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Characterizing Phospholipase A₂-Induced Spinal Cord Injury—A Comparison with Contusive Spinal Cord Injury in Adult Rats

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Abstract

To assess whether phospholipase A₂ (PLA₂) plays a role in the pathogenesis of spinal cord injury (SCI), we compared lesions either induced by PLA₂ alone or by a contusive SCI. At 24-h post-

injury, both methods induced a focal hemorrhagic pathology. The PLA₂ injury was mainly confined within the ventrolateral white matter, whereas the contusion injury widely affected both the gray and white matter. A prominent difference between the two models was that PLA₂ induced a massive demyelination with axons remaining in the lesion area, whereas the contusion injury induced axonal damage and myelin breakdown. At 4 weeks, no cavitation was found within the PLA₂ lesion, and numerous axons were myelinated by host-migrated Schwann cells. Among them, 45% of animals had early transcranial magnetic motor-evoked potential (tcMMEP) responses. In contrast, the contusive SCI induced a typical centralized cavity with reactive astrocytes forming a glial border. Only 15% of rats had early tcMMEP responses after the contusion. BBB scores were similarly reduced in both models. Our study indicates that PLA₂ may play a unique role in mediating secondary SCI likely by targeting glial cells, particularly those of oligodendrocytes. This lesion model could also be used for studying demyelination and remyelination in the injured spinal cord associated with PLA₂-mediated secondary SCI.

Keywords

Phospholipase A₂; Spinal cord injury; Demyelination; Axonal degeneration; Schwann cells

Introduction

There are two mechanisms of damage to the spinal cord after injury: a primary mechanical injury and a secondary injury mediated by multiple injury processes including inflammation, free radical-induced cell death, and glutamate excitotoxicity [1–4]. Increasing evidence shows that phospholipase A₂ (PLA₂) can be induced by at least the three harmful substances mentioned above. PLA₂ is a lipolytic enzyme that hydrolyzes membrane phospholipids to produce free fatty acids and lysophospholipids [5, 6]. These products are precursors of bioactive eicosanoids and platelet-activating factor (PAF) which are well-known mediators of inflammation and tissue damage implicated in pathological states of several acute and chronic neurological disorders [6–8]. PLA₂ contributes to these disorders by attacking cellular membranes, releasing proinflammatory lipid mediators, and generating free radicals [6, 9].

It is believed that PLA₂ may serve as a common molecule in mediating secondary injury because it can be induced by multiple harmful substances that are generated in the injured spinal cord following spinal cord injury (SCI) including inflammatory cytokines such as tumor necrosis factor α (TNF- α) and interleukin 1 β (IL-1 β) [5, 10], free radicals such as those induced by H₂O₂ [11], and excitatory amino acids such as glutamate [9, 12, 13]. Also, PLA₂ and its metabolic products can induce inflammation [5, 7], oxidation [5, 7], and neurotoxicity [7, 14].

PLA₂ is present in the mammalian brain [15, 16] and spinal cord [17]. Our recent experimental results showed that both PLA₂ activity and expression increased significantly following SCI, and that injection of exogenous PLA₂ into the normal rat spinal cord induced tissue damage [18, 19]. In the present study, we investigated the pathogenesis of SCI induced by exogenously delivered PLA₂ in adult rats compared that with conventional contusive SCI, induced by a New York University (NYU) impactor. We found that PLA₂-induced SCI mimicked many features of contusive SCI suggesting that PLA₂ may play a key role in mediating the pathogenesis of secondary injury. However, distinct differences also exist between the two models. In particular, the former induced ventral white matter demyelination followed by remyelination by migrated Schwann cells, whereas the latter induced axon and myelin breakdown as well as cavity formation. Thus, PLA₂-induced SCI

could serve as a unique model to explore mechanisms of demyelination and remyelination associated with secondary SCI.

Material and Methods

Animals

A total of 60 adult female Sprague–Dawley rats (Harlan, Indianapolis, IN), weighing 210–230 g, were used in this study. These included 30 rats for contusive SCI and 30 rats for injections of PLA₂. All surgical interventions and postoperative animal care were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council) and the Guidelines of the Indiana Institutional Animal Care and Use Committee.

Contusive Spinal Cord Injury

Contusive SCI was performed using a NYU impactor as described previously [20]. Briefly, rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and received a laminectomy at the T9–10 level. The SCI was performed at T10 by dropping a 10-g rod (2.5 mm in diameter) onto the dorsal surface of the cord from a height of 12.5 mm. For the shamoperated controls, the animals underwent a T9–10 laminectomy without the weight-drop injury.

Microinjections of PLA₂ into the Normal Adult Rat Spinal Cord

Microinjections of PLA₂ into the normal adult rat spinal cord were described previously [18]. Briefly, a beveled glass micropipette (external diameter, 10–20 μ m), loaded with 4 μ l of PBS or PLA₂ (at a dose of 50 ng; Type III, Cayman Chemical Company, Ann Arbor, MI), was injected bilaterally into the spinal cord (2 μ l/side) at 0.6 mm lateral to the midline and 1.5 mm ventral to the dorsal surface of the cord for 10 min.

Myeloperoxidase Activity Assay

Myeloperoxidase (MPO) activity was measured according to previously reported methods [21, 22] with minor modifications. Briefly, the injured spinal cord segment (1 cm long) was removed and homogenized. The supernatant, after centrifugation at 14,000 $\times g$ for 25 min, was assayed for MPO activity which was determined in duplicate by mixing 0.1 ml of the supernatant with 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml o-dianisidine and 0.0005% H₂O₂. Absorbance at 460 nm was recorded with a Biophotometer (Eppendorf AG, German).

Histological Assessments

At 24 h or 4 weeks after PLA₂ injection or contusive spinal cord injury, animals were perfused, and the cord segments were dissected out, embedded, and sectioned at 20 μ m according to our previous protocols [18, 23]. A set of serial cross sections of the spinal cord were stained for myelin with Luxol fast blue and were counterstained with Cresyl Violet–Eosin. Lesion areas were outlined and quantified using an Olympus BX60 microscope equipped with a Neurolucida system (MicroBrightField, Colchester, VT). Areas of demyelination were quantified using NIH image software.

Toluidine Blue Staining and Electron Microscopy

The toluidine blue staining on semi-thin sections and electron microscopy were described in our previous publication [18]. Briefly, spinal cord segments were fixed overnight in the solution containing 2% glutaraldehyde and 5% sucrose in 0.1 M sodium cacodylate buffer, pH 7.4, followed by 1% osmium tetroxide in the same buffer for 1 h. The tissue was embedded in Spurr's epoxy resin and cured at 70°C. Transverse semi-thin sections (1 μ m)

were stained with a mixture of 1% toluidine blue and 1% sodium borate. Ultra-thin sections (70–90 nm) were collected on copper mesh grids with 600 bars per inch, subsequently counterstained with 4% uranyl acetate in 50% ethanol, and Reynolds' lead citrate, and examined using a Philip CM10 electron microscope.

Immunofluorescence Double Labeling

This method has been described in our previous publication [20]. Briefly, a mixture of rabbit polyclonal anti-GFAP (1:200, Sigma) and mouse anti-SMI-31 (1:2,000, Sigma) antibodies was used to examine astrocytes and axons, respectively. The paired antibody solutions were applied to the sections overnight at 4°C. On the following day, the sections were incubated with fluorescein-conjugated goat anti-rabbit (1:100; ICN Biochemicals, Aurora, OH) and rhodamine-conjugated goat anti-mouse (1:100; ICN Biochemicals) antibodies. Primary antibody omission controls and normal mouse, rabbit, and goat serum controls were used to further confirm the specificity of the immunofluorescence double labeling. Slides were examined with an Olympus Optical Fluoview confocal microscope.

Behavioral Assessments

Behavioral assessments were performed using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale, a 21-point scale (0–21) based on the observation of hindlimb movements of a rat freely moving in an open field [24, 25]. The BBB was evaluated at 1, 2, 3, and 4 weeks after PLA₂ injections or contusive SCI. During evaluation, animals were allowed to walk freely on the open-field surface for 4 min while being observed by two scorers lacking knowledge of the experimental groups.

Measurements of Axonal Conduction

Nerve conduction was assessed with transcranial magnetic motor-evoked potentials (tcMMEP), an in vivo electrophysiological measure of motor pathway function, which was described in our previous publication [18]. The tcMMEP responses were elicited by the activation of subcortical structures with an electromagnetic coil placed over the cranium. Action potentials descend in the ventral spinal cord and synapse onto motoneuron pools in which output signals can be recorded from both of the gastrocnemius muscles.

Statistical Analysis

All data are presented as mean±SEM values. One-way ANOVA with post hoc Tukey *t* test was used to determine statistical significance. A *p* value of <0.05 was considered statistically significant.

Results

1. Pathological changes at 24 h after microinjections of PLA₂ or contusive SCI

Figures 1 and 2 show transverse sections of spinal cords at 24 h after PLA₂ injection or contusive SCI. Cresyl Violet–Eosin staining revealed that both models induced a focal hemorrhagic injury and inflammatory cell infiltration (Fig. 1). However, the PLA₂-induced hemorrhage was mainly confined within the ventrolateral white matter near the injection site, while the hemorrhage induced by contusive injury was widely spread across both the gray and white matter. Both models induced infiltration of inflammatory cells (Fig. 1a, c). This was further supported by the observation that MPO activity, an indicator of neutrophil infiltration, was significantly increased in both the PLA₂-induced (126.9±26.7 U/g Pr) and contusive SCI models (190.3±29.6 U/g Pr), as compared to the controls (*p*<0.01). Luxol fast blue staining showed that PLA₂ microinjection induced confined demyelination localized mainly in the white matter particularly in its ventrolateral portion (Fig. 1b). Notably, the

gray matter remained largely intact. In contrast, contusive SCI induced a centralized damage at the injury epicenter that affected both the gray and white matter with the sparing of only a rim of myelin in the spinal cord periphery (Fig. 1d, dashed line). However, Toluidine blue staining and electron microscopy further demonstrated that PLA₂ injections induced a massive demyelination with many axons that remained intact in the lesion area (Fig. 2a, c). The myelin sheaths were dismantled and lamellae appeared loosely arranged, separated to form large clear spaces, and degenerated into small clusters of vesicles (Fig. 2a, c). Clearly, most of the axons surrounded by these degenerating myelin sheaths appeared to be morphologically normal (Fig. 2a, c). In contrast to the PLA₂ injection, contusive SCI induced both axonal damage and myelin breakdown (Fig. 2b, d).

2. Pathological changes at 4 weeks after microinjections of PLA₂ or contusive SCI

Figures 3 and 4 show transverse sections of spinal cords at 4 weeks after either PLA₂ injection or contusive SCI. Luxol fast blue (Fig. 3a, c) and Cresyl Violet–Eosin (Fig. 3e, g) staining showed that both models induced tissue damage and loss of myelin. Consistent with our observation at the 24-h time point, the PLA₂-induced lesion was confined mainly within the ventrolateral white matter, suggesting that PLA₂ preferentially causes white matter damage. No cavity was found in the PLA₂-induced damage. In contrast, contusive SCI induced damage to both the gray and white matter resulting in a central cavitation. Immunofluorescence staining showed that both models induced glial scar formation surrounding the lesion border (Fig. 3b, d). Interestingly, in the PLA₂-induced damage, astrocytes did not migrate into the lesion area (Fig. 3b) where numerous SMI-31-positive axons were found (Fig. 3f). At high magnification, a clear border between glial fibrillary acidic protein-immunoreactive (GFAP-IR) astrocytes and SMI-31-IR axons was seen (Fig. 3k). In contrast to the PLA₂-induced damage, few SMI-31-IR axons were found within the lesion cavity (Fig. 3h) which was surrounded by a clear border of GFAP-IR reactive astrocytes (Fig. 3d). High magnification of boxed area in Fig. 3d further depicts the lack of SMI-31-IR axons within the lesion cavity in relationship to the GFAP-IR astrocytic glial border where many intact axons were found (Fig. 3l). Toluidine blue staining (Fig. 4a, b) and electron microscopy (Fig. 4c, d) showed that, at 4 weeks post-injury, numerous axons, presumably demyelinated by PLA₂, remained in the lesion area in the PLA₂ injected group. These axons, however, which were remyelinated by Schwann cells, likely migrated from the periphery (Fig. 4a, c). In contrast, at 4 weeks following contusive SCI, most axons were completely degenerated within the lesion area (Fig. 4b, d); however, degenerating myelin segments were frequently visualized, indicating that the process of myelin breakdown is much slower than that of axonal degeneration after contusive SCI. In some areas, scattered axon profiles myelinated by infiltrating Schwann cells were observed (data not shown). In both models, infiltration of macrophages into the lesion area was observed (Fig. 4a, c). Table 1 summarizes the histopathological changes and cellular responses to PLA₂-induced or contusive spinal cord injuries.

3. Electrophysiological and behavioral changes in rats that received PLA₂ microinjection or contusive SCI

In both injury models, the amplitudes of tcMMEP were significantly reduced (Fig. 5 and Table 2). However, contusive SCI induced three types of tcMMEP responses: early, late, or no response (Fig. 5d, f, h), whereas PLA₂ injection induced only two types of tcMMEP responses: early or no response (Fig. 5c, e). About 45% and 15% of the PLA₂-induced and contusion SCI, respectively, had early tcMMEP responses. In both injury models, a decreased BBB motor rating scale was found (Fig. 6). Similar patterns of BBB improvements were observed in the two injury models with no statistically significant differences being found between the two.

Discussion

To simulate the effect of PLA₂ activation after SCI, we directly injected exogenous PLA₂ (50 ng) into the normal rat spinal cord and compared its histopathological, electrophysiological, and behavior changes with those induced after a contusive SCI in adult rats. This dose of exogenous PLA₂ was equivalent to the peak level of PLA₂ observed in the spinal cord at 4 h post-injury [18]. We found that PLA₂-induced SCI mimicked many characteristics of contusive SCI such as hemorrhage, neutrophil infiltration, and demyelination at the early stage and macrophage infiltration, axon degeneration, tissue damage, glial scar formation, and behavioral impairments at the late stage, suggesting that PLA₂ plays an important role in mediating secondary spinal injury and that this injury model may serve as a unique one for exploring the underlying mechanisms of secondary injury.

In addition to its similarities to contusive SCI, PLA₂-induced injury also exhibited its specific characteristics including the lack of cavitation in the lesion area, extensive demyelination that preferentially targets the white matter, enhanced myelination by migrated Schwann cells, and lack of late responses of tcMMEP. These unique characteristics may be related to the lipolytic enzymatic property of PLA₂. Since the white matter and myelin sheath contain high levels of lipids, they are likely more vulnerable to the PLA₂-mediated injury than the gray matter. Overactivation of PLA₂ can hydrolyze phospholipids and induce myelin breakdown. Additionally, the metabolic products of PLA₂, such as lysophosphatidylcholine, can act as a detergent to cause myelin breakdown [26–28]. Indeed, expression of PLA₂ was found to correlate well with the rate of myelin breakdown associated with Wallerian degeneration in both the CNS and PNS although the rates between the two were somewhat different [29].

In the present study, Schwann cell remyelination of residue and/or sprouted axons in the lesion area, although to a limited extent, was found after contusive SCI in adult rats, consistent with previous reports [30]. In contrast, markedly enhanced Schwann cell remyelination occurred in the PLA₂-induced lesion area. Such a phenomenon may be a secondary effect induced by extensive demyelination and loss of astrocytes in response to PLA₂ activation since Schwann cell remyelination in the spinal cord occurs when there is a concurrent loss of astrocytes and oligodendrocytes [31, 32]. It is suggested that demyelination is a sufficient stimulus to excite abundant Schwann cell proliferation and migration [33].

The origin, route, and mechanism of axon remyelination by Schwann cells in the spinal cord after injury are poorly understood. It is suggested that Schwann cells can enter the CNS from peripheral nerves by transit through a damaged subpial glial limiting membrane, a barrier that normally separates central and peripheral elements [31, 34]. Recently, both in vitro and in vivo transplantation studies indicate that CNS precursor cells may be another source of Schwann cells that contribute to remyelination [32, 35]. Although we and others in previous experiments showed that transplanted Schwann cells increased axonal regeneration, were neuroprotective, and improved functional recovery following SCI in rats [36–40], the significance of Schwann cell remyelination after SCI remains to be determined. Similar patterns of BBB improvements observed in the two injury models suggest that the remyelination by Schwann cells was not the only factor for the observed functional recovery in this study.

Similar to our previous finding [41], three types of tcMMEP responses, i.e., early, late, or no response, were found following a contusive SCI. However, in the PLA₂-induced injury model, only two types of tcMMEP responses, i.e., early or no response, were found. The reason for the lack of late tcMMEP response in the PLA₂-induced SCI model remains

unclear. One explanation is that remyelination of demyelinated axons by Schwann cells may contribute to the elimination of the late tcMMEP response in this model.

Although exogenous PLA₂ induced tissue damage and behavioral impairment after its delivery, the underlying mechanisms by which it induces SCI remain to be explored. Recent studies, including ours, have highlighted the following possible mechanisms. Firstly, PLA₂ can damage cell membrane. Phospholipids are the main components of the neural cell bilayer membrane. PLA₂ can directly induce phospholipid degradation and membrane breakdown through hydrolysis of membrane phospholipids, resulting in alteration of membrane function such as fluidity and permeability, behavior of transporters and receptors, and ion homeostasis, and eventually leading to functional failure of excitable membranes and cell death [6, 9, 42]. Secondly, PLA₂ may serve as a key molecule that controls the biosynthesis of several well-known bioactive mediators of inflammation such as eicosanoids (prostaglandins, thromboxanes, leukotrienes, and lipoxins) and PAF [6, 8] in a rate-limiting manner. PLA₂ also induced the expression of cytokines such as TNF- α and IL-1 β in a positive feedback manner [5, 18]. Thirdly, PLA₂ may induce tissue injury by generation of reactive oxygen species (ROS). The metabolism of free fatty acids as PLA₂ metabolites represents a source of ROS. Application of pathophysiological concentrations of free fatty acids has been demonstrated to induce oxidative injury to spinal cord cell cultures [43]. Microinjections of PLA₂ into the normal spinal cord induced expression of 4-hydroxynonenal (4-HNE), a product of lipid peroxidation and marker for oxygen free radical-mediated membrane injury [18]. Mepacrine, a PLA₂ inhibitor, reduced brain injury-induced expression of cPLA₂ and 4-HNE [44]. Fourthly, PLA₂ may induce the release of excitatory amino acids (EAA), such as glutamate. Elevated levels of EAAs have been implicated in the pathogenesis of neural injury and death in many neurological disorders. It was reported that application of PLA₂ to the rat ischemic cerebral cortex resulted in a significant increase in EAA levels, and a PLA₂ inhibitor, mepacrine, significantly decreased the ischemia-evoked efflux of EAA into cortical superfusates, suggesting the involvement of PLA₂ in EAA release [45]. Administration of a PLA₂ inhibitor AACOCF₃ or 4-bromophenacyl bromide inhibited glutamate release in the spinal cord [46]. Inhibition of PLA₂ activity using multiple PLA₂ inhibitors all reduced the efflux of both glutamate and aspartate [47]. Finally, PLA₂ may induce neuronal cell death via apoptosis or programmed cell death. In recent years, apoptosis has been identified as an important mechanism of cell death in many neurological disorders including SCI [48–51]. Several excellent reviews focusing on PLA₂s and apoptosis [52–54] discussed the involvement of multiple isoforms of PLA₂ in apoptosis. Apoptosis is associated with changes in glycerophospholipid metabolism. Cells undergoing apoptosis generally release free fatty acids including AA, which parallels the reduction in cell viability [48, 49], suggesting the involvement of PLA₂ in apoptosis.

In conclusion, our study has demonstrated that exogenously delivered PLA₂ at an amount equal to the peak level of PLA₂ activity in the region of a contusive SCI mimics many characteristics of contusive SCI, suggesting that PLA₂ may play an important role in mediating secondary injury after an initial trauma to the spinal cord. Differences between the two models, however, also exist indicating that PLA₂ plays a distinct role in mediating such damage to the spinal cord particularly to its white matter. Thus, the PLA₂-mediated injury model may be a unique one to elucidate the distinct role and mechanism of PLA₂-induced secondary injury as well as develop novel and targeted repair strategies aimed at improving anatomical and functional recovery following SCI. Experiments along these lines are currently in progress in our laboratory.

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Fig. 1.

Histological comparison at 24 h after either PLA₂ microinjection-induced (**a**, **b**, **e**) or contusive SCI (**c**, **d**, **f**). **a**, **c** Representative sections show the lesion epicenter stained with Cresyl Violet–Eosin. In both groups, hemorrhage and infiltration of inflammatory cells were observed. However, more substantial hemorrhage and tissue damage were found in the group that received contusive SCI as compared to those that received PLA₂ microinjection. **e**, **f** High magnification of boxed areas in **a** and **c** show that, in both cases, infiltration of inflammatory cells (*arrows*) occurred at the lesion epicenter. **b**, **d** Representative sections show the lesion epicenter stained with Luxol fast blue. PLA₂ microinjection induced confined demyelination localized mainly in the white matter particularly the ventrolateral funiculus (**b**, *dashed lines*). Notably, the gray matter remained largely intact. In contrast, contusive SCI induced a centralized damage which mainly affected the gray matter along with the sparing of myelin in the periphery of the white matter (**d**, *dashed line*). **a–d** bar=500 μ M; **e–f** bar=20 μ M

Fig. 2.

Light and electron microscopic comparisons at 24 h after either PLA₂ microinjection-induced (a, c) or contusive SCI (b, d). a, b Toluidine blue-stained semi-thin cross sections showed significant demyelination in the ventrolateral white matter at 24 h after the PLA₂ injection (Fig. 2a, arrows). At the ultrastructural level, the widespread demyelination, induced by PLA₂, was clearly seen (c, arrows). In contrast, the pathological changes at the injury epicenter of contusive SCI were necrotic in nature that contained both axon and myelin breakdown (b, arrows). Such axon and myelin damages were clearly seen at the ultrastructural level (d, arrows). a–b bar=20 μ M; c–d bar=5 μ M

Fig. 3.

Histological and immunohistochemical comparison at 4 weeks after either PLA₂ microinjection-induced or contusive SCI. Luxol fast blue (**a**, **c**) and Cresyl Violet–Eosin (**e**, **g**) staining showed that both models induced tissue damage and loss of myelin. The PLA₂-induced lesion was confined mainly within the ventrolateral white matter (**a**, **e**) with no tissue loss, whereas contusive SCI induced a large centrally located cystic cavity with a thin rim of spared tissue surrounding it. **i**, **j** High magnification of boxed areas in **e** and **g** show numerous darkstained cellular profiles in both lesion areas particularly those after PLA₂-induced injury. **b**, **d** Immunofluorescence staining showed that both models induced reactive gliotic response surrounding the lesion border. Notably, in the PLA₂-induced damage, no GFAP-IR astrocytes were found in the lesion area (**b**) where numerous SMI-31-positive axons were present (**f**) as compared to the lack of SMI-31-IR in the lesion cavity of the contusive SCI (**h**). **k**, **l** High magnification of boxed areas of **b** and **d**. Following PLA₂-induced injury, a clear border between GFAP-IR astrocytes and SMI-31-IR axons was seen (**k**, *dashed line*). In contrast, following contusive SCI, few SMI-31-IR axons were found within the lesion cavity beyond the glial border formed by GFAP-IR reactive astrocytes (**l**, *dashed line*). **a–h** bar=500 μ M; **i–l** bar=40 μ M

Fig. 4.

Light and electron microscopic comparisons at 4 weeks after either PLA₂ microinjection-induced or contusive SCI. (**a, c**) Toluidine blue-stained semi-thin cross sections showed that numerous axons were remyelinated by Schwann cells (*arrows*) in the lesion area induced by PLA₂ injection which is further confirmed at the EM level. **b, d** In contrast, following contusive SCI, most axons were completely degenerated within the lesion area. Notably, numerous degenerating myelin segments (*asterisks*) were observed at this time point indicating that myelin breakdown is a much slower process than axonal degeneration after contusive SCI. In both models, infiltration of macrophages into the lesion area was observed (**a–c**, *arrowheads*). **a–b** *bar*=20 μ M; **c–d** *bar*=5 μ M

Fig. 5.

tcMMEP responses at 4 weeks after either PLA₂ microinjection-induced or contusive SCI. Three types of tcMMEP responses, i.e., an early response (f, 5–7 ms), late response (h, 16–20 ms) and no response (d, absent) were found following contusive SCI. In comparison, only two types of tcMMEP responses, i.e., an early response (e) and no response (c), were found following PLA₂-induced injury. g Comparison of tcMMEP amplitudes between the two injury groups. The amplitudes decreased after both PLA₂ injection and contusive SCI. S sham, ER early response, LR late response, V vehicle.

** $p < 0.01$ vs. sham;

$p < 0.01$ vs. vehicle

Fig. 6. Behavioral changes after PLA₂ microinjection-induced or contusive SCI. BBB locomotion rating scale showed that BBB scores decreased in both the PLA₂-induced and contusive SCI groups, compared with its control group. ***p* < 0.01 vs. sham or vehicle

Table 1Pathological characteristics of PLA₂-induced and contusive spinal cord injury

Characteristics	PLA ₂ -induced injury	Contusive spinal cord injury
Hemorrhage	++	++++
Inflammatory cell infiltration	++	++++
Demyelination	+++	+
Axon degeneration	++	++++
Schwann cell remyelination	++++	+
Astrocytic gliosis	++	+++
Cavity formation	–	+++

Table 2
Comparison of tcMMEP responses between PLA₂-induced and contusive SCI (mean±SEM)

Group	Early response			Late response			Total %
	%	Latency (ms)	Amplitude (mv)	%	Latency (ms)	Amplitude (mv)	
Sham	100	5.70±0.19	28.43±1.23	0	0	0	100
SCI	15	6.15±0.14	2.16±0.25*	20	23.42±0.64	1.07±0.12	35
Vehicle	100	5.98±0.43	26.94±2.22	0	0	0	100
0.05 µg PLA ₂	45	6.09±0.17	2.38±1.49**	0	0	0	45

* $p<0.01$ vs. sham;

** $p<0.01$ vs. vehicle