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Diffusive oxidative stress following acute spinal cord injury in guinea pigs and its inhibition by polyethylene glycol

Jian Luo, Riyi Shi*

Department of Basic Medical Sciences, Institute for Applied Neurology, Center for Paralysis Research, Purdue University, West Lafayette, IN 47907, USA

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Abstract

Spinal cord injury (SCI) results in rapid and significant oxidative stress. We have previously demonstrated that polyethylene glycol (PEG) repairs neuronal membrane and inhibits lipid peroxidation in an in vitro model of SCI. In this study we tested the effects of PEG on oxidative stress in guinea pigs after SCI. Oxidative stress was assessed by lipid peroxidation, protein carbonyl and glutathione content. A compression injury of spinal cord at T10–11 induced a rapid and diffusive oxidative stress. Administration of PEG immediately after injury resulted in a marked decrease in oxidative stress both at the injury site and in its adjacent segments. These results, along with our previous findings, suggest that an early application of PEG can effectively suppress oxidative stress after SCI in vivo.

Keywords: Spinal cord injury; Oxidative stress; Polyethylene glycol; Lipid peroxidation; Protein carbonyl; Glutathione; Guinea pig

Oxidative stress has been extensively explored as one of the central mechanisms of secondary injury in traumatic spinal cord injury (SCI) [7,12,15]. Curtailing oxidative stress constitutes a potentially effective intervention to reduce secondary injury and enhance neurological recovery [7,12]. However, the effort to develop effective treatments to suppress oxidative stress and enhance functional recovery in CNS traumatic injuries has been largely unsuccessful. Recently, we have reported that polyethylene glycol (PEG) immediately seals neuronal membranes and inhibits lipid peroxidation (LPO) in an in vitro acute SCI model [14]. Despite the significant findings in vitro [14,16–18], the same effect of PEG has not yet been confirmed in vivo, which is clinically more relevant. In this study, we intended to evaluate PEG's efficacy in the suppression of oxidative stress in a guinea pig spinal cord compression injury model.

All animals used in this study were handled in accordance with the NIH guide for The Care and Use of Laboratory Animals and the experimental protocol was approved by the Purdue Animal Care and Use Committee. Adult female guinea pigs (350–450 g) were anesthetized with an intramuscular injection of 100 mg/kg ketamine and 20 mg/kg xylazine, and the spinal cord was exposed by dorsal laminectomy aseptically at T10–11. A compression injury was then induced by a constant-displacement, 15 s compression of the spinal cord, using a modified forceps possessing a spacer [3]. The injury site covered T10–11 spinal cord (1 cm long). Such a lesioning procedure had previously been calibrated to produce an immediate and complete loss of compound action potential conduction through the injury and a complete paralysis of the hind limbs [4,5]. When used, PEG (MW ~2000, Sigma-Aldrich) was applied directly to the spinal lesion site [4] within 5 min of injury. Briefly, an aqueous solution of PEG (50%, w/v) was applied with a pipette to the exposed injury site for 2 min and then removed by aspiration. The site of PEG application was then immediately lavaged with isotonic Krebs' solution and any excess PEG and/or Krebs' solution were removed by aspiration. The wounds were closed and animals were kept warm until awaking with heat lamps. Guinea pigs were housed individually and fed ad libitum. The animals were randomly divided into four groups: sham-vehicle, injured-vehicle, injured-PEG and sham-PEG. For the animals in the sham-vehicle and injured-vehicle groups, the cord was exposed to saline solution and lavaged with Krebs' solution, which was subsequently removed. The sham-PEG and injured-PEG groups received PEG exposure and lavage with Krebs' solution. The sham-PEG group was designed to determine if PEG has any non-specific effects

* Corresponding author. Tel.: +1-765-496-3018; fax: +1-765-494-7605.
E-mail address: riyi@purdue.edu (R. Shi).

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on the assays themselves. Therefore, only one time point (1 h after application) was observed in this group to reduce animal usage. All the solutions were prepared and used at room temperature (25 °C).

Spinal cord samples were isolated at 1, 4, 24, and 72 h after surgery ($n = 4–8$ at each time point for each group). Guinea pigs were transcardially perfused with 500 ml oxygenated, cold Krebs' solutions (15 °C) after anesthetization. A total of five cord segments (1 cm long each) including T6–7, T8–9, T10–11, T12–13, and L1–2 were dissected and collected. Each segment was homogenized immediately with a buffer (0.25 M sucrose, 0.5 mM EDTA, 10 mM Tris–HCl, pH 7.4). The homogenate was centrifuged twice for 3 min at $2000 \times g$ and the supernatant was collected for the measurement of oxidative stress. Protein content was determined by bicinchoninic acid (BCA) assay (Pierce, Rockford, IL), with bovine serum albumin as the standard. Three parameters were used to measure the extent of oxidative stress: LPO [14], protein carbonyl [11] and glutathione (GSH) content [1]. Data were expressed as mean ± SD and were analyzed by ANOVA using the statistics program SPSS (11.5, SPSS Inc., Chicago, IL) followed by post-hoc least-significance difference test; $P < 0.05$ was considered statistically significant.

The LPO content in the sham-vehicle group was comparable in all spinal cord segments examined (T6–L2) and remained constant during the 1–72 h after surgery (Fig. 1). In the injured-vehicle group, the compression injury caused a significant increase of LPO not only in the injury site (T10–11), but also in some adjacent segments at 1–72 h post injury (Fig. 1). As an example, the LPO of 24 h after injury is illustrated in Fig. 1, left panel; the LPO increased significantly in T6–L2, with the highest concentrations in T10–11. Interestingly, the LPO in T12–13 was significantly higher than T8–9 ($P < 0.05$) and the LPO in L1–2 was higher than T6–7 ($P < 0.05$). In the injured-PEG group, LPO was significantly reduced in adjacent cord segments as well as in the compressed segment. This general observation had one exception at T6–7 ($P > 0.05$). In the sham-PEG group, application of PEG did not significantly affect the LPO measurement in T6–L2 (data not shown) at 1 h after application. The time course of LPO changes at T10–11 is shown in Fig. 1, right panel. In the injured-vehicle group, the LPO was significantly increased at 1 h after injury, and remained significantly higher up to 72 h post injury ($P < 0.01$ in all comparisons with the sham-vehicle group). In the injured-PEG group, LPO at T10–11 was significantly reduced at 1–72 h post injury (Fig. 1, $P < 0.05$ in all comparisons between injured-PEG and injured-vehicle).

Fig. 2 shows the changes of protein carbonyls. In the sham-vehicle group, comparable levels of protein carbonyls were observed in all segments examined (T6–L2) during the observation period (1–72 h after surgery). There was no significant difference between the protein carbonyls of the sham-PEG and those of the sham-vehicle ($P > 0.05$) at 1 h after PEG application (data not shown). In the injured-vehicle animals, protein carbonyls at the injury site (T10–11) increased significantly at 1–72 h post injury; however, it was not until 72 h post injury that protein carbonyls in adjacent segments were elevated significantly. As shown in

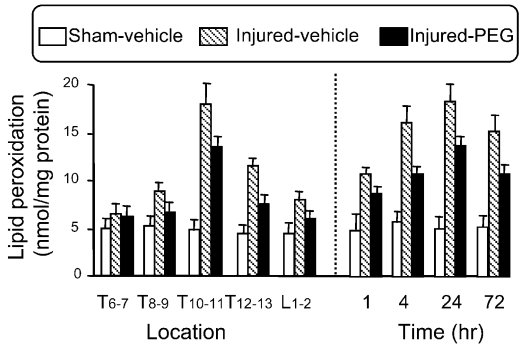

Fig. 1. Spinal cord LPO after compression injury. (Left) Extensive LPO at 24 h after injury and its inhibition by PEG. LPO of the injured-vehicle group was significantly increased in T8–9, T10–11 (injury site), T12–13 and L1–2 ($P < 0.01$ as compared with sham-vehicle for all comparisons). Further, LPO in T12–13 was higher than in T8–9 ($P < 0.05$), and higher in L1–2 than in T6–7 ($P < 0.05$). In the injured-PEG group, PEG treatment significantly decreased LPO at T8–L2 ($P < 0.05$ for all comparisons between injured-PEG and injured-vehicle). (Right) Time course of LPO at T10–11. In the injured-vehicle group, LPO was significantly increased ($P < 0.01$) at 1 h after injury, and maintained at significantly high levels afterwards with the peak at 24 h after injury ($P < 0.01$). Treatment with PEG significantly reduced LPO at all time points ($P < 0.05$ for all comparisons between injured-vehicle and injured-PEG groups).

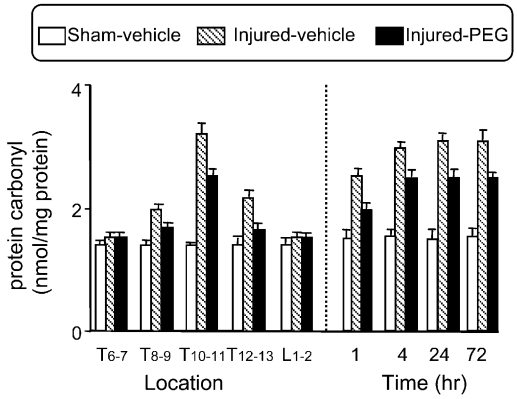

Fig. 2. The increase of protein carbonyls after SCI and the inhibitory effects of PEG treatment. (Left) Seventy-two hours after injury, levels of protein carbonyls were significantly increased at T10–11 (injury site), T8–9, T12–13, and L1–2 ($P < 0.05$, $n = 4–8$) in the injured-vehicle group. Note that protein carbonyls in T12–13 were significantly higher than in T8–9 ($P < 0.01$). Treatment with PEG significantly reduced protein carbonyl levels in T10–11 and T12–13 ($P < 0.01$ for both) in the injured-PEG group. (Right) Time course of protein carbonyls at T10–11. Protein carbonyls were significantly increased at all time points (from 1 to 72 h after injury, $P < 0.01$ for all comparisons with sham-vehicle) in the injured-vehicle group. The injured-PEG group showed significantly lower protein carbonyls at all time points ($P < 0.05$ for all comparisons with injured-vehicle).

Fig. 2, left panel, at 72 h after injury, the protein carbonyls were also significantly increased at T8–9, T12–13, and L1–2 ($P < 0.05$ compared to sham-vehicle in all comparisons). Interestingly, similar to LPO measurement, the levels of protein carbonyls in T12–13 were significantly higher than that of its rostral counterpart (T8–9, $P < 0.01$). Application of PEG immediately after injury significantly inhibited the formation of protein carbonyls in the injury sites and adjacent segments. In the injured-PEG group, at 72 h after injury, the level of protein carbonyls at T10–11 and T12–13 was significantly lower than that of the injured-vehicle group ($P < 0.01$ for both comparisons, Fig. 2, left panel). The time course of protein carbonyls at T10–11 is shown in Fig. 2, right panel. In the injured-vehicle animals, the protein carbonyls at T10–11 were significantly higher than those of sham-vehicle at 1–72 h post injury ($P < 0.01$ for all comparisons). Protein carbonyls were reduced significantly by PEG treatment at all four time points examined in the injured-PEG group ($P < 0.05$ for all comparisons).

GSH content is illustrated in Fig. 3. The animals in the sham-vehicle group exhibited comparable GSH content in all segments examined (T6–L2) during the observation period (1–72 h after surgery). In the injured-vehicle group, the compression injury caused a significant decrease of GSH in both the original injury site and some adjacent segments. For example, at 24 h after injury (Fig. 3, left panel), GSH content at T10–11 (12.85 ± 1.01 nmol/mg protein) was only 70% of the sham-vehicle (18.76 ± 0.94 nmol/mg protein, $P < 0.01$). The adjacent segments, T8–9, T12–13 and L1–2, also had a significant drop in GSH as compared with those of the sham-vehicle ($P < 0.05$). The level of GSH in T12–13 was significantly lower than T8–9 ($P < 0.05$). The injured-PEG group showed a significantly higher level of GSH in T10–11 and T12–13 compared to the injured-vehicle group. Again, in the sham-PEG group, application of PEG did not significantly affect the GSH measurement in T6–L2 (data not shown) at 1 h after application. The time course of GSH is shown in Fig. 3, right panel. GSH contents of the injury site were significantly decreased at 1 h, and continued to decrease up to 72 h after injury in the injured-vehicle group. PEG treatment (injured-PEG group) resulted in significant recovery of GSH content at 1, 4, 24 and 72 h.

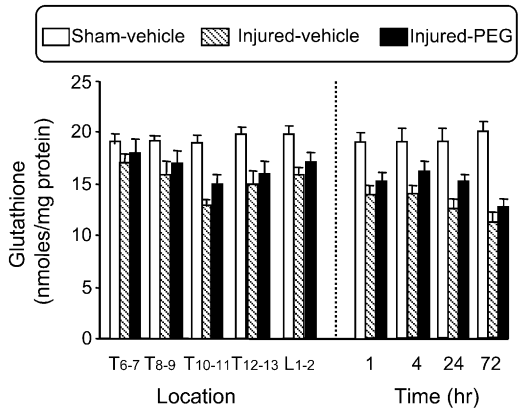

Fig. 3. The decrease of GSH content and the improvement by PEG treatment. (Left) Twenty-four hours after injury, the GSH content of the injured-vehicle group was significantly decreased in T8–L2 ($P < 0.05$), with the lowest levels in T10–11 ($P < 0.01$). Also, GSH in T12–13 was significantly lower than that in T8–9 ($P < 0.05$). The injured-PEG group showed significantly higher GSH levels in T10–11 and T12–13 ($P < 0.05$ for both comparisons with injured-vehicle). (Right) GSH content of T10–11 continuously decreased during a period of up to 72 h after injury ($P < 0.01$, $n = 4–8$) in the injured-vehicle group. In the injured-PEG group, GSH was significantly increased over those of the injured-vehicle at all time points ($P < 0.05$ for all comparisons).

In a previous study [14], we reported that PEG could repair membrane damage and reduce oxidative stress in an in vitro spinal cord compression model. In this study, we have demonstrated that an early application of PEG can have an extended effect in reducing oxidative stress after physical trauma to the spinal cord in vivo. The PEG-induced inhibition of oxidative stress is not due to its non-specific effect on the oxidative assays, because application of PEG to the sham-PEG animals did not affect these assays. This was a remarkable observation and emphasizes the importance of early intervention in modifying the long-term biochemical and functional recovery.

The secondary oxidative stress following in vivo spinal cord trauma is not only long lasting but diffusive, based on this study. Oxidative stress spreads as far as 20 mm, or roughly up to four segments, outside of the original injury site. Furthermore, this spreading lasts at least 72 h post injury. In the sham-vehicle animals, there was no evidence of such a shift in this relevant biochemistry. This finding is consistent with several previous reports. Using an in vitro spinal cord compression model, Blight and his colleagues [13] found a significant subcellular deregulation of the Na^+, K^+ equilibria in the regions adjacent to the injury site. Using a rat spinal cord contusion model, Baldwin and colleagues also found a significant increase of HNE, a toxic LPO by-product, in a region two segments outside of the injury site [2]. Similar diffusive oxidative injury was also observed by Juurlink and colleagues in a rat spinal cord compression model [10]. These findings reveal that secondary oxidative stress resulting from the primary insult is not just limited to the original injury zone but is widely distributed. Given the endotoxic character of many of these chemical intermediates [6], biochemical abnormalities likely lead to the widespread tissue destruction observed after severe SCI.

An observation we found to be quite intriguing is that oxidative stress is often more severe in caudal segments adjacent to the lesion than rostral segments. A similar asymmetric injury zone was also seen in other reports [2, 10]. It is not yet clear if this leads to asymmetric tissue loss, with more severe tissue deterioration in the caudal cord following compression injury. Also not known are the mechanisms underlying this asymmetric spreading of secondary injury.

We emphasize, however, that PEG was administrated

within 5 min after injury in this study. Future experiments need to be carried out to investigate whether a delayed application (i.e. 3–8 h after injury) of PEG, which still resulted in significant restoration of function [5], has any inhibitory effects in oxidative stress.

A conventional strategy to minimize oxidative injury is to increase scavenging of ROS [8,9]. This approach has been proven to be largely ineffective in the context of traumatic CNS injury [8]. We suggest that in order to break the cycle of oxidative injury [19], it is necessary to directly interfere with the generation of ROS by repairing the membranes of damaged cells, as well as reducing ROS using scavengers. In fact, since most of the ROS are extremely short lived and inaccessible to effective scavengers before the damage is done, one could argue that the prevention of ROS formation would be expected to be more effective in reducing ROS related injury. Since PEG alone cannot completely suppress oxidative stress, the combination of PEG and antioxidants may be a more effective strategy to reduce 'secondary injury'. This approach may be particularly effective in CNS trauma since membrane damage and ROS increase are two major mechanisms of injury.

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