STANDARDIZATION OF SPINAL CORD INJURY IN WISTAR RATS

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ABSTRACT

Objective: To standardize an experimental model of spinal cord injury in Wistar rats, computerized weight fall impact equipment were used and the parameters were used determined by the multicenter animal spinal cord injury study – MASCIS . Methods: Thirty rats were used, with age varying between 20 and 25 weeks, and weight ranging from 200 to 300g for females, and from 232 to 430g for males. The impacts were done with weights of 10g starting from 12.5, 25 and 50 mm of height, and the impact speed and compression coefficient were obtained. The impact occurred on the surface of the spinal cord at the level of the tenth thoracic vertebra after laminectomy. Vital signs were monitored and gas analysis was made before and after the spinal cord injury. The lesion volume was evaluated by the quantitative analysis of sodium and potassium ions. Results: Statistically significant correlations were verified among the lesion volume and the mechanical parameters. The lesion volume caused by the fall from 50mm height was superior to that of the 12.5 and 25mm, which didn't differ from each other. Conclusion: The model demonstrated itself to be effective and capable of generating standard spinal cord injuries on Wistar rats.

Keywords: Experimental model. Spinal cord injury. Rats, Wistar.

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INTRODUCTION

The potentially devastating effect of spinal cord injuries on the patient's quality of life is a cause for concern among physicians that care for this kind of patient. The physiopathological changes caused by injury of the spinal cord affect multiple systems, while the extent of changes is related to the severity of the neurological damage.

The neurological lesion can be complete or incomplete; incomplete lesions are those in which the neurological function is preserved at more than two levels below the level of the lesion. The presence of sacral sensibility, distal motor function and motor-evoked sensibility potential indicate incomplete lesion.

According to Holdsworth,[1] complete lesions are those in which there is no neurological function distal to the injury, and that can be reversible or irreversible. Neurological recovery will not occur if the complete deficit persists after spinal cord shock. A complete lesion implies total interruption of nerve communication, even though there is no physical transection of the spinal cord.

Nerve tissue injury occurs due to primary and secondary mechanisms. Primary injury is due to direct or indirect trauma at the level of the lesion; the mechanisms can be by flexion, extension, traction, compression, lateral inclination or laceration by a penetrating object, such as a firearm projectile.[2]

Primary injury is followed by the triggering of the secondary mechanism, which is the release of endogenous mediators that lead to the progression of the neuronal lesion due to physiological and metabolic changes at the injury site.[2,3]

Only through well-planned laboratory investigations was it possible to accumulate the necessary experience to improve our knowledge of the physiopathology of spinal cord injury. This knowledge, combined with the surgical techniques for spine stabilization and the clinical care in specialized units, have made it possible to reduce morbidity and mortality in patients with rachimedular traumatism. In spite of these advances spinal cord injury continues a complex and serious problem, since our capacity to prevent loss or recovery of neuronal function after spinal cord traumatism remains limited. Current methods for evaluation of the physiophathological changes of spinal cord injury in humans are limited and the causes of injuries are multifactorial,[4] hence it is necessary to use experimental models on animals.

We should consider that the animal models differ in many aspects from injuries occurring in humans, both in the mechanism, and topographically, anatomically and in terms of the energy of trauma, but nevertheless are rich and important sources of information.

All the authors declare that there is no potential conflict of interest referring to this article.

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Considering these variables, it is not surprising that a great number of experimental spinal cord injury models are developed.[4] During the last 90 years, several experimental models of acute injury of the spinal cord in animals were developed in an attempt to study and find an effective method to treat acute injuries of the spinal cord in human beings.[5-13]

In relation to the spinal cord injury production mechanism, we observed that the model that uses weight drop is the most readily accepted by researchers and the most similar to the situation experienced by humans during trauma.

Consequently, this study was developed with the aim of standardizing spinal cord injury through the New York University (NYU) Spinal Cord Contusion - IMPACTOR on Wistar rats, by the biomechanical parameters of impact and estimating the volume of injury at the level of the spinal cord lesion.

MATERIAL AND METHODS

SPINAL CORD INJURY MODEL

An analysis was conducted of the injuries produced in 30 Wistar rats, of which 24 were males and 6 females, with age ranging from 20 to 25 weeks and weight from 200 to 300g for the females and 232 to 430g for the males. Lesions were provoked through alternated computerized weight drop impact assays from heights of 12.5, 25.0 and 50.0 mm (10 rats for each type of injury). The rats were anesthetized with intraperitoneal pentobarbital.

Prior to the contusion, the spinal cord was exposed through a laminectomy (with the aid of a surgical microscope). (Figure 1)

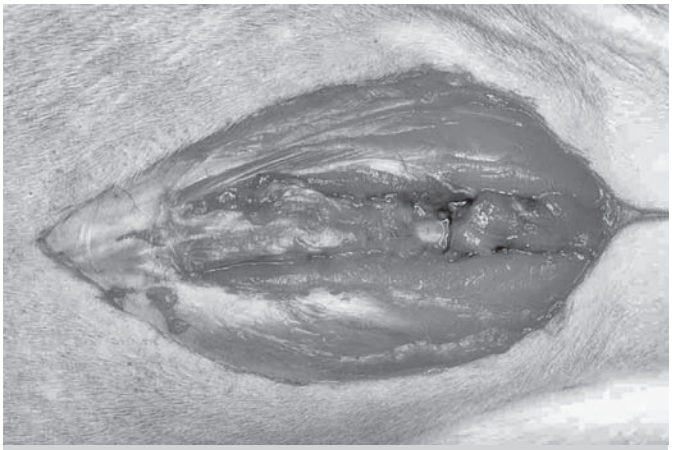

Figure 1 – *Laminectomy at the level of T9-T10 with exposure of the dural sac.*

The weight drop impact test consisted of releasing a 10g impact rod from predetermined heights of 12.5, 25 and 50mm (between the impact rod head and spinal cord surface) in free fall through the guide tube. (Figures 2 and 3)

The lesion volumes were determined by the quantitative analysis of sodium (Na) and potassium (K).[14] Efforts were made to correlate these volumes with the mechanical parameters of the impact assay that generated them and to obtain a regression equation capable of estimating them accurately. The obtainment of a statistically significant correlation ensures that the proposed experimental model is reproducible.

Figure 2 – *Computerized equipment for weight drop impact - Impactor.*

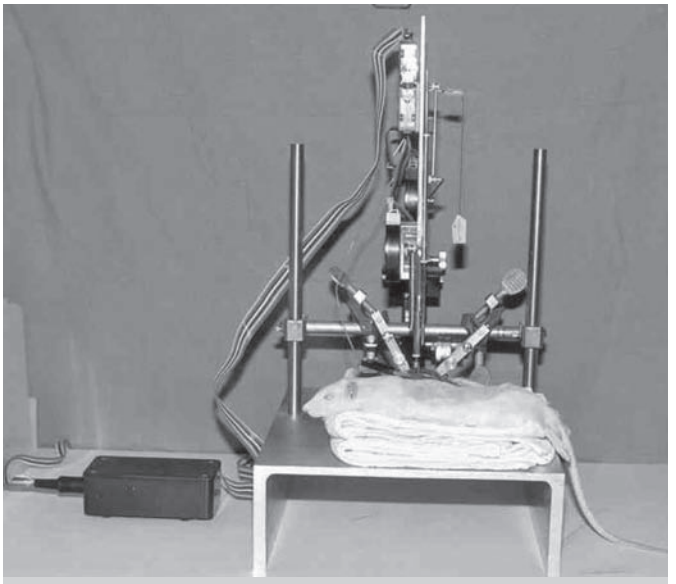

Figure 3 – *Positioning of the rat on the Impactor at the time of trauma.*

The production of lesions, the euthanasia of the animals 48 hours after the procedure and the collection of samples took place at LETRAN – Laboratory for Studies of Rachimedular Traumatism and Peripheral Nerves, and at the Microsurgery Laboratory of the Institute of Orthopedics and Traumatology of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (IOT-HC-FMUSP). The ionic analysis and the comparison of data obtained through the IMPACTOR took place at the Neurosciences Center of Rutgers - State University of New Jersey, New Brunswick, U.S.A.

For statistical analysis they used the Chi-square Test in the comparison among three groups of nominal parameters and for the ordinal parameters they used the Variance Analysis. In cases in which there were significant differences among the groups, these were discriminated by Fisher's protected least significant difference test.

They also conducted correlation tests between and among ordinal parameters. The respective linear regression equation was calculated in cases in which the Correlation Coefficient (r^2) was significant.

They adopted a significance level of 5 % ($\alpha = 0.05$).

The statistically significant results were marked with asterisks.

RESULTS

Immediately after the injury, we observed subdural hemorrhage at the impact site, which was evident in all the animals and appeared more intensive and extensive the higher the height from which the mass was dropped.

No hypotension was observed during the assay or injury of the dura mater after impact.

There was no statistical significance: in the distribution of frequency of gender, descriptive of age (weeks), of weight (g), of pH, of partial pressure of pO_2 (mmHg), of pCO_2 (mmHg), of O_2 saturation (%) and of mean blood pressure (mmHg) of the rats prior to the contusion according to the height of the fall of the impact rod used by group. Comparison by the variance analysis ($A = 0.05$).

Statistical significance was found in the descriptive analysis: of the volume of plasmatic lesion by liter ($p = 0.0001$) (Table 1), of the volume of intracellular lesion by liter ($p = 0.0001$) (Table 2) and volume of tissue lesion by liter ($p = 0.0012$) (Table 3) according to the height of the fall of the impact rod used in each group. A comparison was performed by the variance analysis ($A = 0.05$), with discrimination of differences by Fisher's protected least significant difference test.

Statistical significance was also encountered in the descriptive analysis: of impact speed Imp-S (m/s) ($p= 0.0001$) (Table 4), of the maximum deformation (mm) ($p= 0.0001$) (Table 5) and of the mean compression rate (m/s) ($p=0.0001$) (Table 6) according to the height of the fall of the impact rod, with the performance of comparison by the variance analysis ($A = 0.05$).

Table 1 – *Volume of plasmatic lesion by liter.*

	LVp / l		
	12.5	25.0	50.0
M	36.250	38.764	46.269
SD	3.504	2.805	5.045
SEM	1.108	0.887	1.595
N	10	10	10
Variance analysis	F= 17.441		p= 0.0001*

Discrimination by Fisher's Protected LSD: 12.5 ≠ 50.0 and 25 ≠ 50

Table 2 – *Volume of intracellular lesion by liter.*

	LVi / l		
	12.5	25.0	50.0
M	37.024	39.296	46.180
SD	3.498	3.059	4.956
SEM	1.106	0.967	1.567
N	10	10	10
Variance analysis	F= 14.817		p= 0.0001*

Discrimination by Fisher's Protected LSD: 12.5 ≠ 50.0 and 25 ≠ 50

Table 3 – *Volume of tissue lesion by liter.*

	LVk / l		
	12.5	25.0	50.0
M	40.203	45.243	52.219
SD	4.644	5.185	8.342
SEM	1.469	1.640	2.638
N	10	10	10
Variance analysis	F= 8.818		p= 0.0012*

Discrimination by Fisher's Protected LSD: 12.5 ≠ 50.0 and 25 ≠ 50

Table 4 – *Speed of impact Imp-S (m/s).*

	Imp-S (m/s)		
	12.5	25.0	50.0
M	0.496	0.699	1.000
SD	0.013	0.004	0.013
SEM	0.004	0.001	0.004
N	10	10	10
Variance analysis	F= 5358.1		p= 0.0001*

Table 5 – *Maximum deformation (mm).*

	Cd (mm)		
	12.5	25.0	50.0
M	1.455	1.881	2.458
SD	0.286	0.340	0.217
SEM	0.090	0.107	0.069
N	10	10	10
Variance analysis	F= 31.146		p= 0.001

Table 6 –*Mean rate of compression (m/s).*

	Cr (m/s)		
	12.5	25.0	50.0
M	0.362	0.501	0.733
SD	0.056	0.049	0.059
SEM	0.018	0.015	0.019
N	10	10	10
Variance analysis	F= 116.8		p= 0.0001

Table 7 shows the correlation coefficient and linear regression equations of the lesion volume (LV) according to the compression coefficient (Cr).

Table 7 – *Correlation coefficient and linear regression equations of the lesion volume (LV) according to the compression coefficient (Cr).*

Parameters		Correlation Coefficient R	Regression Equation
Dependent (Y)	Independent (X)	(critical r^2 = 0.49)	Y= A+BX
LVp / I	Cr	0.469	-
LVi / I	Cr	0.504*	Y= 27.2 + 24.8
LVt / I	Cr	0.376	-

Where p= plasmatic
i= intracellular
t= tissular

DISCUSSION

Traumatic injury of the spinal cord is perhaps one of the most, if not the most, incapacitating injury that human beings can suffer, and has caused a great deal of interest in the knowledge of hystopathological, biochemical and functional alterations.

The development of a rational approach in the treatment of acute spinal cord injury in humans requires an experimental model through a quantifiable traumatic mechanism that produces standardized and reproducible injuries. The greatest obstacles in these studies have been the variety of parameters to be controlled in animal models and the lack of a universally acceptable method to produce the lesion.[15]

Since Allen's method[5] was introduced, it has become the most widely used model in the study of traumatic spinal cord injury. This model[5] is also known as the weight drop model, as it consists of the free fall of a known mass from a pre-established height on the surgically exposed spinal cord.

This model, with some improvements, was used by several authors[16-21] and is the one that best resembles traumatic injury in human beings.

Our choice of the New York University (NYU) Spinal Cord Contusion system - IMPACTOR, was due to the fact that the Laboratory for Studies of Rachimedular Traumatism and Peripheral Nerves (LETRAN) is a participant in the Multicenter Animal Spinal Cord Injury Study (MASCIS), which permits the performance of comparable experimental studies.

Rats are a good alternative, according to literature, on account of the pathological characteristics of their spinal cord, and studies using the impact technique affirm that the rat is a valid species for the experimental study of spinal cord injury.[22-24]

Our choice of the Wistar rat is due to its availability in our area and to the low maintenance cost. We did not find any studies utilizing Wistar rats in an experimental study by weight drop with the IMPACTOR.

In this study the rats distributed among the groups that use different impact rod heights presented similar characteristics in relation to sex, age, weight, pH, pO_2, pCO_2, O_2 saturation and blood pressure prior to the contusion, a necessary condition to make them comparable.

In our study we performed a thoracic laminectomy that was sufficient to allow the penetration of the impactor head, with a safety area of 2 mm, always preserving the joint processes and pedicles.

Different weights are used to produce different degrees of trauma, maintaining the same drop height; either the same weight is dropped from different heights, or less often, a combination of both variables is used.

The plasmatic volume of lesion was the one that presented correlation with the biomechanical factors, and a difference was observed between those obtained at 50 mm of height from the others, yet no difference was observed in the volume of injury provoked between 12.5 and 25 mm of height. (Table 1)

In our study we did not observe the phenomenon of multiple impacts, as the IMPACTOR has an adjustment that avoids this second impact.

In our experimental model we did not use the spinal cord protector, as the impactor rod drops directly on the exposed spinal dura mater, thus eliminating the factors that could alter the biomechanical results through the use of the spinal cord protector.

We performed a limited laminectomy; the sufficient to allow the head of the rod to come into contact with the spinal dura mater, and stabilized the spinal column with staples through the spinous processes superior and inferior to the laminectomy.

The weight drop technique exhibits several disadvantages or critiques such as:

1 – The g.m quantification is not a true representation of the energy applied to the spinal cord; a mass of 40 grams falling from the height of 10 centimeters transfers more than 100 times the energy to the spinal cord, than a mass of 5 grams falling from the height of 80 centimeters, even if both are lesions of 400 g.m.[7,18]
2 – The spinal cord is compressed on its dorsal side, different from the previous compressions or circumferences that are observed in injuries in human beings.[25]
3 – Even if some authors have reported that the weight drop technique presents variable results.[8,10]

At the biomechanical parameters of the impact we observed that there is a statistically significant difference, among groups, in the compression coefficient (Cr), in the maximum deformation of the spinal cord (Cd) and in the maximum speed of impact (Imp_S).

The possibility of possessing a standardized model in our laboratory allows countless research prospects such as: the use of neurotrophic factors, blocking of growth inhibitor factors, transplant of peripheral nerves, electro-stimulation, neurogenerator drugs and development of experimental techniques for treatment of spinal cord injury, inclusive of its chronic phase.

CONCLUSIONS

It was possible to perform the standardization of experimental spinal cord injury in Wistar rats according to the parameters determined by the Multicenter Animal Spinal Cord Injury Study - MASCIS.

The experimental model manages to estimate the volume of resulting spinal injury according to the mechanical parameters measured during impact.

No significant difference was proven between the volumes of lesion provoked by falls of mass (10 g) from a height of 12.5 and 25 mm.

The lesion volume provoked by fall of mass from a height of 50 mm was significantly higher than those generated at heights of 12.5 and 25 mm.

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