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Temporal changes of spinal subarachnoid space patency after graded spinal cord injury in rats

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ABSTRACT

Introduction: Disturbances in spinal subarachnoid space (SSAS) patency after SCI have been reported as an incidental finding, but there is a lack of information on its *in vivo* extent and time course. For substances and cells carried in the cerebrospinal fluid (CSF) to reach damaged neural tissue and promote reparative processes, CSF must be able to flow freely in SASS.
Objective: To characterise the extent and time course of SSAS patency disruption *in vivo* in a rat model after graded SCI.
Materials and methods: Anaesthetised rats were subjected to mild or severe cord contusion at T9. Estimation of SSAS patency was carried out at 1 h and 1, 3, 7, 15, 30 and 90 days postinjury, as well as in naïve rats, by quantifying the passage of superparamagnetic beads injected into the CSF at the *cisterna magna* and recovered at spinal level L2. CSF volume recovery was measured simultaneously. Data were analysed by the two-way ANOVA test.
Results: Estimation of SSAS patency revealed nearly complete blockage early after contusion that was unevenly restored entering the chronic stages. Volume of CSF recovered was also significantly decreased early after injury compared to naïve rats, but was fully restored by 1 month postinjury. Overall, although modestly different from each other, changes in both parameters were more pronounced after severe rather than mild injuries for each time point examined.
Conclusions: SCI alters SSAS patency. Its extent is a function primarily of time elapsed after lesion and secondly of injury severity. It is reasonable to expect that disturbances in SASS patency might alter CSF dynamics and impair self-reparative mechanisms and intrathecal therapeutics, making SSAS patency blockage a key target for SCI management.

Introduction

Acute traumatic spinal cord injury (SCI) triggers a series of events that lead to progressive damage, which exacerbates the loss of neurological function. While the occurrence of diverse intramedullary events in the pathophysiology of cord injury is widely recognised, the outcome of targeting such events has been essentially unsuccessful [1–3]. Therefore, it is reasonable to assume that other pathological mechanisms exist that have been underestimated and may account for therapeutic failure. In this regard, extramedullary disturbances could have a role.

The spinal subarachnoid space (SSAS) is the major route of cerebrospinal fluid (CSF) flow. CSF's finely regulated composition ensures spinal cord and nerve root viability in health, and carries to them reparative molecules and cells under pathological conditions [4–9]. Patency of SSAS is vital for CSF free flow. Disturbances of SSAS patency are known to occur after SCI, although its extent and progression *in vivo* have not been fully clarified.

In a morphologic study, we recently reported on the partial or complete obstruction of SSAS after SCI [10]. Although these observations represent a step forward in the knowledge of extramedullary events associated with SCI, the characterization of SSAS patency *in vivo* is a topic pending.

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Accordingly, the objective here was to study the extent and time course of SSAS patency disruptions *in vivo* associated with graded SCI. Under the assumption of a parallelism with our histological findings extent and progression of SSAS patency would be expected to be a function of severity and time elapsed after lesion.

In the present study SSAS patency was estimated using superparamagnetic beads as tracer [11]. Our main finding here is that SCI blocks SSAS patency early after injury with a gradual tendency towards recovery over time.

Materials and methods

Animals and experimental design

Adult female Long–Evans rats, weighing 240–260 g were subjected to graded spinal cord contusion. SSAS assessment was at 1 h and 1, 3, 7, 15, 30 and 90 days postinjury ($n = 8$ per group). Naïve rats ($n = 8$) were used as controls.

Anaesthesia, injury, and cares

For cord injury, animals were anaesthetised with ketamine (80 mg/kg) and xylazine (8 mg/kg) given i.m. A laminectomy was performed aseptically at T9 maintaining meninges intact; SCI was produced using the New York University impactor (MASCIS impactor) by dropping onto the exposed dura the rod weighing 10 g from a height of 12.5 mm or 50 mm for mild or severe injuries, respectively. Postsurgical 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. As an analgesic and 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.

SSAS patency assessment

Estimation of SSAS patency was performed as previously described [11]. Briefly, anaesthetised rats were subjected to a laminectomy at C1/C2 and at L2; animals were then placed on a 45° inclined plane in the cephalic-caudal direction. Under microscopic viewing, 200 μg in 20 μL of an undiluted suspension of superparamagnetic beads (Dynabeads MyOne Streptavidin T1, catalogue # 65601 from Invitrogen™) were injected into the exposed *cisterna magna* at C1/C2 after removing an equal volume of CSF. Immediately after, the dural sac exposed at L2 was torn. All draining CSF was recovered in heparinised capillaries, transferred to Eppendorf tubes, and volume was measured. Any CSF that might have leaked out at the time of dural sac puncture (at the most 10 μL, and many times none at all) was collected with the same capillary from the cavity just bellow the site of puncture and added to the bulk of CSF recovered.

By magnetic separation using a DynaMag™-Spin (Invitrogen™) CSF-suspended streptavidin coated beads were washed to remove any traces of blood contamination. They were then treated sequentially as follows: incubated with biotinylated peroxidase, magnetised and washed to remove excess peroxidase, and then mixed with TMB/hydrogen peroxide for TMB enzymatic oxidation. Reaction was stopped with an H_2SO_4-based solution. Beads were removed magnetically and the yellow solution obtained was measured at 450 nm in a 450 Microplate Reader (BioRad™). The intensity of the colour was directly proportional to the amount of bead-bound enzyme and therefore, a measure of the amount of beads recovered.

Statistical analysis

Data were analysed by the two-way ANOVA test using the InfoStat software (V 2012, from the National University of Cordoba, Argentina). The individual effect and interaction of both injury severity and time elapsed after injury, were analysed for bead and for CSF volume recoveries. Tukey's *post hoc* multiple comparisons test was performed to identify groups that were significantly different. Significance was set at $p < 0.05$.

Results

Overall SSAS patency changed as a function of both independent variables: while the effect of time elapsed after injury was extremely significant ($p < 0.0001$), the effect of injury severity was barely so ($p = 0.0439$). Patency was decreased significantly in rats from 1 h to 15 days after contusions (mild and severe) in comparison with intact animals. Maximum SSAS blockage was observed in rats with severe lesions at 1 day postinjury. On the average, only 65 ng of beads were recovered in this group of rats that is, 0.48% of the average recovery in intact rats, which was 13,596 ng. From the 7th day postinjury on, patency showed a tendency towards recovery in both groups of injured animals as is shown in Fig. 1, especially evident in the chronic stages after injury where some animals showed low, moderate or full recovery. Although not significant, for each postinjury time point examined, average bead recovery was always lower in rats with severe *vs.* mild injuries.

The effect of time elapsed after injury and severity of lesion on the overall changes for CSF volume recovered at L2 was highly significant for both variables ($p < 0.0001$ and $p = 0.0004$, respectively). Volume decreased significantly in injured rats from 1 h (severe injury) to 3 days compared to intact animals. Peak volume reduction was observed 3 days post-severe injury (mean CSF volume recovered was 10.9 μL, that is only 9.4% of that recovered on average in intact rats namely, 115.8 μL). CSF volume recovered in rats from 15 days to 3 months postinjury was similar to that obtained in intact animals. Mean CSF volume recovered at each postinjury time point was less in rats with severe *vs.* mild injuries, in particular, 1 h, 1 day, and 3 days postinjury (Fig. 2).

Discussion

This study was designed to test the hypothesis that contusion to the spinal cord is accompanied by changes in SSAS patency the

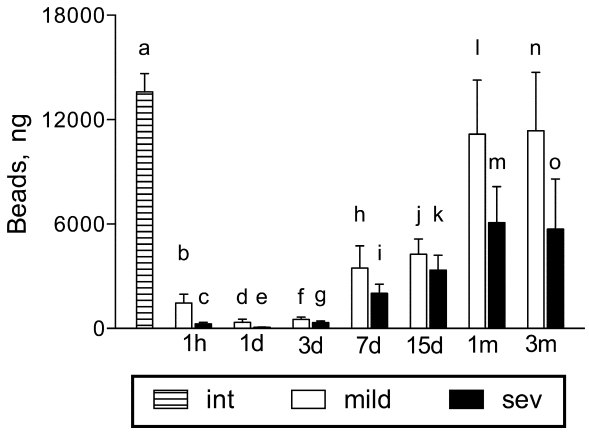

Fig. 1. Subarachnoid patency changes. Mean ± SEM of beads recovered in intact (int), and mildly (mild) or severely (sev) injured rats. Significance ($p < 0.05$): a *vs.* b–k; l, n *vs.* b–g, i; e *vs.* b, h–k, m, o; c, d, f, g *vs.* h, j, k, m, o.