MOTOR AND HISTOLOGICAL FINDINGS IN A MODEL OF SCI: COMPARISON BETWEEN POSTERIOR AND LATERAL CLIPS

ACHADOS MOTORES E HISTOLÓGICOS EM UM MODELO DE TRM: COMPARAÇÃO ENTRE COLOCAÇÃO POSTERIOR E LATERAL DE CLIPES

HALLAZGOS MOTORES E HISTOLÓGICOS EN UN MODELO DE TRM: COMPARACIÓN DE LA COLOCACIÓN LATERAL Y POSTERIOR DE CLIPS

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ABSTRACT

Objective: To evaluate the locomotor and histological impact on the spinal cord comparing lateral and posterior clip placement. Method: Randomized experimental trial. Twenty female Wistar rats, weighing between 250 and 300 grams and aged 12-14 weeks were randomized in two groups according to the placement of the clip: lateral group (N=10) and posterior group (N=10). After exposing the thoracic segment of the spine (T8-T10), a laminectomy was performed at the T9 level under microscopic view. The spinal cord injury was made using a 5 mm long aneurysm clip with a closing pressure of 50 grams. Locomotor behavior was evaluated by the Basso, Beattie and Bresnahan scale in days 1, 7, 14, 21, and 28 after surgery. The area of injury was assessed by histological analysis and measured by a software. Results: The histological evaluation showed a larger mean area of 4.8±1mm^2 of lesion (P=0.03) in the lateral group when compared with the posterior group mean area of 2.3±2mm^2. There was no significant difference between lateral and posterior groups with respect to locomotor scores from day 1 to 28 (P=0.361). Conclusion: The lesion area observed in the spinal cord histology after lateral placement of a clip was significantly bigger than in the posterior placement. The motor evaluation showed similar BBB scores regardless of the type of clamping method.

Keywords: Spinal cord injuries; Spinal cord compression; Histology; Motor activity; Rats.

RESUMO

Objetivo: Avaliar o impacto locomotor e histológico da colocação lateral ou posterior do clipe na medula espinal. Método: Estudo experimental randomizado. Vinte ratas Wistar, que pesavam entre 250 e 300 gramas, com 12 a 14 semanas de vida, foram randomizadas em dois grupos com relação à colocação do clipe: grupo lateral (N=10) e grupo posterior (N=10). Após expor o segmento torácico da medula (T8-T10), uma laminectomia foi realizada no nível de T9 sob visão de microscópio. A lesão medular foi realizada com um clipe de aneurisma de 5 mm de comprimento com pressão de fechamento de 50 gramas. O comportamento locomotor foi avaliado pela escala de Basso, Beattie e Bresnahan nos dias 1, 7, 14, 21 e 28 após a cirurgia. A área da lesão foi avaliada por análise histológica e foi medida por um software. Resultados: A avaliação histológica mostrou média maior da área de lesão medular (P=0.03) na utilização da clipagem lateral 4,8±1 em comparação com a posterior (2,3±2). Não houve diferença significativa entre os grupos lateral e posterior com relação ao escore locomotor do dia 1 ao 28 (P=0.361). Conclusão: A área de lesão medular observada na histologia da medula espinal depois de colocação de clipe lateral foi significativamente maior em comparação com a clipagem posterior. A avaliação motora mostrou resultados similares independente do tipo de método de clipagem utilizado.

Descritores: Traumatismos da medula espinal; Compressão da medula espinal; Histologia; Atividade motora; Ratos.

RESUMEN

Objetivo: Evaluar el impacto locomotor e histológico de la colocación lateral y posterior del clip en la médula espinal. Métodos: Ensayo aleatorio experimental. Veinte ratas Wistar, que pesaban entre 250 y 300 gramas, con 12 a 14 semanas de vida fueron asignadas al azar en dos grupos de acuerdo a la colocación del clip: grupo lateral (N=10) y grupo posterior (N=10). Después de exponer el segmento torácico de la médula (T8-T10), se realizó una laminectomía en el nivel de T9 bajo visión microscópica. La lesión medular se realizó mediante un clip de aneurisma con 5 mm de largo y presión de cierre de 50 gramos. El comportamiento locomotor se evaluó mediante la escala de Basso, Beattie y Bresnahan los días 1, 7, 14, 21 y 28 después de la cirugía. El área de la lesión se evaluó mediante el análisis histológico y su medida por un software. Resultados: El estudio histológico mostró un área media mayor de lesión de 4,8±1mm^2 (P=0,03) en el grupo lateral en comparación con el grupo posterior (2,3±2). No hubo diferencia significativa entre los grupos lateral y posterior con respecto a las puntuaciones del aparato locomotor desde el día 1 al 28 (P=0,361). Conclusión: El área de la lesión observada en la histología de la médula espinal después de la colocación lateral de un clip fue significativamente mayor que en la colocación posterior. La evaluación locomotora mostró puntajes BBB similares sin importar el tipo de método de clipado.

Descriptores: Traumatismos de la médula espinal; Compresión de la médula espinal; Histología; Actividad motora; Ratas.

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INTRODUCTION

Spinal cord injury (SCI) research has advanced substantially in the past two decades.[1,2] Animal models, mostly performed on rats, provide one of the best opportunities to test therapeutic strategies after SCI.

Different types of SCI models in rats have been described in the literature: balloon compression, impact drop compression, spinal cord transection and clip compression (CC). The CC technique uses clips that compress the exposed spinal cord, showing a corresponding neurological and histological correlation between the closing pressure of the clips, time of compression, and degree of neurological deficit.[3-8]

The SCI using the CC type can be performed by placing the clip in two directions in relation to the spinal cord: lateral or posterior. Studies published in the literature that applies the CC as a method of SCI usually do not describe the direction of the clip placement. It was assumed that the severity of neurological symptoms and the histological lesion were different when both techniques were compared, due to the anatomical distribution of the pathways in the spinal cord.

In order to apply the knowledge acquired in the laboratory to clinical practice, it is essential to understand the mechanisms of injury and the pathophysiology of the SCI model used in the experiment. The aim of this study is to evaluate the impact on neurological examination and histology when a SCI was performed using lateral or posterior clipping directions.

METHODOLOGY

A randomized experimental trial developed in the Spinal Cord Injury Laboratory of the Universidade de Caxias do Sul in 2012. Twenty female Wistar rats, ranging from 250 to 300 grams, aged 12-14 weeks, were randomized into two groups according to the direction of clip placement: lateral group (N=10) and posterior group (N=10).

All the procedures were approved by the Ethics Committee in Animal Research at the Universidade de Caxias do Sul, under number 004/11.

The animals were suitably packed into special single cages, and stored in a controlled room. The circadian cycle was respected (light between 07:00 AM and 07:00 PM), the temperature was maintained between 23-26 ^{o}C and the humidity between 62-68%. Water and food were given *at libitum,* and hygiene was performed daily by the laboratory technicians.

Antibiotic prophylaxis was performed with an intraperitoneal injection of 0.1mL of ceftraxion. After trichotomy, the skin was cleaned and antisepsis was performed with aqueous chlorhexidine. Anesthetic procedure was performed with Ketamine, and Xylazine was administered intraperitoneously according to the weight.

Surgical Procedure and Spinal Cord Injury Model

After exposing the thoracic segment of the spine (T8-T10), a laminectomy was performed at level T9 under a microscope (Vasconcelos®), leaving the duramater intact. (Figure 1A) The spinal cord injury (SCI) was made with a 5 mm long aneurismal clip with a closing pressure of 50 grams (Vicca, Porto Alegre, Brazil) placed in the spinal cord for 60 seconds. (Figure 1B) The direction of clip placement was lateral (N=10) and posterior (N=10). (Figure 1C, 1D)

After SCI, the spinal cord was irrigated with saline solution, the muscles and skin were sutured by layers, and the animal was placed under a heating lamp to recover.

After surgery, all rats received 10 mL of intraperitoneal saline solution. Analgesia was performed with 0.7mL of subcutaneous tramadol 12/12 hours (0.5mg/mL) for two days. Additional analgesia was performed with 6 drops of Acetaminophen 200mg/ml diluted in 500mL of water *at libitum* if the rat presented signs of pain (prostration, erected fur, anorexia).

Antibiotic therapy was administered, guided by the presence of signs of urinary or wound infection. Animals with signs of infection received 320mg of Amoxicillin -clavulanate diluted in 500ml of water for at least 7 days, or until 24 hours after the symptoms disappeared.

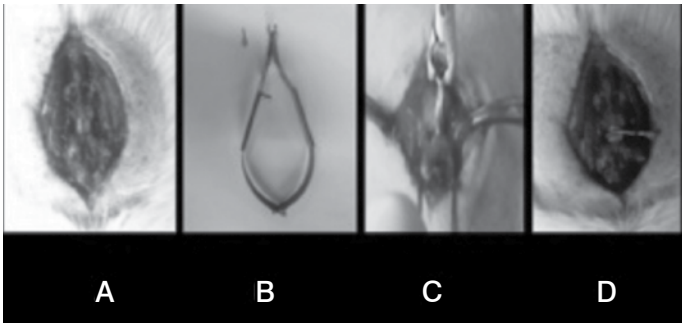

Figure 1. Images of surgical procedure after laminectomy (A). A 5mm long aneurismal clip with a closing pressure of 50 grams (B) was placed on the spinal cord from the posterior (C) or lateral (D) directions.

Due to impaired detrusor function, the bladders of the rats was emptied three times a day by Credé's maneuver. After the partial recovery of the detrusor (2 weeks) the bladders were emptied twice a day.

Locomotor behavior was evaluated by the Basso, Beattie and Bresnahan (BBB) scale[9] at the 1st, 7th, 14th, 21st and 28th day after surgery. Three experienced independent examiners observed the hind limb movements of the rat, and scored the locomotor function ranging from 0 (no movement) to 21 (normal movement). If there was a difference between the scores, the three examiners observed the records of the rat together, obtaining a fourth final score.

Histological Analysis

On the 28th day after surgery, all the rats were sacrificed. The spinal cord was removed and fixed in formaldehyde, and then submitted to morphological and histopathological analysis.

The spinal cord was dehydrated, diaphanized, embedded in paraffin and divided into 4μm slices (Leika – RM 2120 RT) sagittal to the axis of the spinal cord. (Figure 2) Tissue sections were deparaffinized with xylene, stained with hematoxylin/eosin (H&E) and studied using BX43 Olympus microscopy. The images were viewed, captured and measured using the CellSens Dimension program, and the area of lesion was evaluated in cubic millimeters on the sagittal slices (mm^2).

Systematic review

A systematic review was performed in Pubmed and Science Direct, searching for articles from 1995 to 2012 published in English, Portuguese or Spanish. The keywords used were "Spinal cord injury" AND "Rat" AND "Clip". The inclusion criteria were thoracic SCI in rats using CC methodology. The exclusion criteria were: publication before 1995, absence of motor outcome, SCI by a model other than

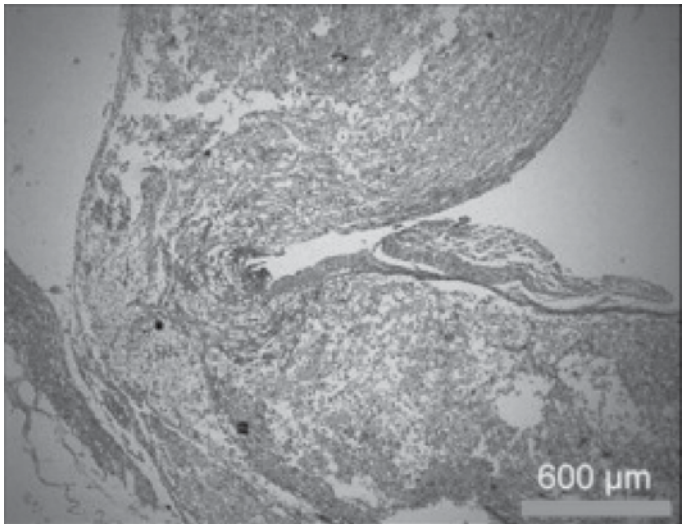

Figure 2. Microscopy view of sagittal image of the spinal cord with the lesion area stained with hematoxylin/eosin.

CC, use of animals other than rats, and SCI performed in segments other than the thoracic level.

The variables studied were age and weight of rats, clipping characteristics such as direction of the clip placement, time of clipping and closure pressure, neurological motor assessment, and histological analysis.

Statistical analysis

The variables included in the study were: weight, locomotor BBB score and area of lesion. All data were stored using Microsoft Excel 2010 (Redmond, WA, USA), and statistical analysis was performed using IBM SPSS 21.0 (Armonk, NY, USA). Parametricity was evaluated using the Shapiro-Wilk test. Variables were described by their means and standard deviation. Area of lesion and weight were analyzed by Student's T test, while BBB scores among the groups were analyzed by ANOVA of repetitive measurements. The level of significance established was $P<0.05$.

RESULTS

The weight of the rats on the day of surgery varied from 221 to 283 grams, with an overall mean of 249.43 grams (Table 1). Comparing the lateral and vertical groups, there was no statistically significant difference in weight ($P=0.87$).

The motor performance of both groups was reduced on the 1st day of BBB, with a mean value of 0.17(±0.49). Comparing motor improvement by the BBB scale from the 1st day to the 28th day, there was a statistically significant improvement ($p<0.001$) in both groups. (Figure 3) There was no overall difference between lateral and posterior BBB scores from days 1 to 28 ($P=0.36$). (Table 1)

Table 1. Weight, BBB scores and Lesion area between the posterior and lateral clip placement groups.

	Posterior	Lateral	P value
Weight at surgery	248.8 (±19.2)	249.9 (±15.2)	0.87¥
BBB Score			0.36£
Day 1	0.2	0.2	
Day 7	2.9	2.3	
Day 14	5.1	3.4	
Day 21	5.9	4.5	
Day 28	7.2	4.7	
Lesion areaΩ	2.3±2	4.8±1	0.03¥

Animal weight- expressed as mean ± standard deviation; BBB- Basso, Beattie and Bresnahan scale (mean); Ω In square micrometers (mm^2); ¥ - Student's T Test; £ - Repeated Measures ANOVA.

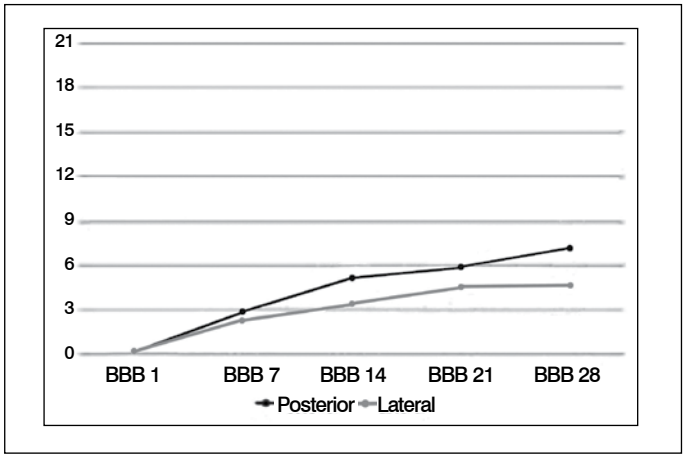

Figure 3. Comparison of locomotor scores between the groups.

Histological Evaluation

The histological evaluation showed a greater lesion area in the lateral group, with a mean of 2.3±2mm^2, when compared with posterior group, with a mean of 4.8±1mm^2 ($P=0.03$). (Table 1)

Systematic Review

The total number of articles retrieved using the keywords determined was 347. (Figure 4) One hundred seventy-eight articles were found in PubMed and 169 in Science Direct. The titles and abstracts of these articles were analyzed, and those that did not discuss the proposed topic were excluded. A total of 15 articles were selected and analyzed in full, according to the exclusion criteria. Five papers did not obey those criteria (1 was published before 1995, 2 did not use the clipping model, 1 did not analyze motor outcome, and 1 was a review article) and therefore were excluded, resulting in the inclusion of 10 articles in this review. (Table 2)

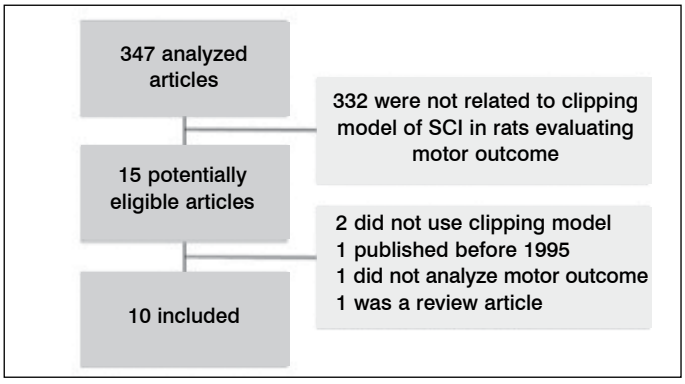

Figure 4. Flowchart of systematic review of the articles.

Table 2. Published articles related to spinal cord injury in rats using clipping models.

Article	Animal		Clip			Injury		
Author	Age at Spinal cord injury (d)	Weight (g)	Guidance Clipping	Clip strength (g)	Time of clipping (s)	Level of spinal cord Lesion	Analysis	Monitoring time (WI)
Gorio *et al.*[13], 2002	NI	180-300	NI	60	60	T3	BBB	4
Gris *et al.*[14], 2004	NI	NI	NI	50 and 35	60	T4/T12	BBB	12
Roussos *et al.*[19],2005	NI	220-280	NI	50	3or5	T9	BBB	7
Poon *et al.*[18], 2007	NI	250-300	Lateral	20,26 and 35	60	T2	BBB	4
Song *et al.*[22], 2009	70-84	160-200	Vertical	10-15	60	T9-T10	BBB	3
Kaminski[25], 2013	60	250	Vertical	50	60	T8	BBB	3
Bao *et al.*[11], 2011	NI	NI	NI	35	60	T4	BBB	8
Hurtado *et al.*[15], 2012	NI	250	NI	35	60	T12	BBB	12
Dayan *et al.*[12], 2012	NI	NI	NI	30	60	T9-T11	BBB	1

d - days; g - grams; s - seconds; WI - weeks after injury.

DISCUSSION

The present study shows that the lateral group had a significantly higher lesion area when compared with the posterior group, although no difference was observed regarding motor recovery.

A better understanding of the SCI mechanism in animal models is crucial to clinical translation. Animal SCI experiments by clip compression is a method used worldwide in neuroscience research, since it reproduces much of the motor impairment found in humans with spinal cord lesion. The present study showed a greater lesion area and more homogeneous BBB scores when the lateral clip placement technique was used, despite the similar motor recovery between the lateral and posterior clip placement.

Most articles published in the last 17 years on experimental SCI using the CC model usually did not describe the direction of clip placement in relation to the spinal cord.[10-21] (Table 2) Among the 10 articles analyzed, the clipping direction was only described in 4, 3 of which performed a posterior clip placement[16,20,22] and 1 a lateral clip placement.[18] The lack of this information is important for adequate analysis and comparison of the results.

The present study shows that the lateral group had a significantly higher lesion area when compared with the posterior group. A possible reason for this finding is that lateral clipping placement blocks blood supply by compressing both the anterior and posterior spinal arteries. On the other hand, posterior clipping may not occlude the anterior spinal cord artery and consequently, the anterior blood supply may not be disturbed, diminishing ischemic consequences.

In the literature, posterior clipping evolves into a lesion with a 2000-2500 μm perimeter[16] and an area of 2.1mm^2.[20] The lesion area in our study was higher than in those, studies due to: (1) the fact that their histological analyses were sagittal and not transverse to the axis of the spinal cord, (2) higher clip pressure, and (3) longer time of clip placement. It is difficult to compare in the literature the value of diameter or area of the spinal lesion obtained by clipping placement, because other variables, such as time of compression and clip pressure, were not homogeneous between the studies.[16,20,22] The volume of lesion in this project using a clip pressure of 50 grams was larger than the perimeter of lesion described with clip pressure of 20, 26 and 35 grams. The present data showed that the clip pressure correlates significantly with the percentage of lesion tissue area.[18]

Usually the BBB score is inversely proportional to the degree of damaged tissue.[18,20] The present study showed a higher lesion area in the lateral group despite the non-statistically results of motor outcome at any day after surgery when a lateral and posterior clip placement group was compared. Among the four articles that described the direction of clipping, only one described the motor outcome values in the results section but was not clearly described by groups, nor by days of analysis.[20] The other three articles have a graphic with the BBB scores, requiring the reader to infer the results.

The motor improvement from day 1 to day 28 observed in the present study after the CC technique using lateral and posterior clip placement highlights the efficiency of CC as an SCI model, the capacity of neural recovery after trauma, and the presence of partial lesion. Spontaneous recovery is also described in the literature.[18,23-25]

CONCLUSION

The lesion area observed in the spinal cord histology after lateral placement of a clip was significantly greater than in the posterior direction of the clip. There was no difference in overall motor performance between the lateral and posterior BBB scores.

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All authors declare no potential conflict of interest concerning this article.

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