QUALITATIVE AND QUANTITATIVE EVALUATION OF RATS' ACUTE INJURIES CAUSED BY ISCHIATIC NERVE SMASHING

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SUMMARY

Rats' ischiatic nerves smashed with different loads were studied with the aid of light microscopy. Weights of 500 g, 1,000 g, 5,000 g, 10,000 g, and 15,000 g were used for 10 minutes in a portable device, especially developed for this study. Morphological and morphometrical analyses of myelinic fibers showed that the injury produced on neural fibers and on neural tissue was directly proportional to the load applied and that a load of 500 g is enough to produce a severe damage, with an important injury of endoneural structures.

Keywords: *Rat, Ischiatic nerve, Smashing, Mechanical load, Morphometrics*

INTRODUCTION

Peripheral nerves injuries are increasingly common in the routine of hospitals' emergency care units due to an increase of urban violence, and traffic, labor-related and domestic accidents [1].Morphological and functional recovery after a nervous injury is seldom complete and perfect, despite of the use of modern and sophisticated treatment techniques. Uncountable factors may influence the regeneration of an injured nervous fiber, such as patient's age, kind of trauma, injury site, denervation time, kind and diameter of affected nervous fibers, the method employed for nervous repair, as well as other individual variables [2].

Most of current knowledge about nerves regeneration has been generated by experimental trials, in which uncountable variables are controlled, assuring results reliability. Thus, in our environment, many experimental studies are being performed, focusing the functional recovery after smashing injuries [3] new alternative techniques for surgical repair[1,4] and the use of physical therapeutic resources (laser, electricity and ultra-sound) that might stimulate nerves morphological regeneration [2,5].

De Medinaceli et al[6] concluded that an injury caused by smashing is a useful modality for the study of peripheral nerves regeneration because it mimics a kind of axonotmesis in which damages are enough to disrupt an axon, leading to a distal Wallerian degeneration, but functional recovery prognosis is good due to preservation of supporting structures, as the satellite cell, basal membrane and supporting connective tissue.

Many authors proposed the use of experimental models for smashing nervous injury to study not only the injury itself, but also regeneration and functional recovery [2,3,5,7,8]. A variety of methods have been used for performing nervous compression in animals, without the required standardization, where each author uses his/ her own method, prevailing haemostatic or other tweezers, which are routinely used in surgical procedures [9]. There are also some more complex devices, such as assays universal machines, among others [2,3,5,7,]. The compression/ smashing application time, as well as the length of an injured segment varies a lot, each author uses his/ her preferences, with no specific logical argumentation to that. The fact is that there was no clear standardization for any of the parameters, which makes very difficult to compare those studies.

Nevertheless, smashing injuries manually produced with the aid of tweezers is not controlled and their severity may vary according to the pressure imposed by the researcher at the moment. This method is not supported in an absolute fashion in literature, since few papers have evaluated the extension of damages caused by those instruments at injury site. This gap motivated the first one, the use of an assay universal machine to produce a controlled injury [2,3] and, secondly, the analysis of a controlled injury, now produced by a dead-load device in order to assess the exact proportionality between load and injury severity, which is still inexistent in literature.

The objective of this study was to categorize, by morphological analysis, and quantify, by morphometric analysis, injuries caused by different smashing loads on rats' ischiatic nerve, using a dead-load device specially designed and manufactured to produce injury, and light microscopy to study it.

MATERIALS AND METHODS

For the purposes of this study, a portable dead-load device was used for smashing rats' ischiatic nerves (Figure 1), which was especially developed as a substitute of the assays universal machine and the spring tweezers used in previous studies and for making smashing process easier, faster and reliable regarding applied load.

Study conducted at the Department of Biomechanics, Medicine and Locomotive Apparatus Rehabilitation, Ribeirão Preto Medical College – USP.

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The research design was approved by the Committee on Ethics in Animal Experiments of the Ribeirão Preto Medical College, University of São Paulo. Twenty five male Wistar rats, weighting 250 - 280 g were used in the experiment. The animals were divided into 5 experimental groups, according to weight used for smashing. The smashing procedure was performed on right ischiatic nerve in all animals, and the left was employed as control. Smashing was performed during 10 minutes, addressing a 5-mm long intermediate segment. In group 1, smashing was performed with a load of 500 g; in group 2, 1,000 g; in group 3, 5,000 grams; in group 4, 10,000 g, and; in group 5, 15,000 g.

Animals were anesthetized with a mix of 10% Ketamin (0.1ml/100 g body weight) and 2% Xylazine (0.07ml/100 g body weight), administered intraperitoneally. The ischiatic nerve was identified and one suture stitch (Mononylon 10-0, Ethicon®), was passed through the epineurium, to identify the site where smashing should be performed, 5 mm below the emergence of the nerve at major ischiatic foramen. Once the nerve was exposed, the animal was positioned on the smashing device, with smashing being performed for 10 minutes (Figure 2). After injury was produced, the animal was removed from smashing device, and surgical wound was sutured and cleaned with antiseptic solution (20% iodinated alcohol), and the animals were placed in appropriate cages for recovery.

For performing histological procedures, all animals were killed 72 hours after the smashing procedure for removing the ischiatic nerves and for subsequent preparation to morphological and morphometrical analyses of the smashed segment. Studied nerves had semi-thin cross sections (05 μm thick) obtained with glass blades through an ultramicrotome MT 6000 – XL (RMC Inc.), transferred to glass slides with one drop of distilled water, dried in heated platinum at 60º and stained with 1% toluidine blue in saturated boric acid; those sections were used for light microscopy studies (Figure 3). Histological procedures employed in this study are the same routinely used for preparing nerves specimens for light microscopy level studies [10]. The segment used in this study was the median segment, with proximal and distal ones being stored as spare material. The semi-thin cross sections of removed nerves' median segments were assessed in a piece of equipment consisting of a light microscope, mounted with a video camera attached to a microcomputer by means of a frame grabber plate. The software used for performing morphometry is the Kontron EletroniK Imaging System KS400, Rel. 2.0.

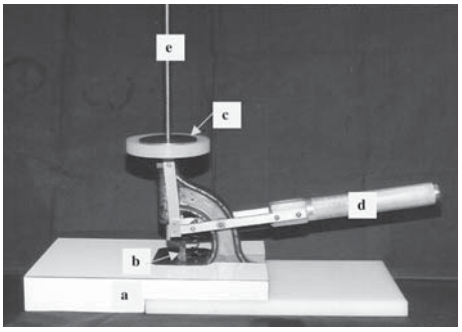

Figure 1 - *Device designed to smash rat's ischiatic nerve. Straight formic base (a); stainless steel base (b); lever (c); gauged plumb masses (d); shaft for attaching gauged masses and nerve compression (e).*

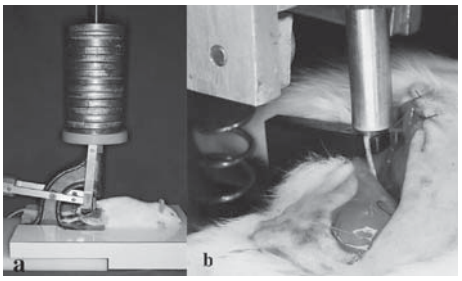

Figure 2 *- Ischiatic nerve smashing on the device. Load applied in a 5-mm long segment, during 10 minutes. (a) animal's positioning overview, (b) detail of ischiatic nerve being smashed.*

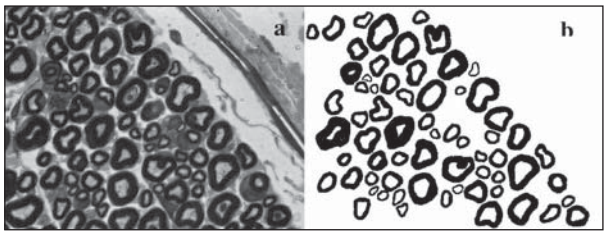

Figure 3 - *Example of microscopic field with 640 x 470 pixels, digitalized on RGB format, from a fascicle of ischiatic nerve. This image was digitalized with an object lens magnification of 100 x with oil immersion, ancillary lens (Optovar) 1.6 x and camera lens 0.5 x. Stain = 1% toluidine blue (a). Binary image (b).*

Digitalized images were observed intending to quantify the presence of hematoma, inflammatory infiltrates, dystrophic axons, foamy cells, intact endoneural vessels, and injured fibers (both small size and large size). Fibers were regarded as injured when presenting evident changes on their myelin sheath, such as the presence of isolated "balls" of myelin, sheath "fractures", very large axons with a very thin sheath.

By the end of the whole morphometric process, the following parameters were assessed in this study: normal and injured myelinic fibers number and density, and, by binarizing myelinic fibers the following parameters could be obtained: area and minimum diameter of myelinic fibers and their related axons, and G ratio.

By using the graphic software Sigma Plot the histograms for fibers and axons frequency distribution and G ratio were generated, which are graphic plots of those structures deployment at studied nerve.

For statistical analysis, the Kolmogorov-Smirnov test was applied to test distribution normality of all available data. Morphometric average data presenting normal distribution were compared to each group by variance analysis for single factor (one way ANOVA), followed by Tukey's post-test. Abnormal distribution data were compared by variance analysis for single factor (one way ANOVA) on Ranks, followed by Dunn's post-test. Histograms for fibers size, axons size and G ratio distribution were compared to each group by means of variance analysis for single factor (one way ANOVA), followed by Tukey's post-test for normal distribution data. For abnormal distribution data, a variance analysis for single factor (one way ANOVA) on Ranks, followed by Dunn's post-test was used. Differences were regarded as significant when $p < 0.05$.

RESULTS

The gross morphological analysis showed that the smashed nerve was visibly larger, with greatly reduced thickness, proportionally to applied load, reaching to the point of leaving it almost translucent. No nerve disrupted during the procedure.

By microscopic morphologic analysis of nerves endoneurium, it was observed the presence of hematoma, inflammatory infiltrate,

Endoneurium	Intact and open vessels	Hematoma	Inflammatory Infiltrate	Injured Fibers		Foamy cells	Axonal Atrophy	Myelin balls	Dystrophic cells
Weight				Small	Large				
500g	+++	++	++	++	+++	+	++	++	+
1,000 g	+++	+	+++	++	+++	+	+++	++	+
5,000 g	++	+	++	+	++	+	++	+	+
10,000 g	+	++	+++	++	+++	++	+++	+++	+
15,000 g	+	+++	+++	+++	+++	+++	++	+++	+

Table 1 - *Morphological evaluation of endoneurium changes by cross system, where one cross (+) means subtle amount, two (++) means moderate, and three (+++) ,means strong.*

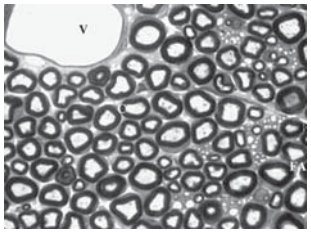

Figure 4 - *image of a control nerve. We can observe the presence of large (A) and small (B) normal fibers, and large-sized fibers showing changes (C), note the presence of capillary vessel at the endoneurium (V) and the amyelinic fiber area (AF).*

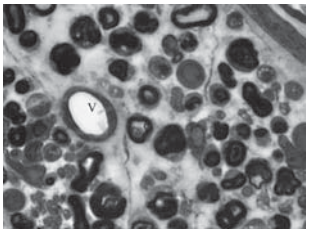

Figure 5 - *Image representing a semi-thin cross section of an ischiatic nerve in smashing group with 500 g, illustrating the presence of intact vessels and inflammatory cells, injury of large-sized myelinic fibers.*

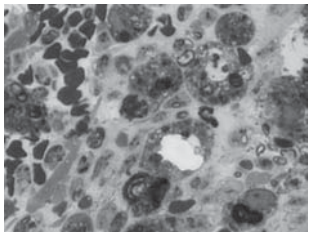

Figure 6 - *Image representing a semi-thin cross section of an ischiatic nerve in smashing group with 1,000 g, illustrating the presence of inflammatory cells, large-sized myelinic fibers injury, and some preserved fibers, presence of hematoma and foamy cells.*

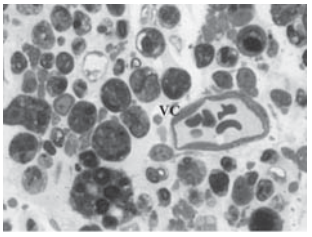

Figure 7 - *Image representing a semi-thin cross section of an ischiatic nerve in smashing group with 5,000 g, illustrating the presence of intact vessels - V and myelin injuries (myelin balls - BM) and inflammatory cells.*

dystrophic axons, foamy cells, intact endoneural vessels, and injured fibers (both small sized and large sized). Those parameters are shown on Table 1. Images for each ischiatic nerve smashing group with related changes can be seen on Figures 4, 5, 6, 7, 8, and 9.

The morphometrical analysis of fascicules was performed, and the morphometry data for the number of normal and injured myelinic fibers in studied groups are shown on Table 2 and Figure 10. Data on normal and injured myelininc fibers density are shown on Table 3 and Figure 11. By comparing the results achieved for number and density of normal and injured myelinic fibers, the statistical analysis showed differences between control group and all groups submitted to smashing ($p < 0.05$). Among the groups submitted to smashing, no difference was seen.

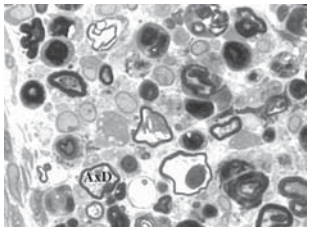

Figure 8 - *Image representing a semi-thin cross section of an ischiatic nerve in smashing group with 10,000 g, illustrating the presence of dystrophic axons- AxD.*

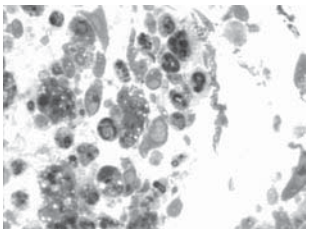

Figure 9 - *Number of normal and injured fibers on Wistar rats' ischiatic nerves according to different loads. Values expressed in average ± MSD. N = number of animals assessed. Load = weight used for smashing. * indicates significant difference compared to control group for the number of injured fibers. .*

Average values for morphometric data of myelinic fibers and myelinized axons (fiber minimum area and diameter, axon minimum area and diameter, and G ratio) are shown on Table 4. By comparing the results achieved for minimum area and diameter of myelinic fibers, the statistical analysis did not show any significant difference between studied groups ($p > 0.05$). By comparing the results achieved for axon area, the statistical analysis did not show any significant difference between studied groups ($p > 0.05$). By comparing the results achieved for minimum axon diameter, the statistical analysis showed a significant difference between control group and the groups smashed with 5000g, 10000g and 15000g ($p < 0.05$). No statistically significant difference was seen when groups smashed with 500g e 1000g were compared to control group, and, also, there was no difference between smashed groups. By comparing the results achieved for G ratio, the statistical analysis showed a significant difference between control group and all smashed groups, and also between groups smashed with 1000g and 15000g ($p < 0.05$).

A statistical analysis of distribution histograms for myelinic fibers diameter of myelinized axons and of frequency distribution for G ratio was performed.

That analysis did not show any significant difference in distribution histograms for minimum diameter of myelinic fibers and their related axons, as well as in the frequency distribution for G ratio among all studied groups, despite evident differences, as shown on distribution graphs below.

When we analyze fibers distribution in all groups combined, we notice that fibers distribution for groups smashed with 500 g and 1,000 g are similar to control, with some distribution deviation to the left. However, when we analyze the distributions for groups smashed with 5,000 g and 10,000 g, those distributions are similar to each other and much deviated to the left, with lost normal bimodal pattern and a large percentage of small-sized fibers. When we analyze the distributions of the groups smashed with 15,000 g, we notice a severe reduction of all classes and a total disarrangement on the

histogram, which differs a lot from all other groups (Figure 12).
Interestingly, by analyzing histograms for axons distribution, all smashed groups are very similar to each other and much different from control. Those histograms are deviated to the left, with a single peak around 1 μm in diameter; that change on histogram pattern is quite suggestive of a strong reduction of axonal diameters (Figure 13).
By analyzing G ratio combined distributions, we notice an enlargement of the basis in all smashed groups, with a strong reduction of the percentage of fibers with 0.6 G ratio (as in control). On the other hand, there was an important increase of G ratio frequencies above and below 0.6, suggesting a large number of fibers showing demyelination and axonal atrophy, respectively. It is also noticeable that the number of fibers presenting axonal atrophy features is higher than the number of fibers with demyelination in smashed groups (Figure 14).

DISCUSSION

Injuries caused by smashing occur relatively frequently under clinical point of view, constituting a reason for studying it deeper than the current details available in medical literature. On the other hand, they are an almost ideal model for experimental use, especially in researches on therapeutic modalities for peripheral nerves injuries in general, because here we don't have the complicating factor of total section followed by suture.
Specialized literature is relatively abundant in publications focusing aspects of peripheral nerves regeneration, using models of smashing injuries. However, there is no smashing injury standard, with each author using different equipment or technique, thus the reproducibility of each method is highly discussible. Chen et al.[7] introduced a controlled smashing injury, using a smashing machine, with which it is possible to fixate the required load for producing injury. The same model was successfully employed by other researchers, in our environment, with a universal assay machine [2,3], and with a controlled-load smashing machine [5]. Nevertheless, after some years using it, it was noticed that the precise injury level was not known, especially employing such different loads as the sequence of 100 g, 500 g and 15,000 g recommended by Chen et al[7]. This was the reason for the present study, of which objective was to qualitatively and quantitatively analyze smashing injuries produced with increasing and strictly controlled loads, using rats as experimental model and histomorphometry techniques.
Rat was the animal selected for the experiment due to its easy handling and to availability as well as to its well recognized use in nervous regeneration studies, and also to the fact that most of existent studies on smashing injuries had been performed in rats as experimental model [1,2,3,5].
Histology, electrophysiology and functional analysis of rats' ischiatic nerve are standard methods to evaluate nervous injuries recovery [1,2,3,5]. We do not have yet in literature a standard method established for inducing or producing a given nervous injury during smashing.
Smashing time was set in 10 minutes, because this is the time that appropriately reproduces what happens in clinical circumstances, such as in car, building, and industrial accidents [3,7]. Also, this length of time has been standardized in our laboratory for all researches [1-3,5]. However, in literature, smashing has ranged from two seconds to 24 hours [9,11-13].
We found numerous factors making the discussion of our results difficult, because literature, although abundant, in general did not addressed the items of our research.
Regarding the load used for inducing smashing, few studies have quantified its application in research. In some studies, the universal assay machine or some other kind of machine has been used, in general, for 10', which can reach up to 10 h of smashing application, and load has ranged from 100g, 500g to 15000g[3,7]. A 15,000-g fixed load was used by Mendonça et al[2], and Monte-Raso et al.[5]. In other studies, a machine was employed to impose a compression of 30 - 80mmHg[11]. Our research used a portable dead-load device for smashing rats' ischiatic nerves, with static load of 500 g, 1,000g, 5,000 g, 10,000 g, and 15,000 g. That device was developed to make smashing process easier, faster and more reliable regarding applied load. Although reproducibility is not reliable, authors

Load	N	Number of normal fibers	Number of Injured fibers
Control	5	2027 ± 294	114 ± 15
500g	5	268 ± 86*	1323 ± 170#
1000g	5	153 ± 32*	1006 ± 169#
5000g	5	285 ± 96*	1602 ± 652#
10000g	5	154 ± 70*	1142 ± 239#
15000g	5	139 ± 72*	1412 ± 366#

Table 2 - *Number of normal and injured fibers on Wistar rats' ischiatic nerves according to different loads. Values expressed in average ' MSD. N = number of animals assessed. Load = weight used for smashing. * indicates significant difference compared to control group for the number of injured fibers.*

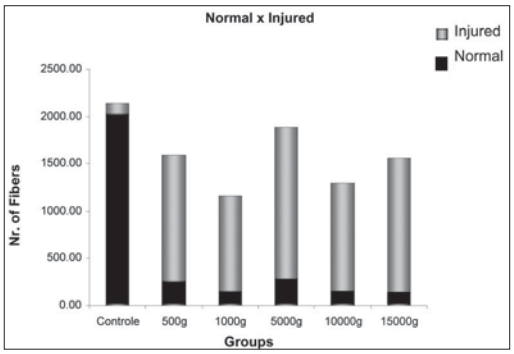

Figure 10 - *Number of normal (blue) and changed (pink) fibers for the different experimental groups. A significant difference was seen between control group and all experimental groups, for both parameters. There was no significant difference between experimental groups.*

Load	N	Normal fibers density (fibers/mm2)	Injured fibers density (fibers/mm2)
Control	5	16426 ± 2102	939 ± 206
500g	5	1529 ± 534*	7531 ± 1645#
1000g	5	1003 ± 78*	6778 ± 1799#
5000g	5	1557 ± 576*	8480 ± 2740#
10000g	5	1062 ± 695*	7306 ± 2231#
15000g	5	493± 321*	5004 ± 2039#

Table 3 - *Normal and injured fibers density on Wistar rats' ischiatic nerves according to different loads. Values expressed as average ± MSD. N = number of animals assessed. Load = weight used for smashing. * indicates significant difference compared to control group for the number o normal fibers; # indicates significant difference compared to control group for the number of injured fibers.*

have produced smashing with the aid of many instruments. Jeweler tweezers nr. 5 have been used by authors such as Kurtoglu et al.13. Other authors also employed tweezers, but did not precisely describe which type [9]. Silk thread was used by Okajima et al.[12].
Even with quantified load, few studies have addressed what kind of injury is produced by each load, intending to standardize the method for inducing nerve smashing, that is, establish a model for nervous smashing injuries. Bridge et al.[8] used jeweler tweezers nr. 5, alternating time and number of times, but not the load, also using knurled haemostatic tweezers, aiming to evaluate and compare the effects of those methods in inducing nervous smashing injury. They observed that despite of the method employed to induce a nervous smashing injury, results were similar to the second injury degree (axonotmesis) with rare factors of a third degree (extraepineural regeneration) in all groups showing similar histology. It seems that in spite of the various methods used to reproduce a nervous injury, the functional and physiological response to smashing action were the same [14]. They developed non-knurled tweezers with pre-determined loads to be applied on the nerve to produce second-degree injuries. In our research, we developed a portable dead-load device for smashing rats' ischiatic nerves, which allowed us to induce a reliable injury, with loads ranging from 500 g, 1,000 g, 5,000 g, 10,000 g, and 15,000 g.
Most studies in literature assessed nerve's histomorphometry two weeks after smashing, which did not enable to compare those results with ours [3,8,15]. In our research, the histomorphometrical evaluation was performed 72 hours after ischiatic nerve smashing.
Some studies in literature addressed the exact site of smashing, but the time for smashed segment analysis does not correspond to our time [8]. Even with the same site for morphometrical analysis, time does not correspond to our study, thus making comparison impossible [15]. Observation of nerve architecture, quality and amount of regenerated nervous fibers, and degree of myelination. The smashing site was also analyzed, but the time for analysis did not correspond to our study.
Some studies in literature addressed the exact site of smashing, with time for smashed segment analysis close to our time [16]. Results on days 1 and 5 for both groups showed the same degree of diffuse edema, degeneration of most axons, and macrophages infiltration [13]. On the second day, a diffuse infiltration of erythrocytes was observed at the epineurium in both groups, myelin degeneration was more prominent in long and medium fibers, small myelinized fibers were protected in both groups. In the present trial, morphological findings ranged from vascular involvement to overt wallerian degeneration, with rupture of isolated axons and related myelin sheaths on loads of up to 1,000 g, rupture of perineural envelope on loads of 5,000 g in only one nerve, of 10,000 g in two nerves and of 15,000 g in five studied nerves. The presence of intact and open vessels was proportionally contrary to the strength of the load applied. The

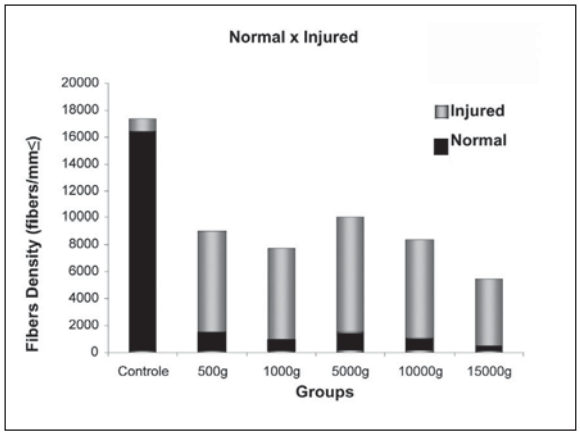

Figure 11 - *Density of normal (blue) and changed (pink) fibers in the different experimental groups. There was a significant difference between control group and experimental groups, for both parameters. There was no significant difference between experimental groups.*

Nerve	Fiber Area (μm^2)	Ø min fiber (μm)	Axon Area (μm^2)	Ø min axon (μm)	G Ratio
Control	24.83 ± 2.71	4.91 ± 0.29	7.39 ± 1.47	2.60 ± 0.29	0.53 ± 0.03
500g	19.98 ± 9.53	3.95 ± 096	4.41 ± 2.49	1.61 ± 0.43	0.42 ± 0.01*
1000g	25.77 ± 17.20	4.23 ± 1.50	7.38 ± 6.44	1.86 ± 0.81	0.44 ± 0.03*#
5000g	17.93 ± 13.11	3.54 ± 1.41	3.80 ± 3.54	1.39 ± 0.64*	0.40 ± 0.02*
10000g	16.05 ± 10.17	334 ± 1.14	3.47 ± 3.23	1.31 ± 0.54*	0.42 ± 0.03*
15000g	24.81 ± 687	4.38 ± 0.69	3.78 ± 1.23	1.49 ± 0.21*	0.37 ± 0.03*#

Table 4 - *Mean morphometric parameters for myelinic fibers of ischiatic nerves of control rats and of those submitted to smashing under different loads. * indicates significant difference of control group compared to other experimental groups. # indicates significant difference between groups.*

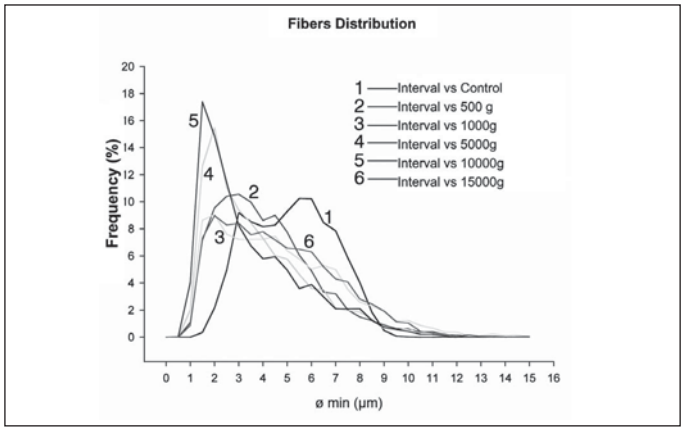

Figure 12 - *Distribution of fibers for all experimental groups.*

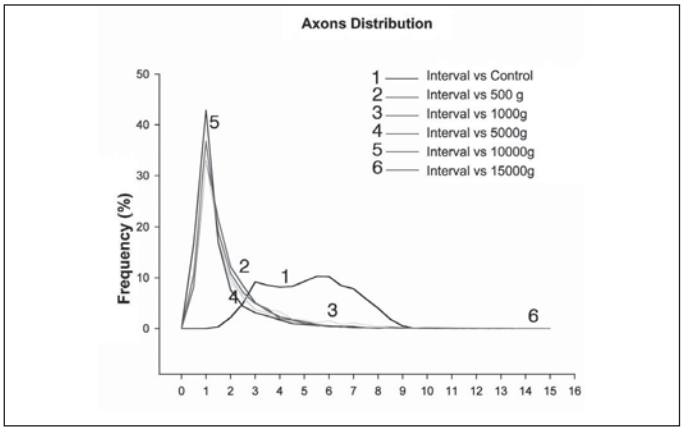

Figure 13 - *Distribution of axons for all experimental groups.*

presence of inflammatory infiltrate, of large injured fibers, axonal atrophy and myelin balls was moderate to intense in all loads applied. Small fibers were strongly injured in the group using 15,000 g for smashing. The presence of foamy cells was stronger on load of 10,000 g and 15,000 g. A subtle presence of dystrophic cells was seen in all smashed groups.

Regarding morphometric findings, this research observed that a higher number of small-sized fibers were spared compared to large-sized ones, as described by Sunderland in 1951, except for the group with 15,000 g, in which both were extremely injured [8], although morphometrical analysis had been performed eight weeks after injury, it was also observed a higher number of small-sized fibers in groups smashed with jeweler tweezers nr. 5. G ratio was significantly higher among all smashed groups when compared to control group, and, also, between groups of 1,000 g and 15,000 g. In the study by Bridge et al[8], G ratio was higher between simple 60-second smashing group (the longest smashing time in the research) and control group. Regarding the number and density of injured and normal fibers, there was no statistical difference, just like the study by Bridge et al[8].

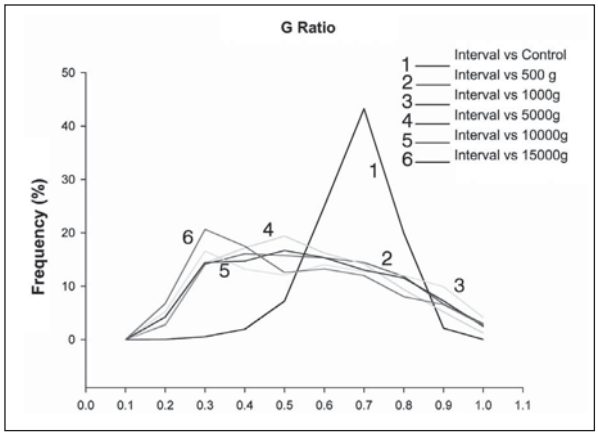

Figure 14 - *G Ratio for all experimental groups.*

Understanding injuries categorization is important to determine treatment for injured patients. Seeddon, in 1943, was the first to categorize nervous injuries into neuropraxis, axonotmesis, and neurotmesis. This classification system was expanded by Sunderland, in 1951, to include two additional injury patterns. A neuropraxis (Sunderland's first degree) involves a local area conveyance blockage, where histological abnormalities may be present, including segmental demyelination with no axonal abnormalities, but recovery is fast and complete. The axonotmesis (Sunderland's second degree) involves axon injury with the presence of Wallerian degeneration distal to injury; recovery is associated to axonal growth at the individual intact endoneural tube. The Sunderland's third degree injury allows for a variable functional recovery, because it is associated not only to axonal injury, but to endoneural scar, which may preclude or inappropriately guide axonal regeneration. In Sunderland's forth degree injury, the nerve, although continuous, has a kind of scar precluding functional regeneration. In neurotmesis (Sunderland's fifth degree) there is a complete trans-section of the nerve without functional recovery. We observed in our study that all loads applied produced neuropraxis and axonotmesis kinds of injuries according to Seddon's classification (1943), and injury severity was dependent on the load used. Thus, the injury produced on a rat's ischiatic nerve by the method employed was mixed. Intending to establish a parallel with Sunderland's classification (1985), all loads applied in this study produced kinds I, II, and III of injury, resulting in a mixed injury; type IV injury was produced with the use of 10,000 and 15,000 g loads. On the other hand, it was observed that the number of fibers featuring axonal atrophy was higher than the number of demyelinated fibers. These data suggest that the most important injury in a smashing model occurs at the axon, characterizing axonotmesis classification.

CONCLUSIONS

1. The amount of injured myelinic fibers on ischiatic nerves of rats submitted to smashing injuries was proportional to the magnitude of applied load, with no significant difference to intact nerves for loads of 100 g and 500 g.
2. There was a prevalence of dystrophic fibers in all groups, except for the 15,000 g group.
3. Large-sized fibers were more affected than the small-sized ones in all groups, except for the 15,000 g group, where both were equally affected.
4. The prevalent kind of injury was axonal atrophy when compared to demyelination.
5. Injuries are mixed, with prevalence of axonotmesis.

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