EXPERIMENTAL STUDY ON THE ACTION OF METHYLPREDNISOLONE ON WISTAR RATS BEFORE SPINAL CORD INJURY

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ABSTRACT

Objective: To evaluate the effects of methylprednisolone used prior to spinal injury, both in relation to possible beneficial effects and to possible associated complications. Materials and methods: The study subjects were 32 Wister rats, divided into 4 groups. Two groups received drugs A (placebo) and B (methylprednisolone) immediately after the injury. Another 2 groups received the same drugs 4 hours before the injury. They were all evaluated over a period of 28 days to verify locomotor function and associated complications. Results: The 4 groups were compared in terms of weight and age. No statistically significant difference was found between the study groups in relation to mean weight and age. In the comparison of intercurrences among the 4 groups a statistically significant difference was found in deaths ($p = 0.047$), where the Drug B T-0 group exhibited a significantly lower proportion of deaths (0%) than that found in the Drug B T-4 group (55.6%). There was no statistical difference among these groups in terms of motor and complication rates ($p > 0.05$ in all the comparisons). Conclusions: the animals treated with methylprednisolone four hours before the injury trauma presented a significantly higher number of deaths than the rats treated with the same drug after the injury.

Keywords: Spinal cord. Methylprednisolone. Wistar Rats

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INTRODUCTION

Spinal injuries are incapacitating, irreversible and involve a high economic and social cost. They have a devastating effect on the victim's quality of life, with patophysiological changes in the cardiovascular, respiratory, gastrointestinal, genitourinary, neurological and musculoskeletal systems. The extent of these changes is related to the severity of the neurological damage. The most frequent cause is traumatism, but spinal injury is also produced by tumors, infection, vascular lesion or even as a complication of therapeutic procedures.

The mechanisms that provoke acute spinal cord injury can be separated in primary and secondary.[1] The primary injury mechanism consists of acute physiological and structural interruption of the axons. Secondary injury results from additional tissue damage, mediated by the inflammatory response, which results in cell death.

Pharmacological treatment after the occurrence of spinal cord injury can contribute effectively to the reduction of the secondary spinal cord injury. The drugs being studied include the corticosteroids, the calcium channel blockers, naloxone, gangliosides, lazaroides, dimethyl sulfoxide and alphamethylparatirosine. Corticosteroids and gangliosides are already used in clinical practice.

Among the corticosteroids, methylprednisolone had its clinical effectiveness proved in randomized, prospective and double-blind clinical trials.[2] Treatment with corticosteroids is based on

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their anti-inflammatory action and on their effectiveness in the treatment of cerebral edema. It is also believed that methylprednisolone acts on the increase of the blood flow, on the stabilization of the cell membrane and on the inhibition of lipid peroxidation with reduction of free radical formation.

Spinal injury can be predicted in some clinical situations, like in the surgical treatment of intraspinal tumors. Many surgeons have employed methylprednisolone prior to surgical procedures with a high risk of spinal cord injury, although there are no studies in literature that justify this indication.

The aim of this study is to evaluate the effects of methylprednisolone administered prior to spinal cord injury in relation to the beneficial effects and complications in a standardized experimental model.

MATERIAL AND METHOD

Thirty-six male Wistar rats, aged 20 weeks and with an average weight of 350g were used in the study. The rats were divided randomly into four groups, according to the drug administered and the start time of treatment in relation to the timing of the spinal cord lesion.

Two groups of rats received intraperitoneal physiological solution and two groups of rats were medicated with methylprednisolone in the dose of 30 mg/Kg via intraperitoneal injection. The drugs were labeled A or B to prevent the researchers from knowing which substance was being administered.

Of the groups that received the drug labeled A, in one it was administered at the time of the injury (Group A-T0) and in the other, four hours before (Group A-T-4). Of the groups that received the drug labeled B, in one it was administered at the time of the injury (Group B-T0) and in the other, four hours before (Group B-T-4). (Table 1)

Table 1 – *Description of the experimental groups.*

Group	Drug	Start time of treatment
Group A, T0	Drug A	Immediately after the injury
Group A, T-4	Drug A	Four hours before the injury
Group B, T0	Drug B	Immediately after the injury
Group B, T-4	Drug B	Four hours before the injury

Source:IOT/HC/FMUSP

The exclusion criteria were: death immediately after the surgical procedure, rats with spinal cord anomalies in the lesion area upon macroscopic evaluation or that presented normal movement in the first evaluation after injury.

The researchers adopted the experimental spinal cord injury model of the Multicenter Animal Spinal Cord Injury Study (MASCIS) standardized for Wistar rats.[3] To produce the spinal cord injury, they used the computerized weight loss equipment NYU Impactor® (New York University Spinal Cord Contusion System). (Figure 1) The injury was caused by dropping a 10 g impact rod from a predetermined height of 25 mm with monitoring of the rod speed, absolute and relative deformation of the spinal cord, instant of effective contact and contact time.

All the rats were anesthetized with 65 mg/Kg of intraperitoneal pentobarbital. A laminectomy was performed for spinal cord exposure. The rats were positioned in the NYU Impactor® in such a way as to allow full contact of the rod tip on the exposed surface of the spinal cord, at the height of the 10th thoracic vertebra. Two clamping jaws were used to fix the spinal column, attached to the spinous processes of the 9th and 11th thoracic vertebrae to reduce deformation of the rat's body at the moment of impact, and consequently, the movement of the spinal column. (Figure 2)

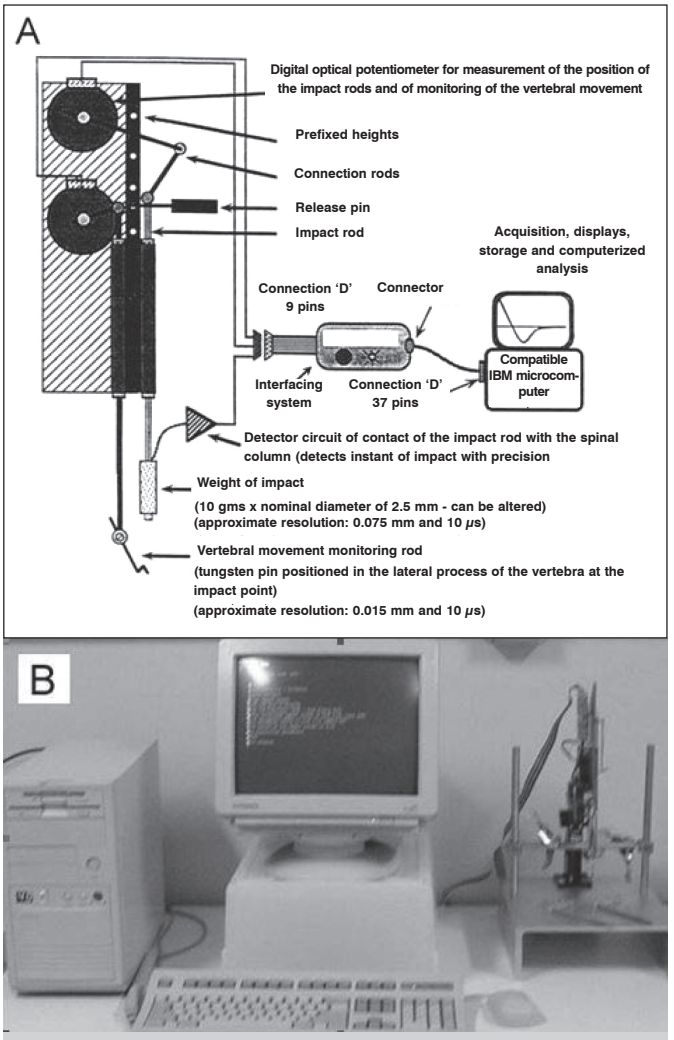

Figure 1 – *Computerized equipment for spinal cord impact by weight drop - NYU Impactor®. A – scaleless schematic diagram and B – Microcomputer and impact device.*

An inspection of the site was conducted after the injury, performing hemostasis with a bipolar electric scalpel in the hemorrhagic cases. Soon afterwards, the wound was closed in plans. The rats were submitted to antibiotic therapy for infection prophylaxis in the surgical wound with 25 mg/Kg of subcutaneous Cefalotin (Keflin Neutro® - Ely Lilly), immediately after the injury and once a day during the next 7 days. In the cases where infection was present, the antibiotic time was extended up to the 10th day and this was considered a complication for statistical purposes.

Recovery of locomotor capacity after spinal cord injury was measured by the Basso, Beattie and Bresnahan scale (BBB).[4] This scale is based on specific observational criteria, with simple and unambiguous definitions of the terms and allows a fast and accurate description of the locomotor performance. (Table 2)

Figure 2 – *A- Laminectomy, with exposure of the spinal cord; B – Clamping jaws on the spinous processes and C – Positioning of the animal in the impact device.*

During the evaluation period by the BBB scale, the rats were observed for mutilations, infections or other alterations.

The evaluation was carried out by two trained observers on the 2nd, 7th, 14th, 21st and 28th days of the postoperative period (PO). They evaluated the rats' locomotor capacity, and made observations on the movement of the joints of the rear leg (hip, knee and ankle), the position of trunk and abdomen, the displacement of the leg (swing) and the mode of contact of the leg with the ground, coordination, toes, contact and release of the leg from the ground, trunk instability and relative position of the tail, in relation to the right and left side.

The evaluation of the rat's locomotor capacity lasted from 4 to 5 minutes during which time the observers extracted the characteristics of the movement executed. The characteristics of consensus between the observers were noted down. They decided on the annotation with the lower score in cases of disagreement.

At the end of the experiment period, all the rats were submitted to euthanasia in conformity with the legislation in force and according to the precepts of the Colégio Brasileiro de Experimentação Animal – COBEA (Brazilian School of Animal Experiments).[5]

The rats were submitted to necropsy examination with observation of lesions possibly associated with autophagy or mutilation, evaluation of macroscopic spinal anomalies included in the exclusion criteria, evaluation of pulmonary alterations such as empyema or condensation, evaluation of the bladder for signs of flaccid neurogenic bladder or alterations suggestive of infection.

The groups were compared in terms of qualitative variables by the Chi-Squared Test. The Kolmogorov-Smirnov Test was applied to test the presence of normal distribution in the quantitative parameters. When comparing the four groups in terms of quantitative variables, the Variance Analysis (ANOVA) technique was used with a fixed factor in the presence of normal distribution of the variables while the nonparametric Kruskal-Wallis test was used otherwise. When comparing the two groups in terms of quantitative variables the Student's T-test was used for independent samples in the presence of normal distribution of the variables while the non-parametric Mann-Whitney test was used otherwise. The significance level of 0.05 (α = 5%) was adopted here while descriptive levels (p) below this value were considered significant and represented by *.

Table 2 – *Operating definitions of categories and attributes of the BBB scale of functional evaluation[4]*

SCORE	OPERATING DEFINITIONS OF CATEGORIES AND ATTRIBUTES
0	No observable movement of the rear limb.
1	Modest (limited) movement of one or of both joints, generally, of the hip and/or knee.
2	Extensive movement of one joint or extensive movement of one joint and modest movement of another.
3	Extensive movement of two joints.
4	Modest movement of all three joints of the rear limb.
5	Modest movement of two joints and extensive movement of the third one.
6	Extensive movement of two joints and modest movement of the third.
7	Extensive movement of the three joints of the rear limb.
8	Pedaling movement without weight bearing or plantar support of the paw without weight bearing.
9	Plantar support of the paw with weight bearing only in stance phase (i.e., when static) or occasional, frequent or consistent stride with weight bearing and no plantar stride.
10	Plantar step with occasional weight bearing and coordination of the fore and rear limbs.
11	Plantar step with frequent to consistent weight bearing and no coordination of the fore and rear limbs.
12	Plantar step with frequent to consistent weight bearing and occasional coordination of the fore and rear limbs.
13	Plantar step with frequent to consistent weight bearing and frequent coordination of the fore and rear limbs.
14	Plantar step with consistent weight bearing, consistent coordination of the fore and rear limbs and predominant position of the rotated leg (internally or externally) during locomotion, at the instant of initial contact with the surface (floor) as well as before the toe-off at the end of the stance phase or frequent plantar stride, consistent coordination of the fore and rear limbs and occasional dorsal stride.
15	Consistent plantar stride and consistent coordination of the fore and rear limbs and no toe-off or occasional release during forward movement of the limb, predominant position of the leg parallel to the body at the instant of initial contact.
16	Consistent plantar step and coordination of the fore and rear limbs during gait and release of the toes occurs frequently during the forward movement of the limb, the predominant position of the leg is parallel to the body at the instant of initial contact and rotated at the instant of release.
17	Consistent plantar stride and coordination of the fore and rear limbs during gait and toe-off occurs frequently during the forward movement of the limb, the predominant position of the leg is parallel to the body at the instants of initial contact and of toe-off.
18	Consistent plantar stride and coordination of the fore and rear limbs during gait and toe-off occurs consistently during the forward movement of the limb, the predominant position of the leg is parallel to the body at the instant of initial contact and rotated in the toe-off.
19	Consistent plantar stride and coordination of the fore and rear limbs during gait and toe-off occurs consistently during the forward movement of the limb; the predominant position of the leg is parallel to the body at the instants of contact and of toe-off and presents the tail pointing downward part of the time or all of the time.
20	Consistent plantar stride and coordination of the fore and rear limbs during gait and toe-off occurs consistently during the forward movement of the limb; the predominant position of the leg is parallel to the body at the instants of contact and of toe-off and presents the tail constantly held high and instability of the trunk.
21	Consistent plantar stride and coordinated gait, consistent toe-off, the predominant position of the leg is parallel to the body throughout the stance phase, consistent stability of the trunk, tail constantly held high.

RESULTS

Of the 36 rats initially included, three were excluded due to death immediately after the lesion.

The 4 groups were compared in terms of weight and age. No statistically significant difference was found between the study groups in terms of mean weight and age.

In motor function no statistically significant difference was found between the study groups in any of the evaluations ($p > 0.05$) (Table 3).

The 4 groups were compared in terms of intercurrences (Table 4).

In the comparison among the 4 groups in terms of intercurrences a statistically significant difference was found in deaths ($p = 0.047$), where the group Drug B T0 presented a significantly lower proportion of deaths (0%) than that found in the group Drug B T-4 (55.6%). (Figure 3)

The animals were regrouped in two new groups according to the drug administered, irrespective of the drug application time, in group A and B. They were compared in terms of weight, age and postoperative measurements (Table 5). There was no statistical difference between these groups in terms of motor activity and in terms of complications ($p > 0.05$ in all the comparisons).

Table 3 – *Motor rates in the different groups.*

Motor Rate – 28th PO – Left side				
mean standard deviation	10.00 ± 6.35	13.50 ± 3.82	13.33 ± 2.25	10.00 ± 2.31
Median	11 (7)	12.5 (8)	14 (6)	10 (4)
minimum – maximum	3 – 21	9 – 21	9 – 15	8 – 12
Kruskal-Wallis test	p = 0,126			
Motor Rate – 28th PO – Right side				
mean standard deviation	10.43 ± 5.88	12.63 ± 3.78	13.83 ± 2.48	10.50 ± 2.38
Median	11 (7)	12 (8)	14.5 (6)	10.5 (4)
minimum – maximum	3 – 21	9 – 21	9 – 16	8 – 13
Kruskal-Wallis test	p = 0.148			

Source: IOT/HC/FMUSP

Table 4 – *Frequency of intercurrence in the groups.*

Variables	**Group A T0**	**Group B T0**	**Group A T-4**	**Group B T-4**
Death – n (%)				
No	7 87.5%	8 100.0%	6 75.0%	4 44.4%
Yes	1 12.5%	0 0.0%	2 25.0%	5 55.6%
Chi-squared test	p = 0.047 * Drug B T0 ≠ Drug B T-4			
Complications – ITU – n (%)				
No	6 75.0%	7 87.5%	7 87.5%	7 77.8%
Yes	2 25.0%	1 12.5%	1 12.5%	2 22.2%
Chi-squared test	p = 0.874			
Variables	**Group A T0**	**Group B T0**	**Group A T-4**	**Group B T-4**
Complications – n (%)				
No	6 75.0%	7 87.5%	6 75.0%	7 77.8%
Yes	2 25.0%	1 12.5%	2 25.0%	2 22.2%
Chi-squared test	p = 0.918			
Complications and/or Death – n (%)				
No	6 75.0%	7 87.5%	4 50.0%	3 33.3%
Yes	2 25.0%	1 12.5%	4 50.0%	6 66.7%
Chi-squared test	p = 0.098			
Relevant findings of necropsy – n (%)				
No	6 75.0%	8 100.0%	7 87.5%	6 66.7%
Yes	2 25.0%	0 0.0%	1 12.5%	3 33.3%
Chi-squared test	p = 0.309			

Source:IOT/HC/FMUSP

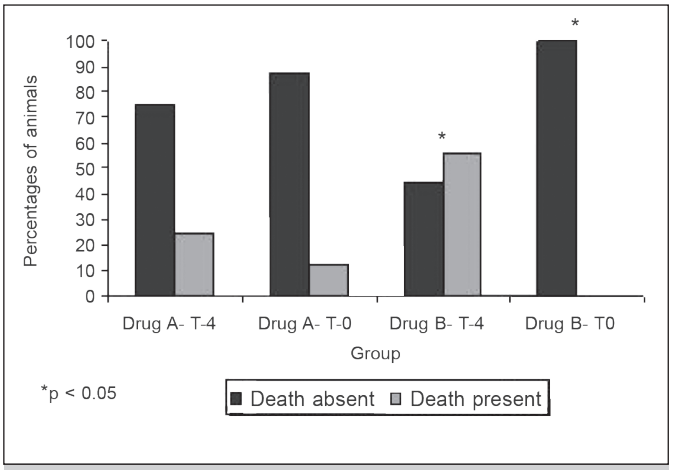

Figure 3 – *Proportion of deaths in the four groups, where group B –T-4 presented a significantly higher proportion of deaths than that found in group B – T0*

Table 5 – *Motor activity of rats submitted to the administration of drug A (Group A) and drug B (Group B), irrespective of the drug administration time.*

Motor Activity – PO28 E		
mean ± standard deviation	11.54 ± 5.03	12.33 ± 3.70
Variables	**Drug A**	**Drug B**
median (n)	13 (13)	12 (12)
minimum – maximum	3 – 21	8 – 21
Mann-Whitney test	p = 1.000	
Índice Motor – PO28 D		
mean ± standard deviation	12.00 ± 4.80	11.92 ± 3.42
median (n)	13 (13)	12 (12)
minimum – maximum	3 – 21	8 – 21
Mann-Whitney test	p = 0.585	

Source: IOT/HC/FMUSP

DISCUSSION

Experimental models and clinical observations of acute spinal cord injury support the concept of secondary spinal injury, in which a mechanical lesion is followed by a series of deleterious events that promote progressive tissue damage and ischemia.[6-8] Therefore, although the primary mechanical lesion is determined by the trauma circumstances and is generally irreversible, there is a succession of biological events that result in the secondary spinal cord injury, which can be reduced by the therapeutic action of neuroprotector drugs.[9,10]

Although there are several substances used to lessen the effects of spinal cord injury after acute traumatism, we chose methylprednisolone to conduct this study as it has shown clinical benefits with improvement of neurological function as evidenced by several authors.[10-17]

Many mechanisms related to the neuroprotector effect of methylprednisolone are mentioned in literature such as the preservation of spinal cord tissue,[18] increase of microcirculation and decrease of the quantity of ATP,[19] decrease of lactate and pyruvate build-up,[20] maintenance of spinal cord blood flow within normal limits,[21] ability to inhibit the oxygen free radicals induced by lipid

peroxidation,[22,23] decrease of lesion volume,[24] improvement of axonal regeneration,[25] reduction of the cascade of secondary effects after the acute trauma,[26] decrease of spinal cord ischemia, inhibiting the increase of vascular permeability at the injured site in the spinal cord[27] and reduction of severe edema, preserving the architecture of the adjacent spinal cord.[28]

Yoon et al.[29] affirm that the model of spinal cord injury, provoked by the NYU Impactor® system, has a very short therapeutic window and that the best results of the use of methylprednisolone occur with a dose of 30mg/kg, applied in the first 30 minutes after the injury. Based on this study, the dose of choice for the use of methylprednisolone was 30mg/kg four hours before the spinal cord injury and immediately after the injury, as described above.

Methylprednisolone use before the trauma is not described and the few studies with drugs before the spinal cord trauma are usually carried out for ischemic lesions, usually found in aorta aneurysm treatments.[30] In this study the participants agreed on the use of methylprednisolone four hours before the traumatism for the drug to obtain an adequate serum level.

Contrary to previous studies, there was no statistically significant difference in the motor evaluations among the groups studied, in all the analyses. It is important to stress that the BBB scale used only contemplates the motor evaluation of rats and many of the studies, mainly the great clinical assays such as the National Acute Spinal Cord Injury Studies (NASCIS),[11,12,31] evaluated not only motor recovery but also the recovery of sensitivity and changes in vesical function.

As regards complications, when the use of methylprednisolone was compared with physiological saline solution, no statistically significant difference was observed. But in comparing the use of methylprednisolone four hours before the trauma and that used immediately after the trauma, it is observed that the use of methylprednisolone four hours before the traumatism had a significantly higher number of deaths. A possible explanation would be that the rats submitted to the application of the drug four hours before the trauma are exposed to a higher level of stress, with release of catecholamines, which might potentialize the harmful effects of methylprednisolone, leading to a higher number of deaths.

CONCLUSIONS

No beneficial effect was observed in the use of methylprednisolone prior to spinal traumatism in terms of motor activity. As far as complications are concerned, the rats treated with methylprednisolone four hours before the trauma presented a significantly higher number of deaths than when compared with the rats treated with the same drug immediately after the traumatism.

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