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REVIEW

Acrolein scavenging: a potential novel mechanism of attenuating oxidative stress following spinal cord injury

Kristin Hamann* and Riyi Shi*,†

*Department of Basic Medical Sciences, School of Veterinary Medicine, Center for Paralysis Research, Purdue University, West Lafayette, Indiana, USA

†Weldon School of Biomedical Engineering, Purdue University, West Lafayette, Indiana, USA

Abstract

It has long been established that oxidative stress plays a critical role in the pathophysiology of spinal cord injury, and represents an important target of therapeutic intervention following the initial trauma. However, free radical scavengers have been largely ineffective in clinical trials, and as such a novel target to attenuate oxidative stress is highly warranted. In addition to free radicals, peroxidation of lipid membranes following spinal cord injury (SCI) produces reactive aldehydes such as acrolein. Acrolein is capable of depleting endogenous antioxidants such as glutathione, generating free radicals, promoting oxidative stress, and damaging proteins and DNA. Acrolein has a significantly longer half-life than the transient free radicals, and thus may represent a potentially better target of therapeutic intervention to attenuate oxidative stress. There is growing evidence, from our lab and others, to suggest that reactive aldehydes such as acrolein play a critical role in oxidative stress and SCI. The focus of this review is to summarize the cellular and biochemical mechanisms of acrolein-induced membrane damage, mitochondrial injury, oxidative stress, cell death, and functional loss. Evidence will also be presented to suggest that acrolein scavenging may be a novel means of therapeutic intervention to attenuate oxidative stress and improve recovery following traumatic SCI.

Keywords: acrolein, aldehyde, hydralazine, lipid peroxidation, oxidative, spinal cord.

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The National Spinal Cord Injury Statistical Center (http://www.spinalcord.uab.edu) reports that there are currently over a quarter of a million people in the United States that are living with spinal cord injury (SCI), with an incidence of 12 000 new injuries each year. The average age at the time of injury is 28.7 years, with most injuries occurring between the ages of 16 and 30. SCI is also associated with tremendous costs in terms of both medical expenses (acute treatment and long term management) and losses in wages, benefits, and productivity. In spite of decades of intense research to promote improved functional recovery and quality of life for these patients, this continues to be a frustrating condition to treat. Unfortunately, successful *in vivo* studies in animals have largely failed to produce similar successes in human clinical trials (Tator 2006). High doses of methylprednisolone demonstrated improved outcome for acute treatment of spinal cord injury in some studies, probably because of its antioxidant properties (Bracken *et al.* 1997, 1998; Bracken 2002; Bracken and Holford 2002; Fehlings and Baptiste 2005; Baptiste and Fehlings 2006). However, the safety and efficacy of this treatment has been recently questioned. Specifically, some more recent clinical trials failed to find improved recovery with methylprednisolone treatment and/or

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Address correspondence and reprint requests to Riyi Shi, Department of Basic Medical Sciences, Center for Paralysis Research, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907, USA. E-mail: riyi@purdue.edu

Abbreviations used: AD, Alzheimer's disease; ADH, alcohol dehydrogenase; ALDH, aldehydyde dehydrogenase; ARE, antioxidant response element; EGR, early growth factor; HNE, 4-hydroxynonenal; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; MESNA, 2-mercaptoethanesulfonate; NFkB, nuclear factor kappa B; PD, Parkinson's disease; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SCI, spinal cord injury.

reported serious complications including pulmonary embolism, respiratory tract infection, urinary tract infections, other infections, and gastrointestinal problems such as a fatal perforated peptic ulcer (George *et al.* 1995; Matsumoto *et al.* 2001; Lee *et al.* 2007; Suberviola *et al.* 2008). Thus, treatment with high doses of methylprednisolone is controversial. One important thing that can be learned from these trials is that attenuating oxidative stress may prove to be a valuable means of therapeutic intervention. However, there is clearly still much to be learned about the pathophysiology of this devastating injury in order to develop new and more efficacious therapies.

Pathophysiology of spinal cord injury

Following traumatic spinal cord injury, the initial, primary insult is only a portion of the total pathology (Young 1993). Mechanical injury sets into play a number of secondary injury processes that result in spreading of the injury to adjacent, otherwise uninjured tissues following the initial insult. In fact, previous studies have demonstrated that secondary injury alone is capable of producing significant damage that is distinct from the effects of mechanical injury (Hamann *et al.* 2008a). Once a patient is presented with traumatic SCI, inhibition of secondary injury processes may be one of the most important means of intervention to prevent further degeneration and thus promote functional recovery. This is because of the absence of significant regeneration within the central nervous system and continued degeneration of already damaged neurons. *In vivo*, secondary injury mechanisms are mediated by a number of factors. These include ischemia/reperfusion injury, inflammation, oxidative stress, glutamate excitotoxicity, elevation of intracellular calcium, and activation of proteases and caspases (Braughler and Hall 1989; Hall 1996; Juurlink and Paterson 1998; Hall and Springer 2004; Kumar *et al.* 2004).

The role of oxidative stress in traumatic spinal cord injury

In particular, oxidative stress is well-established to play a significant role in the pathophysiology of SCI and is a hallmark of secondary injury (Hall 1989, 1991; Hall and Braughler 1993). Traumatic SCI produces mechanical injury to the plasma membrane and thus disrupts the normal ion balances; specifically intracellular calcium and sodium rise. Glutamate excitotoxicity provides additional contributions to the rise in intracellular calcium. Since the electrochemical gradient across the membrane is critical to the function of neuronal cells, Na^+/K^+ ATPases work to reduce intracellular sodium. Reversal of the Na^+/Ca^{2+} exchanger in order to remove sodium may also occur, contributing further to rises in intracellular calcium (Stys *et al.* 1991; Hall 1996). Oxidative stress also contributes to calcium overload (Xiong *et al.* 2007). The subsequent rise in intracellular calcium results in mitochondrial injury and increased production and release of reactive oxygen species (ROS). In addition, calcium activates proteases and caspases, as well as phospholipase, which subsequently releases fatty acids from membranes (Braughler and Hall 1989; Hall and Springer 2004). SCI nearly always disrupts blood supply, and ischemia-reperfusion injury impairs ATP production, increases production of ROS by mitochondria, recruits neutrophils (which produce ROS by respiratory burst) to the injury site, and can stimulate xanthine oxidase to produce ROS (Braughler and Hall 1989; Hall 1996; Juurlink and Paterson 1998; Kumar *et al.* 2004). Lipid peroxidation (LPO) can generate more free radicals as well as reactive aldehydes that bind to and deplete glutathione, resulting in additional oxidative stress.

The central nervous system possesses a number of unique properties that make it particularly vulnerable to oxidative stress. Specifically, the brain and spinal cord contain a relatively large proportion of polyunsaturated fatty acids (PUFAs), such as linoleic acid and arachidonic acid, which are sensitive to peroxidation (Braughler and Hall 1989; Hall 1996). In addition, neuronal cells are rich in mitochondria, which are a potential target and source of ROS. Under conditions of oxidative stress, the endogenous antioxidant system may be overwhelmed as antioxidants such as glutathione, vitamin E, and ascorbic acid are depleted (Hall 1996). However, in spite of strong evidence that post-trauma oxidative stress plays a critical role in the pathophysiology of spinal cord injury (Hall 1989, 1991; Hall and Braughler 1993), conventional strategies aiming to scavenge free radicals have largely failed to produce any safe and effective treatment that can curtail oxidative injury in human clinical trials. Hence, further understanding of the mechanisms of oxidative stress and identification of a novel and more effective target is highly warranted and desirable.

In addition to SCI, ROS and LPO have been associated with numerous disease processes that few other pathological factors can match, including aging, neoplasia, trauma, and ischemia-reperfusion injury (Halliwell and Gutteridge 1999). The mechanism of involvement of LPO has been an area of intense research aiming to prevent, slow down, and even reverse the development of various diseases. This research thus may have important applications in not only spinal cord injury, but in many other diseases as well.

Role of acrolein in spinal cord injury

In addition to the much studied ROS, highly reactive α,β-unsaturated aldehydes, including malondialdehyde (MDA), 4-hydroxynonenal (HNE), and acrolein, are produced as a byproduct of peroxidation of PUFAs in cell membranes (Witz 1989; Esterbauer *et al.* 1991; Uchida *et al.* 1998a; Uchida 1999; O'Brien *et al.* 2005), as well as intracellular

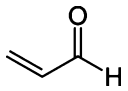

Fig. 1 Structure of acrolein.

enzymatic oxidation of polyamine metabolites (Esterbauer *et al.* 1991; Seiler 2000). Among them, MDA and 4-hydroxyalkenals, including HNE, have received relatively more attention in the literature (Esterbauer *et al.* 1991; Uchida 1999). Indeed, MDA is produced at the highest concentration of the lipid aldehydes (Uchida 1999), and 4-hydroxyalkenals, including HNE, have been implicated in numerous pathological processes (Esterbauer *et al.* 1991; Uchida 1999). More recently, acrolein (structure shown in Fig. 1) has received increasing attention as both a product and initiator of lipid peroxidation (Uchida 1999). Of the α,β-unsaturated aldehydes, acrolein has been shown to be by far the most reactive with various biomolecules including proteins, DNA, and glutathione, and reacts 110–150 times faster with glutathione than HNE or crotonal (Witz 1989; Esterbauer *et al.* 1991; Ghilarducci and Tjeerdema 1995; Uchida 1999; Kehrer and Biswal 2000). Furthermore, one study found that the half-life of acrolein under various environmental conditions was on the order of hours to days (Ghilarducci and Tjeerdema 1995). While the half-life of acrolein in biological systems may be shorter than is predicted by these studies, it is likely to persist in the body for significantly longer than the transient ROS (Esterbauer *et al.* 1991). In addition, acrolein readily forms conjugates with proteins and glutathione that likely have significantly longer half-lives than free acrolein, and have been demonstrated to be highly reactive (Adams and Klaidman 1993; Burcham *et al.* 2004; Kaminskas *et al.* 2004a; Burcham and Pyke 2006). In fact, one study suggested that trapping of acrolein-protein adducts prevented subsequent protein oligomerization and cross-linking, and furthermore that this was a more important means of cytoprotection in cultured hepatocytes than was scavenging of free acrolein (Burcham and Pyke 2006). The pathophysiology of traumatic SCI, and in particular the mechanisms of acrolein-mediated injury, is illustrated in Fig. 2.

Acrolein shows greatest reactivity with thiols, and GSH may be one of the most important targets of acrolein-mediated injury. GSH reacts with acrolein at the third carbon to produce GS-propionaldehyde (Adams and Klaidman 1993), which is subsequently metabolized by aldehydyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) (Mitchell and Petersen 1989). ALDH2, but not ALDH1, is located in glial cells, and neurons contain neither, which suggests that neuronal mitochondria may be particularly vulnerable to acrolein-mediated injury (O'Brien *et al.* 2005). Depletion of GSH may compromise reduction of hydrogen peroxide and lipid peroxides by GSH peroxidase, and these compounds may then react in the presence of iron to form free radicals and subsequent LPO. This is consistent with studies that found glutathione and *N*-acetylcysteine protected against acrolein-mediated injuries *in vitro* (Picklo and Montine 2001; Pocernich *et al.* 2001; Logan *et al.* 2005; Luo *et al.* 2005a) and *in vivo* (Pocernich *et al.* 2001). In addition, acrolein treatment in murine keratinocyte PE cells resulted in increased transcription of antioxidant enzymes and glutathione-*S*-transferase through activation of the Nrf2-ARE (antioxidant response element) pathway, and furthermore this effect was attenuated by treatment with glutathione or *N*-acetylcysteine (Kwak *et al.* 2003). Previous studies have reported depletion of total glutathione with little or no change in the relative proportion of GSSG following brain

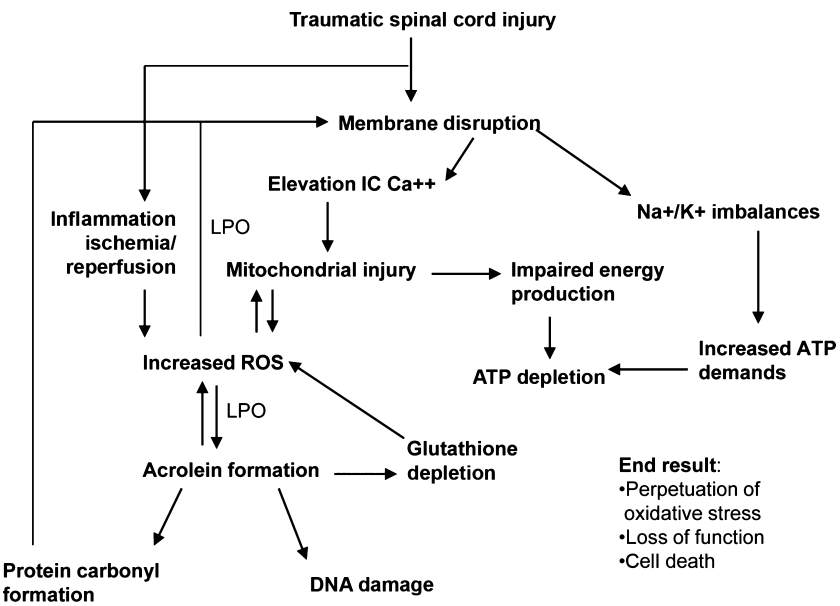

Fig. 2 Pathophysiology of traumatic spinal cord injury and acrolein-mediated injury.

and spinal cord injury *in vivo* (Cooper *et al.* 1980; Lucas *et al.* 2002), which is consistent with the effects of acrolein on spinal cord glutathione concentrations *in vitro* (Hamann *et al.* 2008b). However, the reaction of acrolein with GSH may not decrease the toxicity of acrolein, but rather may be a bioactivation step (Adams and Klaidman 1993). Previous studies found that the reaction product, GS-propionaldehyde, produces superoxide in the presence of xanthine oxidase or aldehyde dehydrogenase more readily than does free acrolein (Adams and Klaidman 1993). This is especially important considering degradation of GS-propionaldehyde by ALDH and ADH has been reported to be relatively slow, and furthermore ALDH activity is inhibited by acrolein (Mitchell and Petersen 1989). Taken together, this suggests that the reaction of glutathione with acrolein is essential to eliminating acrolein; however, this process produces a toxic intermediate and results in depletion of glutathione.

Similarly, in addition to impairing the function of proteins, the reaction of acrolein with amino acids produces reactive products. Acrolein reacts with the free sulfhydryl groups of cysteine residues and the ϵ-amino groups of lysine and histidine residues (Esterbauer *et al.* 1991) to form reactive carbonyl-retaining adducts (Burcham *et al.* 2004; Kaminskas *et al.* 2004b; Burcham and Pyke 2006). A previous study (Uchida *et al.* 1998a) demonstrated by ^{1}H- and ^{13}C-NMR that acrolein reacts preferentially with lysine residues to form N^{α}-acetyl-N^{ϵ}-(3-formyl-3,4-dehydropiperidino)lysine. Some proposed mechanisms of reaction are discussed in detail in the manuscript (Uchida *et al.* 1998a). In addition, the reaction of acrolein with sulfhydryl groups through Michael addition forms an adduct that is more reactive than free acrolein, and may react with nearby amino groups, such as lysine, to form Schiff base adducts, and furthermore can result in cross-linking of proteins (Uchida *et al.* 1998b; Cai *et al.* 2009). These adducts may then undergo other secondary reactions as well (Uchida *et al.* 1998b). Acrolein was also found to react by Michael addition with histidine residues to form N^{α}-acetyl-N^{π}-formylethylhistidine and N^{α}-acetyl-N^{τ}-formylethylhistidine. Another study examined the reaction of acrolein with cysteine residues (Cai *et al.* 2009). Interestingly, they found that cysteine was the most acrolein-reactive residue. However, the reaction of cysteine and acrolein by Michael addition was found to be reversible, which may explain why other studies have failed to identify acrolein-cysteine adducts. In addition, this could allow acrolein to break free from cysteine residues and go on to react with other compounds (Cai *et al.* 2009).

Previous studies suggest that mitochondria may be an important target of acrolein-mediated injury. Specifically, numerous studies report that acrolein treatment results in decreases in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, impaired respiration, and increased production of ROS in mitochondria (Picklo and Montine 2001; Luo and Shi 2005; Luo *et al.* 2005a; Hamann *et al.* 2008b). Treatment with glutathione and *N*-acetylcysteine have been found to prevent acrolein-mediated impairment of mitochondrial respiration (Picklo and Montine 2001). This is consistent with findings that mitochondrial glutathione is decreased following acrolein treatment (Luo and Shi 2005), as well as the hypothesis that glutathione depletion is an important mechanism of acrolein-mediated injury. In addition, it is likely that acrolein impairs mitochondrial respiration by inactivating various proteins. For example, one study found that acrolein treatment decreased activity of aconitase and adenine nucleotide translocase (Luo and Shi 2005), which suggests acrolein impairs function of both the tricarboxylic acid cycle and oxidative phosphorylation. In addition, decreased activity of adenine nucleotide translocase may allow an accumulation of electrons in the electron transport chain and subsequent generation of ROS such as superoxide. However, allopurinol did not inhibit production of ROS, which suggests that xanthine oxidase does not contribute significantly to ROS production by mitochondria (Luo and Shi 2005). In addition, it has been previously demonstrated that acrolein does not induce opening of the transition pore or cytochrome *c* release (Picklo and Montine 2001; Luo and Shi 2005). This was consistent with findings that cyclosporine A failed to prevent the acrolein-mediated decrease in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay in PC12 cells (Luo *et al.* 2005a), and that acrolein-mediated cytotoxicity is primarily by non-apoptotic cell death (Luo *et al.* 2005a; Liu-Snyder *et al.* 2006b). One potential explanation for this finding is that acrolein also significantly impaired ATP production by PC12 cells, and apoptosis is an energy-requiring process (Luo *et al.* 2005a). Thus, the important mechanisms by which mitochondria contribute to acrolein-mediated toxicity include production of ROS and loss of glutathione. These studies illustrate that mitochondria are a rich source of ROS, and thus mitochondrial injury is another important mechanism by which acrolein contributes to oxidative stress.

A number of studies have evaluated the effects of acrolein on various transcription factors, primarily in epithelial cells (Horton *et al.* 1999; Kwak *et al.* 2003; Valacchi *et al.* 2005; Thompson and Burcham 2008). For example, a previous study demonstrated that acrolein-mediated inhibition of proliferation correlated with inhibition of NFκB (nuclear factor kappa B) (Horton *et al.* 1999). Another study demonstrated that inhibition of NFκB correlated with inhibition of IL-8, and thus acrolein had anti-inflammatory effects in bronchial epithelial cells (Valacchi *et al.* 2005). These studies have important implications in exposure to inhaled acrolein and lung cancer, but it is difficult to infer from these findings in epithelial cells the relevance to SCI. This is because, with few exceptions, neuronal cells do not divide. In addition, the inflammatory response differs significantly in the CNS compared to epithelial cells. Another interesting study has

rigorously investigated a number of different transcription factors in response to acrolein in an adenocarcinoma lung cell line, including EGR-2 (early growth response) (pro-death transcription factors), cell cycle control, apoptotic pathways, heat shock proteins, and cyclo-oxygenase (Thompson and Burcham 2008). Again, it is difficult to infer from these studies what the effects would be in neuronal cells. For example, they demonstrated that acrolein induced cytochrome *c* release and apoptosis; however, as discussed in the previous paragraph of this manuscript, numerous studies have demonstrated that acrolein-induced cell death in neuronal cells is primarily non-apoptotic, and does not involve cytochrome *c* release (Luo *et al.* 2005a; Liu-Snyder *et al.* 2006b). Thus, while acrolein-mediated cell death is primarily by apoptosis in epithelial cells, this may not be the case in other cells types. However, it is clear from these studies that acrolein has important effects on a wide variety of transcription factors, and it seems likely that acrolein may also regulate transcription in neuronal cells. Additional studies in neuronal cell types would be necessary in order to assess the relevance to acrolein-mediated injury in the CNS.

Clearly oxidative stress is an important component of acrolein-mediated injury (Luo and Shi 2004, 2005; Luo *et al.* 2005a; Shi and Luo 2006; Hamann *et al.* 2008a,b). In addition to glutathione depletion and production of ROS by damaged mitochondria, there are several other mechanism by which acrolein contributes to oxidative stress. For example, previous studies (Adams and Klaidman 1993) detected formation of hydroxyl radical when GS-propionaldehyde was reacted with xanthine oxidase or aldehyde dehydrogenase. The same study also found that acrolein can form the acroleinyl radical in the presence of xanthine oxidase. While xanthine oxidase may not contribute significantly to mitochondrial ROS formation (Luo and Shi 2005), the significance of this enzyme in cellular production of ROS because of acrolein has yet to be determined. Another important mechanism of acrolein-mediated oxidative stress is disruption of plasma membranes (Shi *et al.* 2002; Luo and Shi 2004; Hamann *et al.* 2008a,b). Injury to the plasma membrane disrupts the normal ion balances, and the subsequent rise in intracellular calcium can lead to additional mitochondrial injury and ROS production. The precise mechanism by which acrolein induces membrane damage is not known. However, damage to membrane bound proteins could play a role. Elevation of intracellular calcium activates calpain (Juurlink and Paterson 1998), and subsequent degradation of the cytoskeleton (Liu-Snyder *et al.* 2006b) may also contribute to axonal degeneration. Production of ROS and subsequent peroxidation of PUFAs in lipid membranes also likely plays an important role in damage to lipid membranes. In addition, oxidative stress further contributes to the rise of intracellular calcium (Xiong *et al.* 2007). Thus, damage to the plasma membrane and oxidative stress are closely related. In addition to oxidative stress, membrane damage also contributes to loss of axonal conductivity, disruption of normal ion balances, and continued degeneration of neurons (Shi and Blight 1996; Shi *et al.* 2000, 2002; Shi 2004; Shi and Whitebone 2006; Hamann *et al.* 2008a,b).

Acrolein has been detected by a variety of methods in its free or protein-bound state, and has been demonstrated to be significantly increased following compression injury in spinal cord injury *ex vivo* (Hamann *et al.* 2008b) and *in vivo* (Luo *et al.* 2005b), following secondary injury in spinal cord *ex vivo* (Hamann *et al.* 2008a), in the brains of individuals with Alzheimer's disease (AD) (Lovell *et al.* 2001), in neurofibrillary tangles (Calingasan *et al.* 1999), and in the Parkinson's disease (PD) brain where it modifies alpha-synuclein (Shamoto-Nagai *et al.* 2007). We recently described a unique model to isolate the effects of secondary injury from those of mechanical injury (Hamann *et al.* 2008a). This study demonstrated that secondary injury by itself is capable of producing significant injury that is distinct from the effects of mechanical injury. We further demonstrated that acrolein is capable of diffusing from the injury site and accumulating and injuring the adjacent, otherwise uninjured tissue. This study was consistent with findings that, following compression injury *in vivo*, acrolein accumulates not only at the injury site, but in adjacent tissue as well (Luo *et al.* 2005b).

The concentration of acrolein in spinal cord has yet to be determined. As such it has yet to be conclusively demonstrated that acrolein accumulates at pathologic concentrations following spinal cord injury, or if it serves as a benign marker for oxidative injury. However, concentrations have been evaluated in other tissue types. Specifically, acrolein concentrations are estimated to reach 80 μM in a model for respiratory tract lining fluids of a smoker (Eiserich *et al.* 1995). In the plasma of patients with renal failure, protein-bound acrolein reaches 180 μM, a six-fold increase (Sakata *et al.* 2003). In the normal and AD brain, acrolein is increased from 0.9 nmol/mg protein (controls) to 2.5 nmol/mg protein (AD brains) in the amygdala, and from 0.7 nmol/mg protein to 5 nmol/mg protein in the parahippocampal gyrus (Lovell *et al.* 2001). In a previous manuscript (Hamann *et al.* 2008b), we estimated these concentrations would correspond to 240 μM in the AD amygdala (86 μM in controls), and 481 μM in the AD parahippocampal gyrus (67 μM in controls). These concentrations intuitively seem high, as concentrations as low as 1 μM have been found to cause significant damage in isolated mitochondria (Luo and Shi 2005), cell culture (Luo *et al.* 2005a), and spinal cord tissue (Luo and Shi 2004). Admittedly, some of the acrolein measured in these studies may represent postmortem and/or artifactual acrolein formation from tissue homogenization and processing. Regardless, previous studies provide significant support for the hypothesis that acrolein accumulates at pathologic concentrations in many disease states. Furthermore, acrolein may reach locally higher concentrations at

certain intracellular locations, such as the plasma membrane and mitochondria.

Acrolein scavenging

The anti-hypertensive drug hydralazine has been shown to bind to and neutralize acrolein (Burcham *et al.* 2000, 2002; Kaminskas *et al.* 2004a) and acrolein-protein adducts (Burcham *et al.* 2004; Kaminskas *et al.* 2004b; Burcham and Pyke 2006). Previous studies identified by ^{1}H NMR the primary reaction product to be (1*E*)-acrylaldehyde phthalazin-1-ylhydrazone, a product of 1 : 1 acrolein-trapping by hydralazine (Kaminskas *et al.* 2004a). They further demonstrated that, in a cell-free system, equi-molar concentrations of hydralazine and acrolein resulted in nearly complete loss of acrolein. The reaction product of hydralazine with acrolein-protein adducts has also been characterized (Burcham *et al.* 2004). Previous studies (Williams *et al.* 1980; Weglarz and Bartosz 1991; Runge-Morris *et al.* 1994) report toxic effects of hydralazine concentrations in excess of 1 mM *in vitro*.

The acrolein scavenger hydralazine has been previously shown to prevent acrolein-mediated cell death and injuries in PC12 cells (Liu-Snyder *et al.* 2006a). In isolated spinal cord *ex vivo*, hydralazine prevented acrolein mediated oxidative stress, membrane damage, and loss of compound action potential (Hamann *et al.* 2008a,b). Hydralazine also prevented compression mediated increases in acrolein-protein adducts in isolated spinal cord *ex vivo*, which correlated well with findings that hydralazine prevented compression mediated oxidative stress and membrane damage (Hamann *et al.* 2008b). Furthermore, in a unique model of secondary injury, treatment with the acrolein scavenger hydralazine not only significantly decreased acrolein accumulation, but also attenuated secondary injury (Hamann *et al.* 2008a). In addition, hydralazine prevented allyl alcohol-induced hepatotoxicity, which is mediated by acrolein, in cultured hepatocytes (Burcham *et al.* 2000, 2004) and in mice *in vivo* (Kaminskas *et al.* 2004b). Furthermore, previous studies report that hydralazine is not an efficient scavenger of superoxide and does not directly influence repair of damaged membranes (other potential mechanisms by which hydralazine could promote improved recovery) (Hamann *et al.* 2008b). Therefore, the mechanism by which hydralazine improves recovery following compression-mediated injuries is likely through acrolein scavenging, and not superoxide scavenging or repair of damaged membranes. Taken together, this evidence further supports the hypothesis that acrolein accumulates at pathologic concentrations following compression injury *ex vivo*, plays a key role in secondary injury mechanisms following traumatic spinal cord injury, and thus represents a potential novel target of therapy to promote improved recovery following spinal cord injury.

One of the major limitations of the acrolein scavenger hydralazine is that therapeutic concentrations may not be achieved *in vivo*, as the concentration following IV administration at the anti-hypertensive dose is estimated to peak at 0.5–1.0 μM, and its half-life is only about 30 min to 1 h (Reece 1981). It has been estimated that acrolein concentrations may exceed this following SCI *in vivo* (Hamann *et al.* 2008b), and as described previously hydralazine reacts in an equi-molar manner to neutralize acrolein (Kaminskas *et al.* 2004a). In addition, its vasodilatory effects would be highly undesirable in a patient that suffered spinal cord injury, as they would likely be suffering from neurogenic shock. However, studies using hydralazine *in vitro* provide valuable proof-of-principle that acrolein-scavenging is an effective means of curtailing oxidative stress. We mentioned in a prior manuscript (Hamann *et al.* 2008b) that topical application of acrolein scavengers may be a potential means of therapy, as well as developing a more efficient acrolein scavenger. Since the hydrazine group on hydralazine is responsible for its acrolein-scavenging abilities but not the anti-hypertensive effects, these studies will provide valuable information for future drug design and development.

In addition to hydralazine, there are several other hydrazines that have received less attention than hydralazine with regards to acrolein-scavenging, but may overcome some of these limitations (structures shown in Fig. 3). Dihydralazine is similar structurally and pharmacologically to hydralazine, but possesses two hydrazine groups. Thus, it could potentially be twice as efficient as hydralazine at trapping acrolein. It has been shown to trap acrolein in a cell-free system and protect against acrolein-induced lactate dehydrogenase (LDH) release in cultured hepatocytes (Kaminskas *et al.* 2004a). Phenelzine is a monoamine oxidase inhibitor that, like hydralazine, possesses a hydrazine group. Previous studies demonstrated that phenelzine reacts with 3-aminopropanal to form a hydrazone, and protects against 3-aminopropanal and acrolein induced LDH release *in vitro*, as well as ischemia-reperfusion injury in gerbils *in vivo* (Wood *et al.* 2006). Phenelzine is unlikely to produce more efficient acrolein-scavenging than the 1 : 1 scavenging of hydralazine, since hydralazine and phenelzine both possess one

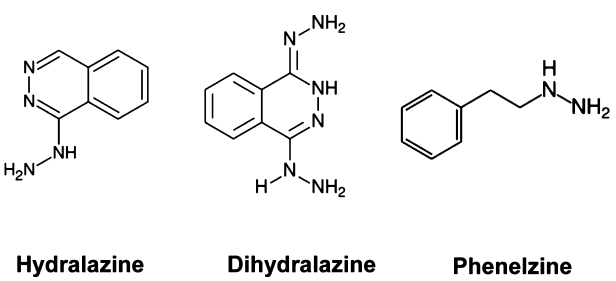

Fig. 3 Structures of hydralazine, dihydralazine, and phenelzine.

hydrazine group. However, it has the advantage that it is not a vasodilator and can be safely administered at higher concentrations following SCI. Following oral administration of 30 mg/day in people (0.5 mg/kg for a 150 lb person), its concentration in serum peaked at 19.8 ng/mL (.085 μM), but the half-life was 11.6 h (based on FDA-approved clinical trials by Pfizer). In addition, doses of 15 mg/kg subcutaneously have been administered to gerbils following ischemia-reperfusion brain injury (Wood *et al.* 2006), which could reasonably be expected to produce serum concentrations much higher than those following 0.5 mg/kg oral dosing.

Some other acrolein-scavenging drugs have been evaluated with less success. Previous studies (Burcham *et al.* 2002, 2004; Kaminskas *et al.* 2004a) found that hydralazine was a far more efficient acrolein scavenger than methoxyamine, aminoguanidine, pyridoxamine, and carnosine (other well known aldehydes scavengers). While MESNA (sodium 2-mercaptoethanesulfonate) is an efficient acrolein scavenger in a cell-free system, previous studies (Kaminskas *et al.* 2004a) found that it did not protect cultured hepatocytes against acrolein-mediated LDH release as well as hydralazine. They hypothesized that its ability to enter and protect cells was impaired by its low lipophilicity, which was consistent with a human clinical trial that found it remained in the vasculature following IV administration (Kaminskas *et al.* 2004a).

Some additional difficulties in pharmacological scavenging of carbonyls such as acrolein-modified protein have been previously discussed (Burcham 2008). In particular, the fate of hydralazine-trapped acrolein-protein adducts in tissue is not clear; for example if they are degraded or if they accumulate in tissues, and furthermore what is the impact of tissue accumulation. It has been previously suggested that accumulation of hydrazine-trapped acrolein-protein adducts in tissue could potentially induce adverse reactions such as autoimmune disease (Burcham 2008). Indeed, hydralazine is well known to be an important cause of drug-induced lupus erythematosus, which may be because of its modification of DNA and subsequent formation of anti-nuclear antibody (Parodi *et al.* 1981; Runge-Morris *et al.* 1994; Kumar *et al.* 2004). Additional studies would be necessary in order to evaluate the metabolism of hydrazine-trapped acrolein-protein adducts, the effects of tissue accumulation, and the risk-benefit ratio of acrolein scavenging under various disease conditions.

Summary and conclusions

Previous studies provide significant evidence that acrolein plays an important role in the pathophysiology of SCI. Acrolein's long-lived potential to perpetuate oxidative stress provides one possible explanation for the ineffectiveness of ROS scavengers in clinical trials, and it thus represents a novel and potentially more effective target for attenuating oxidative stress. Furthermore, oxidative stress plays a critical role in the pathophysiology of not only spinal cord trauma, but many other diseases as well, including AD, PD, ischemia-reperfusion injury, trauma, inflammation, and neoplasia. Thus, acrolein could potentially play an important role in these diseases as well. Indeed, acrolein has been found to be significantly increased in the AD brain (Lovell *et al.* 2001) and PD brain (Shamoto-Nagai *et al.* 2007). In addition, acrolein is a component of cigarette smoke that has been implicated in lung cancer (Feng *et al.* 2006) and is a known carcinogen (Esterbauer *et al.* 1991; Feron *et al.* 1991; Cohen *et al.* 1992; Kehrer and Biswal 2000). Because of its well-established toxicity, relatively long half-life, and role in perpetuating oxidative injury, acrolein scavenging represents a novel mechanism of preventing oxidative injury and potential treatment for not only spinal cord injury, but many other diseases as well.

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