

ORIGINAL ARTICLE

# Effect of hypovolemia on traumatic spinal cord injury

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**Objectives:** Experimentally evaluate the effect of hypovolemia in acute traumatic spinal cord injury.

**Methods:** Twenty adult male Wistar rats were submitted to traumatic spinal cord injury through spinal cord contusion by direct impact. Ten animals were subjected to bleeding of 20% of their estimated blood to simulate a hypovolemic condition after spinal cord contusion and 10 animals were used as control. The animals were evaluated before, 1, 3, 7 and 14 days after the production of the spinal cord injury through behavioral tests (inclined plane test and motor assessment).

**Results:** The spinal cord contusion associated with hypovolemia had a negative influence on functional outcomes of the spinal cord injury. The animals submitted to hypovolemia after spinal cord contusion had lower scores in behavioral tests (inclined plane test and motor assessment), presenting a slower recovery of the motor function.

**Conclusion:** In the experimental model used, the group of animals with hypovolemia after traumatic spinal cord injury had slower recovery and lower intensity in behavioral tests.

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## INTRODUCTION

Spinal cord injury is still a devastating injury because of limited capacity of adult mammalian central nervous system axon to regenerate. Besides the development of research approaches to repair spinal cord injuries, little can be done to undo or repair the initial damage to spinal cord tissues. Active approach to repair spinal cord lesion and functional restoration is a major challenge, and all efforts have been made to reduce the secondary injury triggered by the primary injury that increases and worsens the primary lesion and clinical outcome.<sup>1,2</sup>

Traumatic injury of spinal cord comprises two steps: the primary phase is a result of mechanical damage of the cord due to the kinetic energy transfer to the spinal cord<sup>3</sup> causing structural disruption of axons, nerve cell bodies, support structures (glial cells), hemorrhage and changes in the spinal cord vascularization.<sup>4</sup> Within few minutes of the injury, a secondary phase begins, which causes the expansion of the primary lesion.<sup>5</sup> Despite of the inability of the current treatments to act on the recovery of the primary lesions, a range of therapeutic proposals have been presented aimed to reduce harmful effects of secondary lesions and a lesion extension of the spinal cord.<sup>6</sup>

The central nervous system division is didactic; the spinal cord and the brain have morphological and functional characteristics that allow the analogy of pathophysiological phenomena that occur in these structures.<sup>7</sup> Thereby, the vascular disorders resulted from traumatic brain injury could be extrapolated to the spinal cord.

The perfusion decreases in the injured area reduce the oxygen supply, neurons energy and the glial cells, causing damage to the cell membrane, leading to increased permeability and subsequent penetration of fluids, blood components and harmful substances to

neurons.<sup>8</sup> Meguro and Tator<sup>9</sup> demonstrated that the association of spinal cord injury to other traumas presented severe neurological deficit, lower neurological recovery and increased mortality when compared with pure spinal cord injury. They concluded that the increased incidence of hypotension may be related to a less favorable neurological outcome.

The study was designed considering the reported effects of hypovolemia on traumatic brain lesion, the similarities between brain and spine cord tissues and the lack of studies focused on the effects of hypovolemia on traumatic spinal cord lesion. We hypothesized that hypovolemia would trigger deleterious effects on traumatic spinal cord and its outcome.<sup>10</sup>

## MATERIALS AND METHODS

The experiments were carried out according to the guidelines of Brazilian Society of Neuroscience and Behavior that comply with National Institutes of Health Guide for care and use of laboratory animals. The study was submitted to the State University of Maranhão Ethics Committee (UEMA), as decided by the Veterinary Medicine Course Agricultural Science Center no 034/2014.

A total of 20 male Wistar rats weighing 260–320 g were used in the study.

The animals were kept in a temperature-controlled room ( $24 \pm 2$  °C) with a 12-h light/dark cycle (lights on at 0600 hours). They were kept in cages, containing four animals each, and fed with standard *vivarium* diet and *ad libitum* water.

The animals were randomly divided into two experimental groups (A and B) with 10 animals in each group. In group A, the spinal cord injury was induced through direct contusion, and in group B after the spinal cord injury the animals had a 20% reduction in their blood volume through the blood drawing.

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### Spinal cord contusion

Animals were anesthetized with an intraperitoneal injection of 100 mg kg<sup>-1</sup> ketamine (cristália) and 10 mg kg<sup>-1</sup> xilazina (zoovet), followed by subcutaneous administration of 24 000 UI kg<sup>-1</sup> penicilin, 2.5 mg kg<sup>-1</sup> banamide (meglumine) and analgesia with dipyrone 15 mg kg<sup>-1</sup> intramuscular weight.

After shaving the skin, a posterior incision in the skin and underlying muscles was performed at the level of T8–T12 vertebra. Muscles were retracted, and with the use of a microscope a total laminectomy and exposure of duramater were performed between T9 and T10.

The spinal cord injury was produced by direct contusion and through a rod connected to a device designed for this purpose. The rod of 2 mm diameter and 10 g slid over a stainless steel tube of 3 mm diameter, fixed to a support with perforations in every inch to control the releasing time of the rod. The tube was positioned over the laminectomy site and exposure of the duramater (T9–T10); the spinal cord was injured by the impact of the rod that slid in free fall into the steel tube from a height of 15 cm.

After the surgical procedure and contusion of the spinal cord, the paraspinous fascia, muscle and skin were closed with 4–0 nylon suture (Ethicon, São Paulo, Brazil). The animals were placed in a warming comforter and body temperature was maintained at ~37 °C until they were fully awake, at which time they were returned to their home cages, 4 rats per cage. Postoperative care included regular bladder expression during the period of evaluation (14 days).

All animals were kept alive for the functional evaluation (for 14 days), and then euthanasia was performed. The disposal of the animals was frozen and sent for incineration by those responsible for biological waste University of Maranhão.

### Hypovolemia

Hypovolemia was induced in group B (10 animals) randomly selected after the production of spinal cord injury. Hypovolemia was performed in order to simulate the hemorrhage after the spinal cord injury. The amount of blood removed from the animal was 20% of the estimated blood volume for the Wistar rats, corresponding to 5.4 ml per 100 g per weight.<sup>11</sup> Initially, the procedure was to remove 5% of the blood volume of the animal and then 2.5% in every 2 min until the removal of the estimated total, which was 20% of blood volume.

The approach to the carotid artery was through the 2–3 cm cervical access between the mandibular and external region. The carotid artery was cannulated with 28-inch gelco for withdrawing blood. After the withdrawal of blood, which corresponded to 20% of the blood volume animal, the artery was connected to the proximal and distal portion, and the suture made by planes using a 4–0 nylon suture (Ethicon, Brazil).

### Evaluation methods

Behavioral parameters were evaluated preoperatively 1, 3, 7 and 14 days after the experimental procedure. The hypovolemia effects on the experimental traumatic spinal cord injury were assessed using behavioral evaluations. (A) The inclined plane test consisted of placing the animal on a board coated by rubber whose surface could be adjusted to stay at various angles, establishing the largest angle in which the animal was able to maintain its position for 5 s. The ability of the animals to keep themselves on the board at different angles was tested at angular intervals of 10°, starting from 10° until 90°. (B) Motor assessment by the behavior combined score test was performed before the production of the lesion, and 1, 3, 7 and 14 days after the traumatic spinal cord injury. The inclined plane test was performed as described by Rivlin and Tator.<sup>12</sup> In this test, the rat was placed on a mat in such a way that its body axis was perpendicular to the axis of an inclined board, which could be adjusted to a slope of varying grade. The angle of the inclined plane was the maximum angle of the plane at which a rat could maintain for at least 5 s.

The behavior combined score test (evaluation of spinal cord injury according to the Kuhn and Wrathall scale<sup>13</sup>) was performed to the motor evaluation. This evaluation method assigns values for the motor function coordination, toe extension of the back leg, withdraw extension, withdrawal from pain, ventral response and snatch the bar with the back leg (Figure 1).

### Statistical analysis

Data were analyzed through the statistical program SPSS for Windows 17.0 (São Paulo, Brazil, 2009). The dependent variable of the plan degree of inclination was evaluated by analysis of variance, using two factors (group and time) and the interaction between them. Functional evaluation variable, ordinal variable, was analyzed by a nonparametric test of Kruskal–Wallis, and the comparison between the times was taken by the Dunn test. Evaluation of the group effect was performed by the Mann–Whitney test. In all tests, the level of significance was set at 5% ( $P < 0.05$ ).

## RESULTS

### Inclined plane test

The evaluation through the inclined plane test performed by analysis of variance assessed the effect of two factors, in other words group (spinal cord injury or spinal cord lesion + hypovolemia), recovery time (1, 3, 7 and 14 days) and interaction between them. A statistical difference was observed ( $P < 0.05$ ) in relation to groups, recovery time and the group × time interaction.

The inclined plane test used a parameter that provided a numerical variable related to animal functional activity, and statistical difference was observed ( $P > 0.0001$ ) in all evaluation periods after performing the spinal cord injury. Group B animals who suffered hypovolemia after spinal cord injury had lower mean force than the animals of group A (spinal cord injury without hypovolemia). In the evaluation before production of spinal cord injury, significant difference was observed between groups ( $P = 0.213$ ), indicating the homogeneity of the groups.

### Functional evaluation

The evaluation through the combined score of behavior provided values for different measured motor functions: motor scale, toe extension, withdraw extension, withdrawal from pain, ventral response and snatch the bar with the back leg, in different evaluation periods: preoperative, 1, 3, 7 and 14 days after the experimental procedure. The sum of the scores of different motor functions provided functional assessment.

During preoperative evaluation, no difference of the evaluated parameters was observed in both experimental groups.

Assessment of motor scale showed scores ranging from 0 (no movement of the back leg) to 6 (ability to walk with normal movement of all members), and significant difference was observed between the experimental groups from the evaluation of the 3rd day after the injury. The highest scores were observed in the experimental group without hypovolemia, and the difference was observed in all evaluation periods. The toe extension reflex evaluation could be noticed when the animal was lifted by the tail, presented in the review of the 3rd and 7th day, with higher score in the experimental group without hypovolemia after spinal cord contusion.

The extent withdrawal evaluation presented at the 3rd and 14th day statistically higher score in the group without hypovolemia after spinal cord injury. The withdrawal from pain evaluation presented at the 3rd and 14th day statistically higher score in the group without hypovolemia after spinal cord injury.

The ventral response presented in the evaluation of the 1st, 3rd and 14th day statistically higher score in the group without hypovolemia after spinal cord injury. Snatch the bar with the back leg presented in the evaluation of the 3rd and 7th day statistically higher score in the group without hypovolemia after spinal cord injury.

Functional assessment represented the sum of the scores of the different motor functions evaluated and showed statistical difference in all periods of observation with statistically higher score in the group without hypovolemia after spinal cord injury.

<p><b>a Motor scale: coordination of motor function test.</b></p> <p>0 - no movement with the back leg, cannot stand the body weight over it;          1 - scarce perceived movements, cannot stand the body weight over it;          2 - frequent or vigorous movement in MMII, cannot stand the weight;          3 - can stand the weight over the back leg, may take one or two steps;          4 - stand the entire weight of the body, can take consistent steps using the distal portion of the back leg, hip flexion maintained;          5 - walks with a slight deficit;          6 - walks normally moving all the joints of the back leg.</p>
<p><b>b Toe extension: back leg toe extension reflex when the animal is lifted by the tail.</b></p> <p>0 - no extension;          1 - minimum extension, flaccid fingers;          2 - normal extension of the fingers;          3 - hyperextension with trembling fingers or trembling leg.</p>
<p><b>c Withdraw extension: withdrawal reflex of the back leg against the body when it is manually extended.</b></p> <p>0 - no withdrawal flexion, flaccid back leg;          1 - weak or slow motion against the body;          2 - normal withdrawal of the back leg against the body;          3 - trembling hyperflexion of the back leg.</p>
<p><b>d Withdrawal from pain: reflex to withdraw the leg against the body in response to an applied stimulus in the leg hypothenar eminence.</b></p> <p>0 - no response from the tight leg;          1 - leg abrupt movement without removing the leg against the body;          2 - normal, rapid withdrawal of the leg against the body;          3 - hyper flexion of the back leg, with tremors.</p>
<p><b>e Ventral response: is the reflection of the animal which straightens returning to the usual position, when placed on its back on a flat surface.</b></p> <p>0 - animal remains in the supine position;          1 - slow return to the prone position, over half a second;          2 - average return to the prone position;          3 - fast return to the prone position, in half a second.</p>
<p><b>f Hold the bar with the back leg: perception reflex, in response to contact of the back leg to a small diameter bar.</b></p> <p>0 - no response when the leg touches the bar;          1 - the back leg responds to touch, but cannot grab it;          2 - the back leg successfully grabs the bar, but weakly;          3 - the back leg strongly grabs the bar and pulls it against its body.</p>

Figure 1 Adaptation of the combined behavioral score used in rats with spinal cord injury.<sup>13</sup>

It is observed that in the pre-test period no significant differences were noticed ( $P > 0.05$ ) between the groups, indicating that in the initial conditions the groups were homogeneous. In different periods of evaluation after injury production, the experimental group with hypovolemia after traumatic spinal cord injury showed lower scores of the different evaluated motor function. A lower functional activity and lower evaluation scores in the experimental group in which hypovolemia was induced after traumatic spinal cord injury were observed.

Functional assessment in relation to the evaluation time was performed using the Kruskal–Wallis test and Dunn’s test, which revealed significant differences ( $P < 0.05$ ) in the responses of animals at the evaluation time, indicating the speed in the progress of animals’ evolution.

It was observed that the group without hypovolemia improved their answers earlier than the group with hypovolemia.

## DISCUSSION

Our results showed that the traumatic spinal cord injury followed by hypovolemia has a negative effect on acute traumatic spinal cord

injury functional recovery. All behavioral evaluations showed significant reduction in functional activity in the experimental group in which hypovolemia was induced after spinal cord injury.

This experimental model aimed to simulate hypotension, due to spinal cord injury association with hypotension and hypovolemia, as well as its occurrence often, followed by multiple traumatic injuries. The arterial blood pressure was not monitored during the experiment, although a significant amount of blood was withdrawn to produce hypovolemia, thereby determining hypotension, and this hypovolemia induction model quoted in publications of experimental studies using rats.<sup>11</sup> Our findings corroborate the clinical and experimental evidence that hypotension may contribute to exacerbate secondary injury after acute spinal cord injury.<sup>10</sup>

The treatment of traumatic spinal cord injuries should approach not only the injured vertebral segment but also sectors with no direct connection with the morphological spinal column, which have an important role in the pathophysiology of his injury.<sup>14</sup> Among these, there is the maintenance of blood pressure, and this topic has not been widely applied in experimental studies.

Hypotension is regarded as being one of the five major predictors of the injury prognosis that ends the worst prognosis and can be modified by therapeutic measures.<sup>15</sup>

Our study only used behavioral tests in the result evaluation. This methodology evaluation provided a simple way to observe the effects of hypovolemia in traumatic spinal cord injury. The conducted experimental study can serve as a pilot study and opens the possibility of further studies with more specific and sophisticated methodology to assess the hypovolemia effects on the traumatic spinal cord injury at vascular, histological and molecular level.

It was observed in this work that, although the statistical analysis has been performed by different variables, ordinal and numerical, and applied parametric and nonparametric tests to assess motor behavior and inclined plane inclination degree, both were clearly sensitive to show the motor behavior changes over time, presenting strongly correlated results. When comparing the LM<sub>s</sub>H (spinal cord lesion without hypovolemia) group and the LM<sub>c</sub>H (spinal cord with hypovolemia) group with hypovolemia, it was observed that both converge to the same result, in other words the animals from the LM<sub>c</sub>H group when compared with the animals from the LM<sub>s</sub>H group presented significantly lower motor recovery, <5% ( $P < 0.05$ ), becoming slower over time.

The reduction in blood volume after traumatic spinal cord injury induced significant changes in the animals motor functions showing slower motor recovery. These results highlight the role of hypovolemia after traumatic spinal cord injury, so that this parameter should be considered as a preventive measure to protect the spinal cord after and prevent the expansion of secondary injury.

## CONCLUSION

Traumatic spinal cord injury associated with hypovolemia had a negative effect on the functional assessment of the animals, which showed higher neurological deficit and lower functional recovery, according to behavioral testing.

## DATA ARCHIVING

There were no data to deposit.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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laboratory in post-graduation in Pharmacology. Thesis Part was conducted in Graduate Program in Science College.

## AUTHOR CONTRIBUTIONS

Conception, study design, acquisition, interpretation and statistical analysis, technical procedures, macroscopic examination and manuscript writing: O de Cassia Sampaio. Conception, study design, acquisition, interpretation, statistical analysis and text critical review: HLA Defino. Conception, study design and text critical review: EA Del Bel Belluz Guimaraes.

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