Spatial and Temporal Morphological Changes in the Subarachnoid Space after Graded Spinal Cord Contusion in the Rat

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Abstract

Spontaneous repair or treatment-induced recovery after spinal cord injury (SCI) is very limited and might be related to extramedullary alterations that have only briefly been documented. Here we report on the morphological changes of the spinal subarachnoid space (SAS) in a clinically relevant model of SCI. Anesthetized rats were subjected either to mild or severe spinal cord contusion at T9. Spine blocks from the site of injury and adjacent segments were harvested at acute (1 h and 1 day [d]), subacute (3 and 7 d), and chronic (1 and 3 months) stages post-injury. Histopathology and morphometry at each decalcified vertebral level were assessed. At acute and subacute stages, reduction of SAS lumen was observed after both mild and severe injuries. Acutely, after severe injuries, SAS occlusion was associated mainly with cord swelling and subarachnoid hematomas; a trend for dural sac constriction was observed for mild injuries. At 7 d, cord swelling diminished in both instances, but dural sac constriction increased for severe injuries. At early stages, in the epicenter and vicinity, histopathology revealed compression of neurovascular elements within the SAS, which was more intense in severe than in mild injuries. In the chronic stage, SAS lumen increased notably, mostly from cord atrophy, despite dural sac constriction. Myelograms complemented observations made on SAS lumen permeability. Post-traumatic arachnoiditis occurred mainly in animals with severe injury. In conclusion, early extramedullary SAS changes described here might be expected to produce alterations in cerebrospinal fluid (CSF) dynamics and cord blood perfusion, thereby contributing to the pathophysiology of SCI and becoming novel targets for treatment.

Key words: arachnoiditis; CSF; dural sac; pathophysiology; SCI

Introduction

THE POOR SPONTANEOUS REPAIR and regenerative capabilities of the spinal cord (SC) after trauma have encouraged exhaustive search for causes that explain such lack of healing.¹ Despite the abundant information available on the mechanisms of secondary injury involvement in the worsening of the primary traumatic lesion,² it is likely that other pathophysiological aspects have remained overlooked.

In this context, there is limited histopathological information regarding the changes that occur in the extramedullary spinal structures and spaces surrounding the SC after spinal cord injury (SCI). Most anatomical studies are related to the SC parenchyma.³

Because the spinal subarachnoid space (SAS) holds neurovascular structures essential for neurological function, and because it is

the main pathway for cerebrospinal fluid (CSF) flow, it is fundamental to acquire further knowledge regarding changes in such unique extramedullary space in relation to traumatic cord injury.

Current studies on alterations in CSF dynamics associated with SCI report on the relationship between arachnoiditis and late progressive neurological deterioration associated with post-traumatic syringomyelia.^{4–6} However, there is a need for systematic studies on this topic at early stages of the lesion, at the point when secondary mechanisms of injury and attempts for endogenous repair occur.

Therefore, this study was designed to characterize morphological changes of the spinal SAS in a clinically relevant model of SCI. We hypothesized that contusion to the SC should be accompanied by changes in the SAS as a function of intensity and time elapsed after lesion. We believe that such changes could be associated with the pathophysiology of post-traumatic cord damage.

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Methods

Experimental design

Adult female Long–Evans rats, weighing 240–260 g, were subjected to graded SC contusion. For morphology, rats were killed at 1 h and 1, 3, 7, 30, and 90 days post-injury; intact rats were used for control ($n=8$ /group). All animals were used for both qualitative and quantitative studies. Myelography of uninjured rats, and those injured at 1, 7, and 30 days post-injury ($n=2$ /group) was performed to complement morphological studies.

Animal experiments were conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. The present study was approved by our Institutional Animal Care and Use Committee.

Anesthesia, injury, and care

For cord injury, animals were anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg) given i.m. A laminectomy was performed aseptically at the base of T9 spinous processes maintaining meninges intact; then the SCI was produced using the New York University impactor (MASCIS impactor) by dropping the 10g rod from a height of 12.5 mm for mild lesions, and from 50 mm for severe injuries. Post-surgical care included manual expression of bladders twice a day until bladder function returned. Food and water were provided *ad libitum*. As prophylactic for infections, 8 mg/kg of ciprofloxacin lactate (Bayer, Mexico City, Mexico) were given subcutaneously every 12 h, starting at the end of surgery and for 7 consecutive days. To prevent self-mutilation, acetaminophen (Cilag, Mexico) was given in the drinking water at an approximate dose of 64 mg/kg/day for 1 week.

Morphological assessment

At the above indicated times, rats were deeply anesthetized with pentobarbital and perfused by intracardiac puncture with 200 mL saline solution, followed by 400 mL 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Spine blocks from T8 to T12 were dissected out and post-fixed for 4 days in the same fixative at 4°C. These blocks were then placed in a 12% ethylenediaminetetraacetic acid (EDTA) aqueous solution (pH 6.5) for decalcification at room temperature. The EDTA solution was changed daily until vertebrae became soft (usually after 10 days). Decalcified spines were then placed in phosphate-buffered saline (PBS) solutions of sucrose ascending concentrations (7.5 %, 15 %, and 30 %) at 4°C, over a period of 3 days. Spine blocks were cut into individual vertebral segments from T9 to T11. Each segment was in turn frozen and cut into transverse 6 μ m thick sections, and stained with the standard Masson method. Sections were obtained at the intervertebral discs; the epicenter occurred at T10 (between vertebrae T9 and T10).

Using morphometry, SAS lumen cross-sectional area, SC cross-sectional area, and perimeter of the dural sac (an indicator of dural sac constriction or dilation) were measured for each segment. Digital images were taken with a camera (Evolution MP color from Media Cybernetics V 6.1, Silver Spring, MD) affixed to a microscope (Olympus BX51 TRF from Olympus Corporation, Tokyo Japan) using $\times 4$ objective. Measurements were performed after manual outlining in one stained section of each vertebral level; quantification was automatic (Image-Pro Plus image analysis software from Media Cybernetics V 6.1, Silver Spring, MD). Slides were assessed blindly with respect to the injury condition.

Myelography was performed administering into the *cisterna magna* of above indicated anesthetized rats 150 μ L of the non-ionic Optiray™ 320 contrast agent after removing 150 μ L of CSF using in both instances a # 30 gauge needle. After 5 min, lateral and ventrodorsal images were obtained by placing rats on a Raytheon

RME 325R X-ray generator platform. At the end of the study, rats were euthanized with an overdose of pentobarbital.

Statistical analysis

Raw data obtained by morphometry were analyzed by the two-way ANOVA test using the InfoStat software (V 2012, from the National University of Cordoba, Argentina). Each dependent variable (SAS lumen area, SC area, and dural sac perimeter) was analyzed for the individual effect of injury severity and time elapsed after injury, as well as for the interaction of both independent variables in which case Tukey's post-hoc multiple comparisons test was performed to identify groups that were significantly different. Statistics were performed separately for each spine level from T9 to T11. Differences were considered significant when $p < 0.05$. Mean and standard error of the mean were plotted as the percent change relative to the mean of intact rats as a function of both injury condition and time elapsed after injury.

Results

In comparison with intact specimens (Fig. 1), at acute and sub-acute stages (1 h to 7 days post-injury), partial, and in some cases

FIG. 1. Intact specimen at T10. The dural sac (arrows) defines the epidural (ed) and subarachnoid (*) spaces. (A) In the subarachnoid space, nerve roots are free and vascular elements are patent. (B and C) Detailed view of A showing in B a pial plexus vessel (&) and a nerve root (r), and in C a branch of a radicular artery (#). Masson's stain. Scale bars: (A) 0.5 mm; (B) 100 μ m; (C) 50 μ m. Color image is available online at www.liebertpub.com/neu

FIG. 2. Specimens of the site of injury at the acute stage. **(A)** Panoramic view of a mild injury showing the subarachnoid space (*) in **(B)** partially obliterated and free of hematomas; the spinal cord (SC) shows scattered petechial hemorrhages. **(B)** Detailed view of **A** showing an uncompressed nerve root (r), and a patent blood vessel (&). **(C and D)** Panoramic and detailed views of a specimen of severe injury exhibiting greatly diminished lumen of the subarachnoid space, which contains a large hematoma (+); cord shows hemorrhages and infarcts, as well as diffuse swelling. Arrows indicate the dural sac. Masson's stain. Scale bars: **(A and C)** 0.5 mm; **(B and D)** 100 μ m. Color image is available online at www.liebertpub.com/neu

almost complete, occlusion of the SAS lumen was observed (Figs. 2 and 3). Subarachnoid hematomas were present in most specimens with severe lesions, but were a less frequent finding after mild injuries (Figs. 2 and 3). Blood vessels and nerve roots contained within the SAS frequently appeared compressed between the dural sac and the cord. Neurovascular compression appeared to be more intense in severe than in mild injuries (Figs. 2 and 3), and closer to the site of injury rather than away from it. At the acute stage, the SC parenchyma showed hemorrhages, swelling, and infarcts (Fig. 2); at the subacute stage, necrotic and hemorrhagic cord lesions were observed (Fig. 3).

At the chronic stage (1–3 months post-injury) histopathology showed restitution of the SAS lumen; atrophy of the cord with intramedullary cysts of various sizes; and post-traumatic arachnoiditis characterized by cord tethering (fibrous binding of the SC to the dural sac), subarachnoid cysts, and nerve roots traps (Fig. 4B). Signs of arachnoiditis were much more evident in severe than in mild injuries.

Morphometric analysis revealed changes in SAS lumen dimensions, SC cross-sectional areas, and dural sac perimeters

according to the time elapsed after injury, the severity of lesion, and the spinal level involved. In general, the greatest changes were seen for severe injuries, at the epicenter and adjacent segments. Statistical significance of these changes is indicated in Figure 5.

At the acute and subacute stages after injury, SAS lumen was reduced after both mild and severe injuries in relation to intact specimens, but was increased in the chronic stages post-injury in relation to measurements performed at early stages (Fig. 5 A–C).

From 1 h to 3 days, the cord area increased compared with intact specimens; at 7 days it was similar in all injured and intact rats; in the chronic stage this area was decreased (Fig. 5 D–F). Changes in the dural sac perimeter were minor at acute and subacute stages; this parameter was decreased in the chronic stages post-injury (Fig. 5 G–I).

SAS was better defined in the lateral than in the ventromedial myelograms. Lateral images showed a nonhomogeneous SAS thickness, which was thinner in the central thoracic region. Columns of contrast medium were not symmetrical, at times appearing more intense either ventrally or dorsally. In intact as well as chronic

FIG. 3. Specimens of the site of injury at subacute stage. (A–C) Images of a mild injury showing the subarachnoid space lumen (*) free of hematomas, uncompressed nerve roots (r), as well as patent pial plexus (&) and radicular (#) blood vessels; the spinal cord (SC) shows necrosis and hemorrhage. (D–F) Images of a severe injury demonstrating almost complete occlusion of the subarachnoid space, compressed roots (r), as well as non-patent radicular and pial vessels. (E) Hematoma (+), between the dural sac (arrow) and the pia mater (arrowheads) shows early signs of fibrosis (blue stain). (F) Compressed nerve root (r) between the dural sac (arrows) and the pia mater (arrowheads). Cord shows abundant devitalized tissue. Masson's stain. Scale bars: (A and D) 0.5 mm; (B, C, E, and F) 100 μ m. Color image is available online at www.liebertpub.com/neu

(30 days post-injury) mildly and severely injured rats, SAS was permeable to contrast medium. For acute (1 day) mild and severe injuries, and subacute (7 days) severe injuries, contrast medium flow was blocked several vertebral segments cranial to the site of injury. For subacute mild injuries, the column of contrast medium reached the site of injury (Fig. 6).

Discussion

Here, we report on a systematic histological study in rats subjected to graded SC contusion that provides information on poorly documented spatial and temporal changes occurring in the spinal SAS.

Early changes in SAS lumen

During the early stages post-injury, reduction of SAS lumen is the most outstanding extramedullary event. Even though this event is observed the first week after injury, magnitude, apparent cause,

and craniocaudal extension varies according to the severity and time elapsed after injury.

For severe injuries, diminished SAS lumen appears to be associated with an increase in SC volume (swelling) from 1 h to 3 days post-injury, whereas at 7 days, when cord swelling subsides, it is associated with a constriction of the dural sac, judging from its decreased perimeter. The presence of subarachnoid hematomas, and in some cases nerve root swelling, also contributes to the reduction of SAS lumen.

In contrast, for mild injuries, SC swelling tends to be lower and constriction of the dural sac tends to be higher than in severe injuries. In addition, subarachnoid hematomas are smaller and less frequent. Previous reports have mentioned spinal SAS obstruction in early stages of both complete and incomplete SCI, but have not described the cause and long-term evolution of SAS permeability.⁷

Although unrelated to diminished SAS lumen, others have described SC edema in response to SCI, apparently as a function of severity.^{8,9}

FIG. 4. Specimens of the site of injury at chronic stage. (A) Mild injury at 3 months post-injury showing cord atrophy and recovery of subarachnoid space lumen (*). Nerve roots (r) are free in the space and appear of normal size. There is no cord tethering. (B) Severe injury at 3 months post-injury; dural sac constriction (arrow) and severe cord atrophy results in an increased subarachnoid space lumen (*); the cord shows tethering, nerve roots show (r) atrophy and entrapment; a subarachnoid cyst (@) in the tethering area is evident. Epidural space (ed). Masson's stain. Scale bars: 0.5 mm. Color image is available online at www.liebertpub.com/neu

FIG. 5. Morphometric changes after cord injury. (A–C) Subarachnoid space lumen cross-sectional area. (D–F) Spinal cord cross-sectional area. (G–I) Dural sac perimeter. Plots represent the mean $\pm$ S.E.M. ($n=8$) of the percent change relative to the mean dimensions for intact rats, at several time periods from 1 h to 3 months, for mild and severe cord injury, at different spine levels (T9 [A, D, and G], T10 [B, E, and H], and T11 [C, F, and I], with epicenter at T10). * $p < 0.05$.

FIG. 6. Representative images of lateral myelograms. (A) Control radiography of intact rat without contrast medium. (B) Myelography showing contrast medium subarachnoid space (SAS) permeability in an intact rat. (C) Myelogram showing blockage of contrast medium several segments cranial to the site of injury; image corresponds to a severely injured rat at 1 day post-injury. (D) Image exhibiting contrast medium reaching the site of injury in a rat with mild injury at 7 days. (E) Myelogram showing SAS permeability of the contrast medium beyond the site of contusion in a rat with severe injury at 30 days. *site of injury. Arrows indicate the end-point of the contrast medium column at both dorsal and ventral aspects; in B, dorsal end-point is out of frame.

Possible implications of early changes in SAS on CSF flow and blood circulation to nerve tissue

Reduction of SAS lumen (the major CSF flow pathway) occurring the first week after injury must hinder or suppress spinal CSF circulation, as suggested by the myelograms taken 1 and 7 days post-injury. It is notable that changes in dynamics of CSF flow at early stages after SCI have largely been ignored as part of the mechanisms involved in

the pathophysiology of acute SCI, even though CSF is essential to normal function of the central nervous system (CNS) as well as in multiple neuropathological conditions.¹⁰

CSF intimately contacts the CNS tissue with which it exchanges materials bidirectionally. It carries numerous substances that provide nutrition, microenvironment adjustments (e.g., pH and osmolality), trophic support, regulation of immune responses, and other signaling mechanisms.¹¹ CSF also carries and supplies oxygen to the nerve tissue.¹² Furthermore, CSF flow plays an important role in replacing the lymphatic vessels that the SC and nerve roots lack.¹³

Even though we did not study it, our observations suggest that inadequate CSF turnover must be occurring, interfering with self-reparative mechanisms such as the proper functioning of the choroid plexus-CSF nexus, which receives information through the arrival of molecules generated at the site of the lesion, and responds by synthesizing a variety of biologically active substances (e.g., trophic factors, hormones, and carrier proteins) that travel through the CSF to target cells in injured neural tissues for support repair processes.^{13–15}

As a consequence of SAS lumen occlusion, blood vessels and nerve roots traveling in SAS are compressed, more intensely so in severe than in mild injuries.

It is known that blood supply to the SC comes from radicular arteries and from pial vascular plexus, both elements enclosed in the SAS.¹⁶ Pial vascular plexus poses a high compensatory capacity to protect the cord from circulatory insufficiency when radicular arteries are obstructed.¹⁷ Although we did not study blood circulation, it is reasonable to speculate that the extramedullary vascular events we describe here, in addition to intramedullary vessel damage known to occur after SCI,^{18–20} should result in a greater deficit in cord blood perfusion and contribute importantly to the pathophysiology of secondary damage after traumatic SCI, considering that the CNS is extremely vulnerable to hypoxic-ischemic insults. SAS occlusion could not only disturb blood supply to neural tissues. It is presumable that also venous drainage of the SC and nerve roots is impaired.¹⁶

Taking into account that nerve roots in the SAS are nourished by CSF as well as by blood,²¹ early closures of SAS could contribute to chronic radiculopathy reported after SCI.^{22,23}

Late changes in SAS lumen

Recovery of SAS lumen in the chronic phase of injury is associated mainly with atrophy of the cord. An unexpected finding in this late phase is the substantial constriction of the dural sac in rats with severe injury. Both cord atrophy and dural sac constriction occur at the same location in the spine, in mild and severe injuries, although this is greater in the latter case.

Late morphological SAS lumen recovery and SAS permeability to contrast medium observed in myelograms, suggest that CSF flow is restored. However, we hesitate to propose that this flow has normal characteristics. Taking into account the pathological changes observed at the site of injury, mainly in severe injuries, including cord tethering and irregular surface of the cord by pial fibrosis and nerve root traps, it is possible that CSF flow dynamics are altered as suggested by others.^{24,25}

These pathological changes, defined as post-traumatic arachnoiditis, have been associated with late neurological worsening related^{26,27} or not²⁸ to syringomyelia. Arachnoiditis results from the evolution of acute meningeal inflammation to late scarring. Subarachnoid hematomas, like those observed here after severe injuries,

possibly potentiate acute post-traumatic meningeal irritation/inflammation leading to more intense chronic arachnoiditis.^{29,30}

Study limitations

It is reasonable to expect that our histological findings do not exactly represent the *in vivo* SAS configuration, because of the lack of CSF and intrathecal pressure. Trying to correct for this potential bias, we chose to measure the length of the dural sac instead of the area it surrounds, as the length remains constant regardless of its folds. Imaging studies *in vivo*, such as MRI and ultrasound, could be useful regarding this topic, but would not allow for fine morphological details as those described in this study.

Future research

The evidence shown here for spatial and temporal intensity-dependent changes of SAS after cord injury warrants further studies. CSF circulation cannot be attributed exclusively to SAS lumen, but also involves fluid pressure and dural sac elasticity, both of which, together with other *in vivo* dynamic measurements (e.g., MRI, SAS permeability) should be useful for a more comprehensive understanding of the impact of extramedullary alterations in the pathophysiology of SCI.

Conclusion

SCI results in SAS alterations that are a function of intensity and time elapsed after injury, as well as the spinal level involved. Main findings of this work include that 1) there is SAS lumen reduction at acute and subacute stages after injury; neurovascular elements contained in SAS appear compressed; 2) reduction of SAS lumen in severe injury is mainly associated with cord swelling and subarachnoid bleeding; 3) at the chronic stage, regardless of dural sac constriction, SAS lumen is restored because of atrophy of the cord; histopathology shows changes consistent with chronic arachnoiditis; 4) myelograms are consistent with morphological findings; and 5) in general, the greatest changes were seen for severe injuries, at the epicenter and adjacent segments.

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Author Disclosure Statement

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