Through this process, acrolein plays a critical role in oxidative stress and poses a threat to many biochemical processes.

Acrolein detection

The detection of free acrolein is technically challenging because of its high reactivity and volatility. Acrolein is capable of undergoing spontaneous polymerization, Diels-Alder condensations, oxidation, and reduction due to the presence of the carbonyl and vinyl functional groups on the molecule (Shi et al., 2011a). Based on this current understanding of the nature of acrolein, it is reasonable to estimate that the majority of acrolein exists in acrolein-protein adducts or is metabolized by endogenous antioxidants such as GSH.

Acrolein-lysine compound levels in the rat SCI model have been detected in many studies using immunoblotting techniques (Uchida, 1999; Hamann et al., 2008b; Park et al., 2014). These techniques allow the quantification of acrolein adducts to correspond with *in vivo* acrolein levels directly at the tissue of interest. However, although immunoblotting is a highly accurate method, it is complicated and time consuming. Also, animals must be sacrificed in order to harvest tissue for immunoblotting. Thus, this method requires more experiments and increased costs. Therefore, a noninvasive method of detecting acrolein or acrolein metabolites would benefit the investigation of acrolein-mediated injury in SCI research.

A promising new method for detecting acrolein levels noninvasively is through acrolein metabolite quantification in urine samples. Specifically, N-acetyl-S-3-hydroxypropyl-cysteine (or 3-hydroxypropyl mercapturic acid, 3-HPMA) has been determined to be a major metabolite of acrolein and is also chemically stable. Because the binding ratio between acrolein and 3-HPMA is 1:1, measuring 3-HPMA level from urine is a reliable biomarker for quantitation of acrolein (Zheng et al., 2013). Therefore, the measurement of the acrolein metabolite 3-HPMA in urine is used to correlate the amount of acrolein present in the rat SCI model. This method uses liquid chromatography (LC) to separate the diverse chemical species in urine and then tandem mass spectroscopy (MS) to quantify the amount of 3-HPMA in the sample.

The detection of 3-HPMA *via* LC/MS/MS methods using urine samples is a beneficial technique due to its noninvasive nature and ability to be performed quickly. However, there are differences between the results of dot blotting and 3-HPMA detection when the two methods are used in tandem. For example, elevated levels of 3-HPMA are only detected for three days following SCI. With dot blotting, these elevations can be detected up to two weeks following injuries (Zheng et al., 2013; Park et al., 2014). This may be due to the limited diffusion of acrolein into the blood stream. Also, urine assays can only indicate that there is a whole-body increase in acrolein levels; this elevation cannot be pin-pointed to a certain tissue. Using the two methods together offers a more accurate view of the production of acrolein following SCI. Reliable acrolein detection is imperative for studying acrolein elevation in relation to neurological injury models as well as the efficacy of therapies that target endogenous acrolein.

Acrolein scavenging

Since acrolein may play an important role in secondary injury after traumatic primary SCI, acrolein scavenging is an effective method to examine the role of acrolein in SCI by reducing acrolein levels and observing possible mitigation of detrimental effects. Many studies have shown that attenuating oxidative stress, particularly in the case of methyprednisolone, could be a valuable means of therapeutic intervention for SCI treatment (Bracken et al., 1997; Bracken et al., 1998; Fehlings and Baptiste, 2005). However, because of its serious side effects, including pulmonary embolism, respiratory tract infection, urinary tract infection, and gastrointestinal problems, methylprednisolone is not an attractive therapeutic for SCI (George et al., 1995).

The FDA approved anti-hypertensive drug, hydralazine, has been shown to bind to and neutralize acrolein (Burcham et al., 2000; Burcham et al., 2002) and acrolein-protein adducts (Burcham et al., 2004; Burcham and Pyke, 2006). Hydralazine is a strong nucleophile with a hydrazide functional group with which acrolein and acrolein-protein adducts react through Michael addition (Kaminskas et al., 2004b). The reaction byproducts between hydralazine and acrolein were identified as (1E)-acrylaldehyde phthalazin-1-ylhydrazone (E-APH) and (1Z)-acrylaldehyde phthalazin-1-ylhydrazone (Z-APH) (Zhu et al., 2011). The primary reaction product is E-APH which reduces the cellular toxicity of acrolein and is excreted from body safely. Specifically, hydralazine reacts in an equi-molar manner to form E-APH, therefore in a cell-free system, equi-molar concentration of hydralazine nearly eliminates the acrolein (Kaminskas et al., 2004b).

Using a method developed recently (Wang et al., 2011), it has been shown that hydralazine can reach therapeutic concentrations after systemic application within just 2 hours and its bioavailability through systemic application was more than 90% compared to about 40% through oral application (Hamann et al., 2008a). Since the spinal cord tissue

has a specific gravity value of 1 (Hamann et al., 2008a), the hydralazine level in the spinal cord is calculated to be about 20 μmol/L which is in the range of therapeutic concentrations, 25 μmol/L (Liu-Snyder et al., 2006b). In fact, 5 mg/kg of hydralazine achieves these effects, which is lower than the suggested upper limitation for safe dosing of hydralazine for human pediatric patients (7.5 mg/kg) (Hamann and Shi, 2009). During tests with application of 5–25 mg/kg of hydralazine, no significant side effects were observed (Zheng et al., 2013). Therefore, it is evident that a low dosage of hydalazine can be reached quickly in the central nervous system (CNS) and reduces the acrolein concentrations after SCI safely.

It has been shown that the acrolein scavenger, hydralazine, can prevent acrolein-mediated cell death and injuries in PC12 cells (Liu-Snyder et al., 2006b). In *ex vivo* spinal cord tests, hydralazine alleviated acrolein-induced membrane damage, oxidative stress, and loss of compound action potential (Hamann et al., 2008a, 2008b). In addition, in isolated spinal cord *ex vivo*, hydralazine reduced compression-mediated accumulation of acrolein-protein adducts, which correlated with evidence that hydralazine prevented compression mediated membrane damage and oxidative stress (Hamann et al., 2008a). Taken together, these results support that acrolein accumulation can safely be attenuated by acrolein scavenging with hydralazine. Hydralazine can further be used as a tool to investigate the mechanistic role of acrolein in secondary injury following SCI.

Mechanisms of acrolein-mediated dysfunction in the CNS

Membrane damage and the increased presence of acrolein due to traumatic SCI have been implicated in cell dysfunction in the CNS. Acrolein has been shown to induce cell death in rat PC12 cells *via* MTT assays, a measurement of large mitochondrial function. Cell toxicity was greater when caused by acrolein than HNE or malonaldehyde (MDA), other aldehydes produced by LPO (Liu-Snyder et al., 2006a). It is noteworthy that in this study acrolein concentrations of 75 μmol/L were used, which is probably greater than the concentration produced *in vivo* after SCI. Levels of both adenosine triphosphate (ATP) and GSH were shown to decrease upon application of acrolein in the study, which is consistent with the theory of the role of acrolein in endogenous antioxidant depletion in the CNS. The cytotoxic effects of acrolein on the PC12 cells were attenuated by the application of hydralazine. The acrolein scavenger decreased membrane damage and GSH and ATP reductions. Hydralazine also demonstrated HNE and MDA scavenging abilities, though it was the strongest scavenger of acrolein (Liu-Snyder et al., 2006a). Acrolein mediated cytotoxicity, and its attenuation by hydralazine, further support the critical role of acrolein in neuronal dysfunction after traumatic SCI.

Cell dysfunction can also be noted in the abundant mitochondria of neurons in the CNS. In normal cell function, electron leakage during respiration in mitochondria causes reactions with oxygen to form ROS. However, the endogenous antioxidant system controls this normal ROS production to prevent oxidative stress (Fukui and Moraes, 2008). Following membrane damage, intracellular ionic concentrations of calcium, sodium, and potassium are altered, disrupting the function of the abundant mitochondria in the CNS. In this case, the metabolism of the cell shifts from respiration to ROS overproduction (Borgens and Liu-Snyder, 2012). Mitochondrial antioxidants are no longer able to keep up with ROS production and are depleted rapidly, increasing oxidative stress, lipid peroxidation, and consequently the production of acrolein.

The vicious cycle of acrolein production by LPO and antioxidant depletion is facilitated by the overproduction of ROS in the mitochondria of the CNS post-trauma. Luo and Shi demonstrated this in their findings of *ex vivo* mitochondrial impairment after acrolein exposure without injury (Luo and Shi, 2004). Further, this finding suggests that without the initial presence of increased concentrations of ROS, acrolein can initiate oxidative stress and neuronal damage. Also, mitochondrial damage by acrolein was found to be both concentration and time dependent. Increasing concentrations of acrolein significantly decreased mitochondrial function. This trend was also seen as the time of exposure to acrolein was increased from 15 to 240 minutes. Mitochondrial deficiency has also been supported *in vivo* by the findings that following SCI, mitochondrial function significantly decreased and ROS levels were increased within 1 hour and up to 24 hours after injury (Azbill et al., 1997). The increase of ROS following injury is an expected result following mitochondrial dysfunction due to the high levels of ROS normally produced in mitochondria.

In addition to mitochondrial impairment, acrolein has been shown to cause demyelination of axon and, consequently, the loss of axonal conduction. The myelin sheath provides an electrical insulator, promoting fast conduction in axons. When these oligodendrocytes are removed or damaged, ion channels are exposed and action potential generation is compromised. Shi and colleagues demonstrated the retraction and splitting of the myelin sheath from axons in the presence of acrolein using coherent anti-stokes Raman scattering (CARS) and simultaneous two photon excitation fluorescence (TPEF) (Shi et al., 2011b). The combination of these techniques enabled imaging of myelin with the labeling of potassium channels, indicating the exposure of the potassium channels in axons induced with injury by acrolein application. The length of the nodes of Ranvier was found to increase significantly with the application of acrolein and significant splitting was seen at the paranodal regions of axons (Shi et al., 2011b). Similar demyelination was also seen when spinal cord injury was induced (Sun et al., 2010). Myelin thickness has also been shown to be reduced following SCI (Totoiu and Keirstead, 2005). This thickness reduction was found to be chronically progressive. Further, when an injured spinal cord was incubated with a non-injured spinal cord, the non-injured spinal cord experienced myelin damage (Hamann et al., 2008b). Therefore, the damage must have resulted from biochemical mechanisms which transferred from the injured spinal cord to the non-injured spinal cord. Also,

this damage was attenuated by the application of hydralazine, an acrolein scavenger that is not an effective ROS scavenger (Hamann et al., 2008b). This indicates that acrolein plays a major role in the damage accrued during secondary injury post-SCI, rather than ROS playing the major role.

Beyond morphological damages induced by both acrolein and injury to the spinal cord, electrophysiological impairment is also seen. A reduction in compound action potential coinciding with demyelination of axons was seen following compression injury of the spinal cord (Ouyang et al., 2010). Upon the application of 4-AP, a potassium channel blocker, compound action potential amplitudes recovered significantly. This implies that potassium channel exposure is a key event in demyelination to impair axonal conduction. This research has shown that acrolein is able to induce myelin damage as well as potassium channel exposure and could also be a key factor in the pathogenesis of SCI.

The role of acrolein-mediated cell damage in the CNS has been widely investigated and shown to impair mitochondria, induce demyelination, expose potassium channels, and as a result, cause the loss of axonal conductance and, ultimately, cell death.

Endogenous level of acrolein and its reduction in SCI rat model

Despite overwhelming evidence of acrolein toxicity *in vitro* and acrolein generation in *ex vivo* compressed spinal cord studies in the last decade, the evidence of endogenously-generated acrolein in the CNS disorder *in vivo* model has only been demonstrated recently.

In humans, contusion is the most common type of SCI (Jung et al., 2008b), so the contused SCI animal model is clinically relevant. Using this SCI rat model, Park and colleagues have found novel results that the level of acrolein is elevated and persists in spinal cord tissue for up to 2 weeks after SCI (Park et al., 2014). The 3-HPMA levels in urine from different severity SCI rat models also significantly increased 1 day after SCI. Specifically, the 3-HPMA level in the severely injured rats was higher compared to that of the moderate SCI, which was still elevated compared to the uninjured controls (Zheng et al., 2013). These results support that the acrolein concentrations are increased in the spinal cord following traumatic SCI and the elevation of acrolein correlates with the severity of SCI. Although acrolein was reported to be increased in spinal cord compression injury in guinea pig (Luo et al., 2005), this is the first time increased acrolein has been found in rat contusive SCI.

It has also been shown that intraperitoneal (IP) injection of hydralazine could reduce acrolein levels in the contused-SCI rat model. Specifically, hydralazine injections of 5 mg/kg body mass can significantly reduce acrolein levels in the spinal cord from different severities of contusive SCI rat at 1 day post injury based on both immunoblotting and 3-HPMA detection (Zheng et al., 2013). These findings further support the neuroprotective role of hydralazine for the effective treatment for neurotrauma and neurodegenerative diseases by trapping acrolein.

The combination of immunoblotting and 3-HPMA urine analysis, which was discussed previously as a newly established acrolein level quantification method, has led to significant discoveries involving acrolein in the contusive *in vivo* SCI rat model. The discoveries not only indicate that elevated endogenous levels of acrolein correlate with the presence of injury, but also the severity. No less valuable is the evaluation of treatment by acrolein scavenging that can be conducted with these methods. Even further, immunoblotting and 3-HPMA detection for acrolein evaluation can be extended to investigate the role of acrolein in sensory and motor dysfunction.

Acrolein-mediated motor dysfunction after SCI

Many studies have demonstrated the adverse effects of contusion injury to the spinal cord on motor function. However, the effects of acrolein, independent of contusion injury gives greater insight into the importance of the role of acrolein in SCI. To investigate if acrolein itself is sufficient to generate neurological deficits without physical impact, Park and colleagues injected a small amount of acrolein directly into the right side of normal spinal cord and the same amount of saline was injected as a control into the left side of spinal cord (Park et al., 2014). In this study, it was found that administration of acrolein to the contralateral side resulted in significant motor deficits, while the saline injection side showed no motor dysfunction. In addition to motor deficits, 8 weeks after acrolein injection site showed noticeable scar tissue compared to the saline injection site. These results support that acrolein alone can generate neurological deficits in live animals.

Additionally, Park et al. (2014) investigated the neuroprotective role of acrolein scavenging on motor function recovery following contusive SCI. In this study, hydralazine solution was administered to one group through a daily intraperitoneal injection for 2 weeks after surgery. For functional recovery quantification post-SCI, Basso-Beattie-Bresnaham (BBB) open field locomotor testing was performed (Basso et al., 1996). The hind limb locomotor score of the hydralazine treatment group was significantly increased compared to the SCI group starting 1 week after SCI and it was maintained through the last period. This finding also validates and further supports the notion that hydralazine is an effective neuroprotective treatment not only *in vitro*, but in a live animal model of SCI as well (Park et al., 2014).

It has also been observed that hydralazine can significantly reduce cyst formation, a severe form of tissue damage in the spinal cord 4 weeks after treatment, following SCI. This acrolein-mediated scar tissue has been shown previously in a rat spinal cord transection injury (Rooney et al., 2009). Therefore, it is reasonable to expect that hydralazine-mediated cyst reduction can promote the reduction of scar formation, which is a major mechanical and chemical barrier for neuronal regeneration following SCI (Park et al., 2010). Reducing cyst formation will likely promote the survival of motor neurons after traumatic SCI which may help preserve motor function.

Acrolein-mediated sensory dysfunction after SCI

Although there are many of reports about the role of ROS in neuropathic and inflammatory pain following SCI (Kim et al., 2004; Gris et al., 2004; Wang et al., 2004) there is little information regarding the possible role of free acrolein or accumulation of acrolein-protein adducts. Luo and Shi (2004) have demonstrated that even micro-molar concentrations of acrolein can promote the generation of ROS. These results support that acrolein can induce pathological pain through oxidative stress and inflammation.

There is evidence that acrolein is a neuropathic pain inducer following SCI *via* the transient receptor potential ankyrin1 (TRPA1). Specifically, TRPA1 is a sensor for endogenous products from metabolism and oxidative stress-derived substances. As one of the strongest electrophiles, acrolein is a specific agonist for the TRPA1 channel (Trevisani et al., 2007). The expression level of TRPA1 is up-regulated in the spinal dorsal horn and primary afferent fibers following SCI (Venkatachalam and Montell, 2007).

Gating of the TRPA1 channel occurs by the reaction of of electrophilic ocmpounds, including acrolein, with the nucleophilic free sulfhydryl groups of cysteine and E-amino group of lysine in the cytoplasmic N-terminal of the channel, which promotes the conformational changes (Andrade et al., 2012). Additionally, the reaction between cysteine and acrolein is reversible which allows acrolein to break free from cysteine-acrolein adducts and react with other molecules. Therefore, an increase in the expression levels of acrolein after SCI could influence neuropathic pain modalities through activation of TRPA1 channel following SCI.

It has been observed that mechanical and cold allodynia can occur in the SCI rat model, which starts 1–2 weeks post contusive SCI and lasts several weeks (Due et al., 2014). Also, investigations have shown increases in acrolein expression level at least 2 weeks after SCI, which is in coincidence with neuropathic pain behaviors (Due et al., 2014; Park et al., 2014). In addition, Due and colleagues quantified changes in gene expression level of TRPA1 by using associated DRG cells (L_{3-6}). The data showed that, seven days after SCI, the mRNA levels of TRPA1 were increased in dorsal root ganglia (DRG). This may implicate acrolein's contribution to the chronic neuropathic pain even when its concentration returns to normal physiological levels after contusive SCI. In addition, acrolein is also known as a pro-inflammatory agent, which can stimulate the release of chemokines (Kirkham et al., 2003). It has been shown that TRPA1 activation is enhanced by inflammatory factors (Jung et al., 2008a). Therefore, acrolein might activate TRPA1 channels through both direct binding and indirect mechanisms *via* pro-inflammatory signaling.

In addition to an increase in acrolein levels after SCI, the emergence and maintenance of neuropathic pain is mediated by increased overall excitability state of DRG sensory neurons (Due et al., 2014). The data shows that SCI itself induces a decrease in the current threshold to generate action potential in the small and medium diameter of sensory neurons and an increase in the number of DRG sensory neurons that respond to acrolein after SCI. Moreover, acrolein can induce an increase in sensory neuron excitability at single DRG cell level and the acrolein-mediated effect on neuronal cell sensitivity is greater in the SCI animal model compared to the sham injury. This is further supported by the enhancement of mechanical and cold allodynia *via* the direct exogenous injection of acrolein into the spinal cord. Combined with the electrophysiological results that acrolein exposure to sensory DRG can increase neuronal sensitivity in small and medium diameter sensory neurons, these *in vivo* acrolein injection results strongly support the role of acrolein as an endogenous pro-nociceptive agonist.

As a pro-nociceptive agonist and pro-inflammatory factor, acrolein could be a suitable therapeutic target. The involvement of acrolein in neuropathic pain after SCI was further investigated by treating with safe doses of hydralazine. Results showed that systemic application of 5mg/kg of hydralazine significantly attenuated mechanical allodynia and thermal hyperalgesia following SCI (Park et al., 2014). The analgesic effects of hydralazine were maintained for several weeks and were observed even in 14 daydelayed administration of hydralazine. Hydralazine might not only prevent acrolein binding to TRPA1 channel, but also reduce the acrolein-mediated inflammatory events by removing acrolein after SCI.

These data strongly suggest that acrolein could be a key factor in the emergence of neuropathic pain following SCI. As such, with the significant analgesic effect of hydralazine, acrolein scavenger treatments may be a novel and effective therapeutic strategy for neuropathic pain management to improve the quality of life for SCI victims.

Alternative acrolein scavengers

Throughout this review, we have discussed the use of hydralazine as an acrolein scavenger. However, one of the limitations of using hydralazine is that its half-life is only a few hours (Reece, 1981), while on the other hand, the half-lives of acrolein and its protein adducts are longer than this. In addition, hydralazine is clinically used as an antihypertensive medication which directly induces vasodilation, which would be highly undesirable in a SCI patient (Tuncel and Ram, 2003).

In addition to hydralazine, there are other drugs that have potential use in acrolein scavenging. Their chemical structures can be seen in **Figure 1**. Phenelzine has a hydrazine group that reacts with acrolein at the same molecular ratio as hydralazine (Wood et al., 2006). Because the half-life of phenelzine is much longer (11.6 hours) than that of hydralazine, it could provide additional neuroprotection (Shi et al., 2011a). As a monoamine oxidase inhibitor, phenelzine can affect blood pressure temporarily, although it is not a direct vasodilator (Sheehan et al., 1980). Hypotensive effects are life threatening side effects to SCI patients so phenelzine might be used as an alternative to hydralazine. Zheng and colleagues have demonstrated that phenelzine can neutralize the acrolein after SCI (Zheng et al., 2013), and be administered in higher concentrations than hydralazine without hypotensive side effects (Unpublished data). However, the safety of phenelzine for acrolein scavenging should be established through further experiments. Also, dihydralazine has two hydrazine groups, and as such it could potentially be twice as efficient as hydralazine for acrolein scavenging (Kaminskas et al., 2004a). As

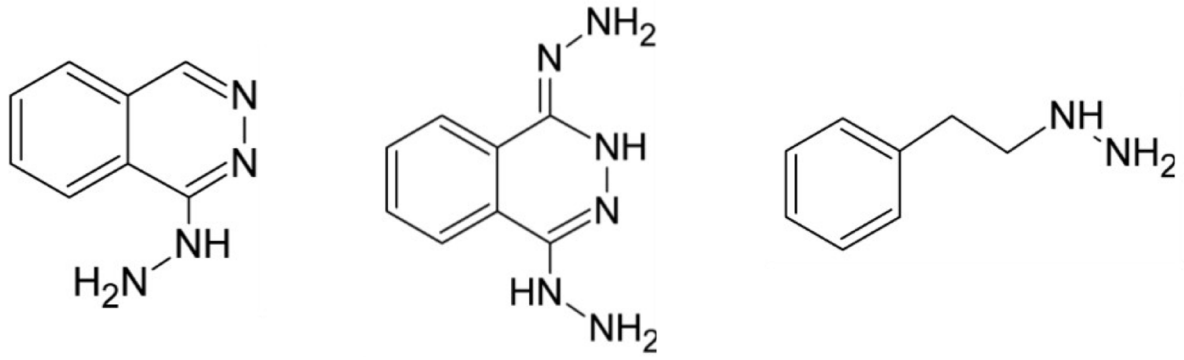

Figure 1 Chemical structures of acrolein scavengers, hydralazine, dihydralazine, and phenelzine (left to right).

such, both phenelzine and dihydralazine can be considered alternative acrolein scavengers, although more studies must be done to investigate their safety and efficacy.

Summary and conclusion

There is strong evidence that acrolein plays an important role in secondary cascade events after primary traumatic SCI. Furthermore, acrolein, with its high reactivity, long half-life, ability to produce free radicals, cytotoxicity and elevated concentrations in neurological disease state, has been associated with motor function deficits after SCI.

In addition, as a potent pro-nociceptive agonist, acrolein enhances neuronal excitation of DRG sensory neurons derived from a SCI animal model. Although further studies are needed to determine where acrolein influences the elevation of sensitivity on nociceptor through TRPA1 and its contribution to neuropathic pain behavior after SCI, acrolein is a novel therapeutic target for SCI induced neurological deficits.

Although there are multiple studies that the acrolein scavenger, hydralazine, can attenuate neuronal damage in tissue culture and isolated spinal cord, this is the first demonstration that acrolein is a novel therapeutic target for neurodegenerative malfunction such as motor function deficits and neuropathic pain after SCI. Additionally, hydralazine has the ability to exert an acrolein-scavenging effect and offer neuroprotection in live SCI animal models. Furthermore, because the half-life of hydralazine is a few hours and injections of hydralazine were only administered once a day, it is possible that more frequent administration of hydralazine daily may boost its strength in suppressing acrolein *in vivo*, not only in the initial stage when acrolein is elevated, buteven in the chronic stages of neuropathic pain after SCI.

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