SPINAL CORD INJURY EXPERIMENTAL MODEL AND MOTION EVALUATION PROTOCOL IN WISTAR RATS

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ABSTRACT

Objectives: To determine a standardized spinal cord contusion model and a method for motor assessment in rats with paraplegia. Methods: This study used 20 Wistar rats divided into 4 groups according to level of severity of spinal cord injury; standardized intermediate lesions were made through system MASCIS IMPACTOR (Multicenter Animal Spinal Cord Injury Study): group 1, 12.5mm (mild injury); group 2,25mm (moderate injury); group 3,50mm (severe injury; in the group 4 the animals suffered no injury (control group). Motor function was assessed after 48 hours, using the scale proposed by Basso, Beattie and Bresnahan. Results: Using the model, we observed that the mild contusions (12.5mm height) were effective, and the animals presented acute urinary tract infection one week after the injury. Moderate contusions (25mm height) were effective, and the animals presented urinary infection until 2 weeks after injury. The severe contusions (50mm height) were effective, and the animals presented urinary infection for 3 to 4 weeks and autophagy. Conclusion: The model of spinal cord injury using the system MASCIS IMPACTOR and the functional assessment proposed by Basso, Beattie and Bresnahan is reproducible and can be used, enabling information exchange among different researchers.

Keywords: Spinal cord injuries. Paraplegia. Contusions.

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INTRODUCTION

Experimental models have been used to accumulate experiences and consequently to improve and refine knowledge about spinal cord injury physiopathology.[1-3]

This knowledge applied to clinical and surgical treatments is used to reduce morbidity and mortality in patients with spinal cord injury.[4]

It is important to have a standardized assessment applied in experimental studies to determine the effects of each therapeutic intervention that one wishes to study. Some assessment criteria have been presented in literature, and the scale proposed by Basso, Beattie and Bresnahan, BBB, is that used in this study.[1,2,4]

Defined as a scale of 21 points that observes recovery of locomotion in the hind limb after thoracic spinal cord injury in rats, this scale represents a detailed and organized categorization of the recovery of movements in the lower limb following the spinal cord lesion. Each index represents a single and sequential stage of behavioral recovery.[5]

The objective of this study is to evaluate, using the BBB scale, the severity of lesions at different levels of height made using a standardized experimental spinal cord injury model known as a the "Mascis Impactor".[4]

The study subjects were 20 young male adult Wistar rats, with mean age 20 of weeks, weighing around 350g, from the Centro de Bioterismo da Faculdade de Medicinada USP, divided into four groups of five animals.

The group divisions were performed as follows:

Control group: we performed only approach and laminectomy at the level of T9

Group 2: 12.5 mm representing mild injury

Group 3: 15 mm representing moderate injury

Group 4: 50 mm representing severe injury.

The procedures and the accommodation of the animals were performed at the Laboratório de Estudos do Traumatismo Raquimedular e Nervos do Instituto de Ortopedia e Traumatologia da USP. The animals were anesthetized with intraperitoneal pentobarbital, in the concentration of 45mg/kg, in the lower third

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of the abdomen, then submitted to dorsal trichotomy, antisepsis with chlorhexidine, and longitudinal incision in the topography of T7 to T12 (Figure 1), dissection by planes and exposure of the posterior components of the dorsolumbar spine. (Figure 2) The laminae from T9 and T10 were resected in the caudocranial direction. (Figure 3)

SPINAL CORD CONTUSION

The lesions were produced in compliance with the international protocol *MASCIS* (Multicenter Animal Spinal Cord Injury Study), a model that produces spinal cord contusions at different levels, which determines the degree of severity. (Figures 4, 5, 6, 7 and 8) The injury levels range from 6 mm of height, which is considered a mild injury, to 70 mm of height, which simulates an extremely severe lesion. Intermediate lesions were produced with 12.5 mm representing mild injury, 25 mm moderate injury and 50 mm of height representing severe injury. The lesion procedure was followed by the observation of standardization parameters such as clinical aspects of the injury site and graphs. (Figures 9 and 10)

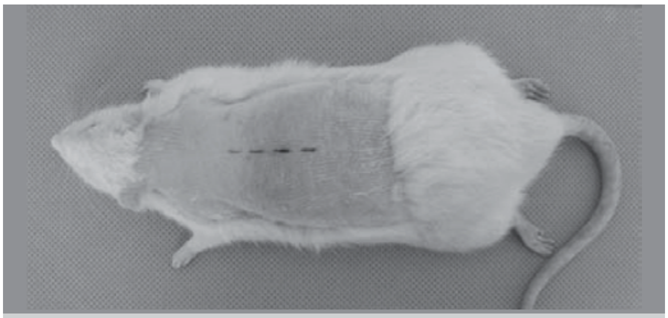

Figure 1. *Planning of incision.*

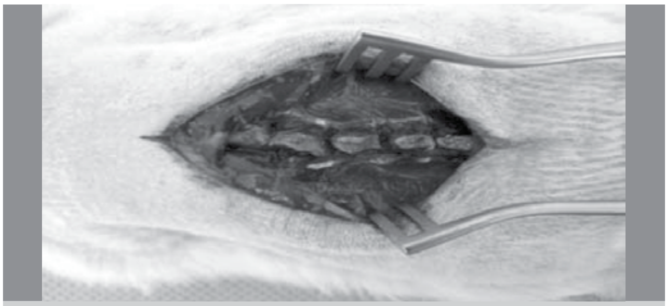

Figure 2. *Exposure dorsolumbar region.*

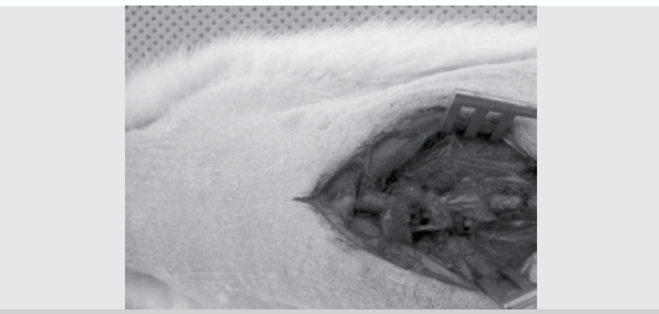

Figure 3. *Exposure spinal cord at T9..*

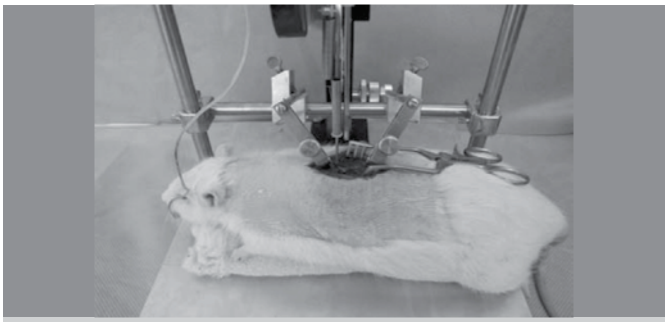

Figure 4. *Animal positioned.*

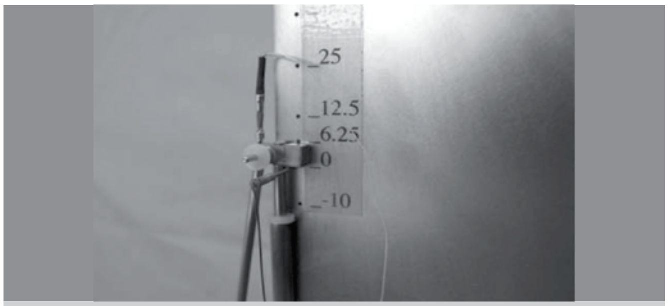

Figure 5. *Initial contact neutral position.*

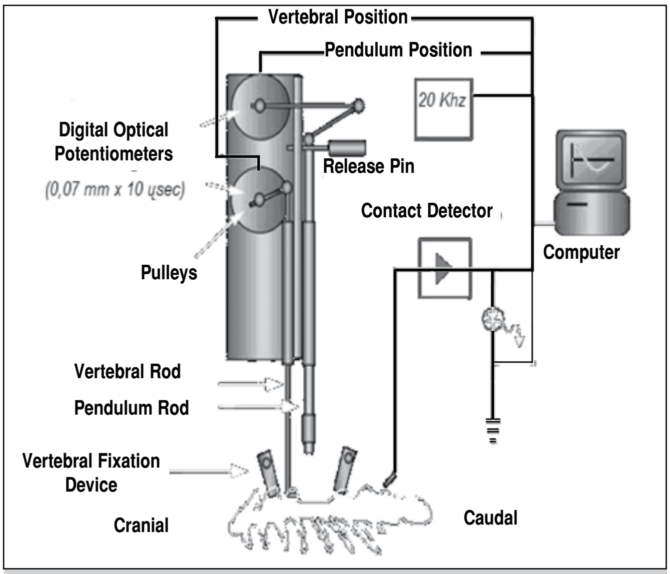

Figure 6. *Schematic representation of the model used.*

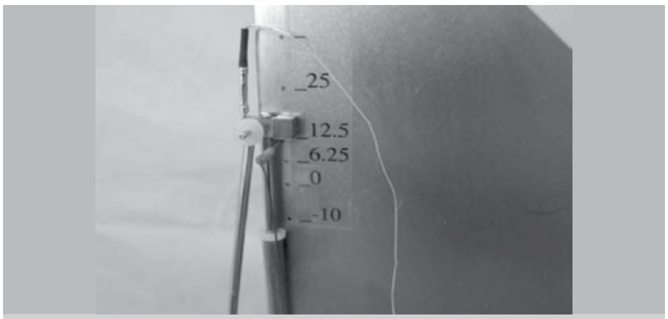

Figure 7. *Positioning 12.5 mm.*

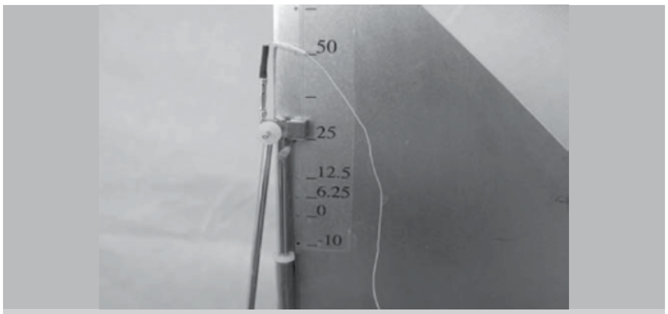

Figure 8. *Positioning 25 mm.*

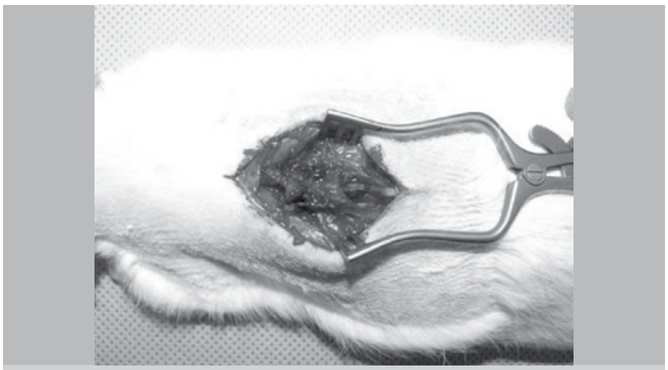

Figure 9. *Post-contusion aspect.*

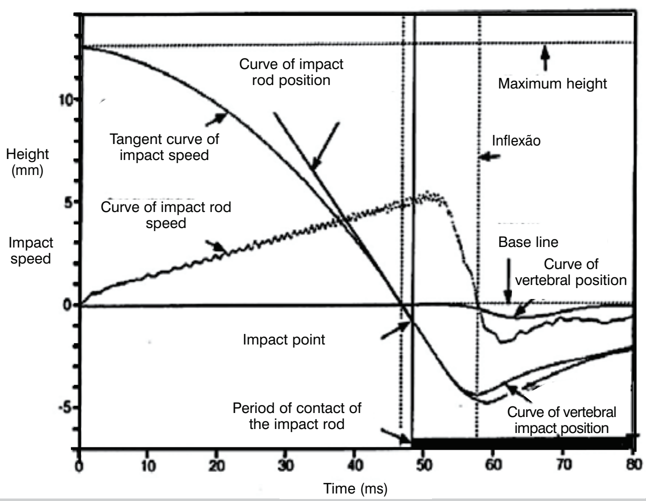

Figure 10. *Graphic parameters.*

The injury site was irrigated with saline solution at room temperature, followed by the inspection, cauterization, myorrhaphy and skin suture. (Figures 11 and 12) A dose of 25mg/kg of cephalothin was administered subcutaneously.[4]

The animals were kept in cages (60x40cm) in groups of 3 or 4, and provided with feed and water *ad libitum*. The massages for vesicle emptying were performed daily, in addition to the evaluation of possible complications, such as urinary infection. Motor assessments were conducted after 48 hours following the motor assessment protocol proposed by Basso, Beattie and Bresnahan, "BBB", which corresponds to a scale of 21 points, according to the motility of the pelvic limbs and tail of the animal, in a standardized place with dimensions of 90 cm of diameter and 7 cm of height without the presence of noise. (Charts 1 and 2).

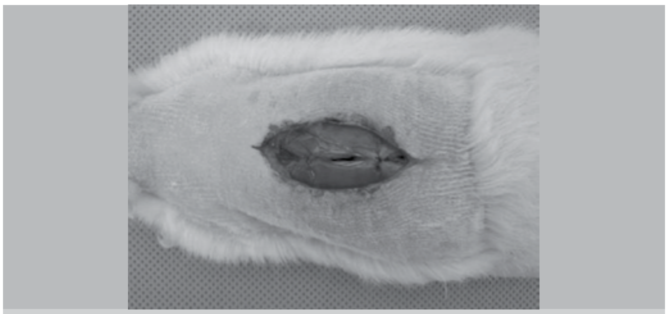

Figure 11. *Myorrhaphy.*

Figure 12. *Cutaneous suture.*

RESULTS

The data obtained show statistical differences among the groups and also that the contusions are effective and performed in a standardized manner, since no statistical differences were noticed in the same group, according to Chart 3 and Figure 13.

DISCUSSION

Several experimental models have been described in literature for the study of standardized spinal cord injuries in animals.[1-6] In our protocol we opted for the "MASCIS IMPACTOR" model, which uses weight drop from a standardized height causing spinal cord contusion in rats, after which they are assessed in their locomotor function.[1,2,7] In this experimental study we used Wistar rats due to the low cost and ease in their obtainment.

The functional assessment methods are not yet totally standardized, with several descriptions in literature.[3,8,9]

Within the standardized evaluation of spinal cord injury, to provide a safe exchange of data among the different research groups, anatomopathological, biochemical and imaging diagnosis alterations, among others, are being analyzed.[10-11]

In our study we opted for the functional assessment model described by Basso, Beattie and Bresnahan (BBB), which uses a standardized locomotor assessment scale. We opted for the methodology adopted in the Multicenter animal spinal cord injury (MASCIS), which uses the BBB scale after spinal cord injury at standardized levels of height (12.5, 25, 50 mm).

The results presented by the groups exhibited differences in

Chart 1. *BBB protocol scores*.

Score	Characteristics	Remarks
0	No rear limb (RL) movements are observed	
1	Light movements of 1 or 2 rear limb joints	Light – Below 50% of the joint capacity
2	Extensive movement of 1 joint and possible slight movement of other joint in the rear limb	Extensive – Above or equal to 50% of joint capacity
3	Extensive movement of 2 rear limb joints	Two joints = hip and knee, usually
4	Light movements of all 3 joints	Three joints = hip, knee and ankle
5	Light movements of 2 joints, extensive movement of the 3rd joint of the rear limb	
6	Extensive movements of 2 joints and delicate movement of the 3rd joint of the rear limb	3rd joint = ankle
7	Extensive movements of the 3 joints	
8	Light movements, without bearing the body weight or the foot resting without bearing the body weight	Rhythmic extension of 3 joints of the rear limb, the trunk is sideways
9	Arch support with bearing of body weight while immobile or occasional, frequent or consistent bearing of body weight with dorsal support	Weight bearing = contraction of muscle. Extension of the rear limb during arch support of the foot or raising of the immobile pelvis
10	Step bearing the body weight occasionally, without coordination between the fore and rear limbs	Occasionally > 5th and below or equal to 50%. Steps – arch contact with weight bearing, the rear limb advances to reestablish arch contact. Coordination – simultaneous movements between the rear limb and the fore limb, alternating between the sides.
11	From frequent to consistent strides with weight bearing without coordination between the rear and fore limbs	Frequency – 51 to 94% of the time Consistent – 95 to 100% oft he time
12	From frequent to consistent strides with bearing of body weight and occasional coordination between the fore limbs and rear limbs	60 to 50% of coordinated locomotion
13	From frequent to consistent strides with weight bearing and frequent coordination between the rear limb and the fore limb	51 to 95% of coordinated locomotion
14	Consistent coordination of the stride with arch support and predominant position of the foot and of rotation in initial contact and in raising, frequent plantar strides, consistent coordination between the fore limb and occasionally with dorsal support	Rotation =>internal and external rotation of the rear foot when it is supported and raised
16	Consistent coordination of stride with arch support, predominant parallel position of the foot in initial contact and when raised	Frequent freedom of the 1st toe ≥ More than half of the strides are taken without hearing the scratching sounds
17	Consistent coordination of stride with arch support, predominant parallel position of the foot in initial contact and when raised	
18	Consistent coordination in stride with arch support and consistent release of the 1st toe. Parallel position of the foot in initial contact and when raised	Consistent scratching of four toes for a period of 4 minutes
19	Consistent coordination of stride with arch support, consistent release of the 1st toe. Parallel position of foot in initial contact and when raised, and the tail is down most of the time	Tail down =>tail touches the ground during the steps
20	Consistent coordination of stride with arch support, consistent release of the 1st toe. Parallel position of foot in initial contact and when raised, the tail consistently raised and instability of trunk.	Raised tail =>does not touch the ground. Instability of trunk =>lateralization of trunk when turning quickly (loss of balance)
21	Consistently coordinated in gait, consistent movement of the 1st toe. Parallel position of the foot in support and when raised. Tail up Consistent stability of trunk	Consistent stability of trunk without wobbling or falling; movement of pelvis and tail coordinated with locomotion

Chart 2. BBB Protocol (subcomponents)

CATEGORY	TYPE	CHARACTERISTIC
Movement of rear limb	Hip (R/L)	O – no movement S – limited movement E – extensive movement
	Knee (R/L)	O – no movement S – limited movement E – extensive movement
	Ankle (R/L)	O – no movement S – limited movement E – extensive movement
Position of Trunk	Side	≥ Lying on one of the sides
	Central	≥ Central position (normal)
	Support	> Uses tail to support the body
Abdomen	Supported	=> Abdomen resting on the ground
	Parallel	=> Abdomen parallel to the ground (normal)
	High	=> Abdomen and pelvis raised
Position of the Foot	Light movements (R/L)	=>Support of foot without bearing weight =>Support of foot bearing weight
Walking	Weight (R/L)	0 – never O – Occasionally (below or equal to 50% of the time) F – frequently (51 to 94% of the time) C – consistent (95 to 100% of the time)
	Coordination	0 – never O – Occasionally (below or equal to 50% of the time) F – frequently (51 to 94% of the time) C – consistent (95 to 100% of the time)
	Mobility of the 1st toe	0 – never O – Occasionally (below or equal to 50% of the time) F – frequently (51 to 94% of the time) C – consistent (95 a 100% of the time)
Predominant position of foot	Initial contact (R/L)	I – internal rotation E – external rotation P – parallel (normal)
Predominant position of foot	Initial contact (R/L)	I – internal rotation E – external rotation P – parallel (normal)
	Raising of foot	I – internal rotation E – external rotation P – parallel (normal)
Instability of body	Yes/No	Yes – Wobbling, imbalance
Tail	Raised/ Lowered	Raised– the tail is above the ground
		Lowered – the tail touches the ground

Chart 3. Score evaluated according to model proposed by "BBB".

Animal / Group	1	2	3	4	5	Total
Control	21	21	21	21	21	105
12.5 mm	17	16	17	17	15	82
25 mm	8	8	7	7	5	35
50 mm	0	0	0	0	1	1

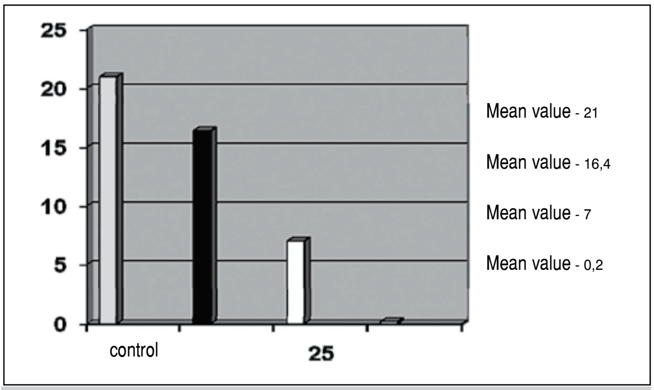

Figure 13. *Mean value of the BBB index in each one of the groups studied.*

the functional assessment of the injury at each standardized height, which confirms the objectivity and reliability of the data from the BBB scale.

It is important to emphasize that this assessment method presents reliability in the exams carried out by different observers as demonstrated by Basso *et al.*[2]

CONCLUSION

It is concluded that the spinal cord injury model using the MASCIS IMPACTOR system and the functional assessment model proposed by Basso, Beattie and Bresnahan "BBB" is reproducible and can be used by observers with previous training in the evaluation of animals with spinal cord injury, favoring the exchange of information among the different researchers that use the same scale.

REFERENCES

1. Cristante AF, Barros Filho TEP, Oliveira RP, Marcon RM, Rocha ID, Hanania FR *et al*. Antioxidative therapy in contusion spinal cord injury. Spinal Cord. 2009;47:458-63.
2. Basso DM, Beattir MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field test in rats. J Neurotrauma.1995;12:1-21.
3. Tarlov IM. Spinal cord compression, mechanism of paralysis and treatment. Springfield, IL: Charles C Thomas; 1957.
4. Rodrigues NR. Padronização da lesão experimental em ratos Wistar [tese]. São Paulo: Faculdade de Ciências Médicas, Universidade de São Paulo; 1999.
5. Haguigui SS, Espejo SS, Sepejo MAP, Rodriguez F, Clapper A. Radiofrequency as a lesioning model in spinal cord injury. Spine. 1996;34:214-9.
6. Fernandes E, Pallini R, Marchese R, Tulomonti G. Experimental studies on spinal cord injuries in the last fifteen years. Neurol Res. 1991;13:138-59.
7. Basso DM, Beattie MS, Bresnahan JC, Anderson DK, Faden AI, Gruner JA *et al*. MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. J Neurotrauma; 1996;13:343-59.
8. Faconer JC, Naroyama PA, Bhattachojee M, Liv SJ. Characterization of a experimental spinal cord injury model using waveform and morfhonetic analysis. Spine. 1996;21:104-12.
9. Black P, Markowitz RS, Cooper V, Mechanic A, Kushner H, Damjanov I *et al*. Models of spinal cord injury: Part 1. Static load technique. Neurosurgery.1986;19:752-62.
10. Vialle LRG, Fischer J, Marcon JC, Vialle E, Luizzi R, Torres LSB. Estudo histológico da lesão medular experimental em ratos. Rev Bras Ortop.1999;34:85-9.
11. Girardi FP, Khan SN, Cammisa FP Jr, Blanck TJ. Advantages and strategies for spinal cord regeneration. Orthopedic Clin North Am. 2000;31:465-71.