EXPERIMENTAL INTRANEURAL HEMATOMA MODEL IN RATS: EVALUATION OF FUNCTIONAL RECOVERY AND NEURAL HISTOMORPHOMETRY

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SUMMARY

Emergence of intraneural hematoma with involvement of peripheral nerves can occur after trauma or coagulation disorders. The decision for expectant management or decompressive surgical techniques is still controversial.

Forty male Wistar rats were divided into 4 groups. In group A, an intraneural injection of autologous blood was provided at the right sciatic nerve. In group B, after the hematoma creation, a longitudinal epineurotomy was performed. In the group C (sham-operated), the sciatic nerve was exposed without hematoma. In group D. immediately after the hematoma creation, an interfascicular neurolysis was performed. Nerve function recovery was assessed using the Bain-Mackinnon-Hunter Sciatic Function Index (SFI). At the end of the study, the animals were sacrificed and a specimen of the sciatic nerve at compression midpoint was removed for morphometric analysis.

Group A displayed an initial SFI of –28.43, with full functional recovery on the fifth day. Immediate drainage of the hematoma by longitudinal epineurotomy (group B) promoted recovery of normal sciatic function on the first day (SFI -14.42). Addressing the hematoma via interfascicular neurolysis resulted in an initial SFI of –23.69 and recovery of normal sciatic function on the third day. The morphometric variables indicated an improvement of ischemic parameters following both types of surgical intervention.

Keywords: *Nerve compression syndromes; Peripheral nervous system diseases; Hematoma; Microsurgery; Animal models.*

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INTRODUCTION

Clinical conditions, such as compressive syndromes or limb trauma, that lead to functional or anatomical compromising of the peripheral nerve are quite frequent in medical practice. Surgical approach is essentially based on the kind of trauma - open or closed - and on nervous injury degree. In practical and feasible terms with microsurgery we can find four peroperative situations: nerve integrity; integrity associated to contusion and presence of intraneural hematoma; nervous section, and; section associated to neural substance loss.

In situations associated to extensive contusions, particularly at limbs' closed trauma, sensitive and motor changes can occur on a compromised nerve's territory not necessarily with nervous section. As seen on microscope, fibers may look normal or present with tissue contusion with the presence of intraneural hematoma.

In other cases, especially observed in patients with coagulopathies, that hematoma can emerge spontaneously. When this occurs in little expansible compartments, such as the carpal tunnel, this may determine significant functional loss due to nervous compression. Hematoma formation at the carpal tunnel, either resulting from trauma or associated to coagulopathies, is favored by the persistence of a median artery, which is a branch of the anterior interosseous artery[1]. McCormack[2] identified the persistence of this vessel in 4.43% of the anatomical dissections.

The optimal treatment is not established yet when nerve continuity exists associated to intraneural hematoma. Therefore, we developed an experimental study using sciatic nerves of rats intending to simulate the emergence of an intraneural hematoma. The evolution upon expectant approach or surgical decompression treatment was made by means of Walking Track functional analysis and neural histomorphometry[3,4,5,6,7].

MATERIALS AND METHODS

Forty (40) male Wistar rats weighting, in average 300 ± 30 g were used as experimental model. Anesthesia was induced with sodium pentobarbital (30-50mg / kg of body weight) via intraperitoneal. Trichotomy was made on right gluteus and left inguinal regions.

Surgical Procedure

At ventral decubitus, an incision of approximately 1.5cm on the posterior surface of the right limb was performed. With an operating microscope, gluteus muscles were retracted, exposing the right sciatic nerve from its origin to the first

Study conducted at the Laboratory of Medical Investigation (LIM-4) of the Discipline of Plastic Surgery, Medical College, University of São Paulo (FMUSP)

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subdivision (fibular branch). In group C (CONTROL), surgical procedure was finished upon this step.
In groups A, B and D, with the animal lying on their back, a longitudinal left inguinotomy was performed, exposing femoral vessels. Dissection and catheterization of the left femoral vein were provided followed by aspiration of 0.3 ml of blood. By means of a Butterfly®- modified needle number 27 (9 mm long and 0.4 mm wide externally) the intraneural injection of 0.2 ml of autogenous blood was applied, producing an intraneural hematoma on the right sciatic nerve (Figure 1).
In group A, surgical procedure was finished after the emergence of intraneural hematoma. This group intends to simulate the evolution of the conservative treatment.
In group B, after hematoma emergence, a longitudinal epineurotomy was performed in order to drain the hematoma. This procedure was performed immediately after hematoma emergence.
In group D, after intraneural hematoma emergence, as previously described in other groups, internal neurolysis (interfascicular neurolysis) was performed by microsurgical technique with epineural opening and interfascicular blunt dissection.
Following surgical procedures, muscles and skin were closed by planes.
At procedures completion, animals were kept in separate warm cages until all vital functions were fully reestablished. In the subsequent days, rats received water and food ad libitum.

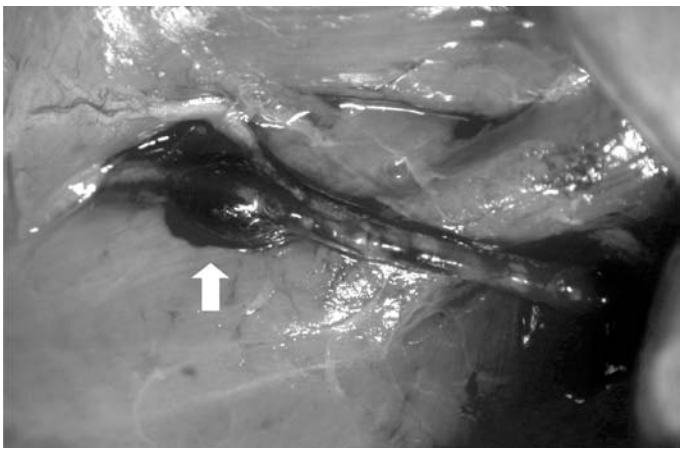
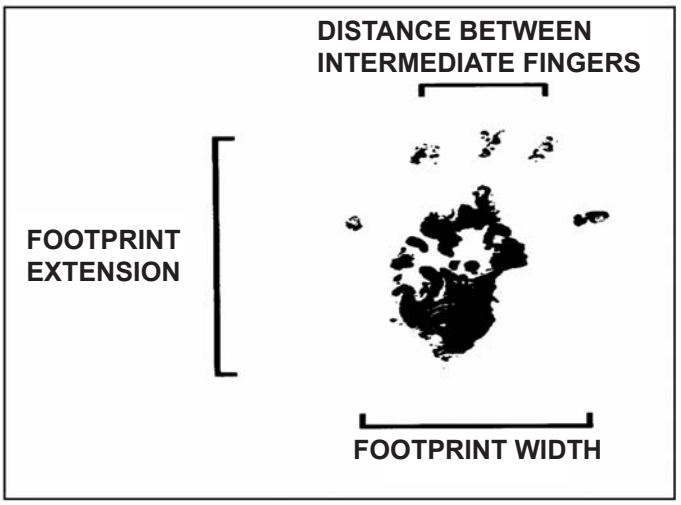


Figure 1 - *Intraneural hematoma on right sciatic nerve*

Nervous function assessment by Walking Track method

Walking Track (WT) is a functional analysis method for nerves, which assesses gait changes resulting from injuries causing limping to the operated paw, by footprint. For nerve function analysis, the Bain-Mackinnon-Hunter's Sciatic Function Index (SFI)[3,4,5,6,7] was used. This index uses footprint extension (distance between the 3rd finger end and calcaneus), footprint width (distance between the 1st and the 5th fingers), and distance between intermediate fingers (2nd and 4th) of the posterior paw (Figure 2). Values obtained by this formula are regarded as a rate of the functional status of the sciatic nerve expressed as functional deficit. Variations from 0 to ± 10% are within normality deviations for the formula, with zero percent representing normal function and -100% representing total function loss. Intermediate values correspond to partial function deficits.



SFI=-38.3(LFE-NFE/NFE)+109.5(LFW-NFW/NFW)+13.3(LIFD-NIFD/NIFD)-8.9
SFI: Sciatic function index
LFE/NFE: Experimental (R) and normal (L) footprint extension
LFW/NFW: Experimental and normal footprint width
LIFD/NIFD: Distance between experimental and normal intermediate fingers

Figure 2 - *Parameters and Bain-Mackinnon-Hunter's formula for calculating the Sciatic Function Index*

WT was obtained by using an aisle of 8.2 x 42 cm with open ends. Rats had their posterior paws (operated and control ones) impregnated with black Indian ink. They were then set free to ambulate over a sheet of white paper with the same dimensions of the aisle mentioned above. When the animals stepped with dorsum, footprints were regarded as "immeasurable".
Footprints were selected for analysis per quality and clearness of their prints, being verified on the operated paw (right) and self-control paw (left). Measurements were always made by only one investigator who was blinded to which measurements belonged to each group.
Measurements were made at the immediate postoperative time, and subsequently at 2-day intervals for each rat during the first week. After that period, periodic assessments were made at every 4 days, and subsequently to the first month, on half-monthly basis up to the 61st day, obtaining one SFI for each day. Values found were applied on the empirical SFI formula.
The initial statistical assessment of the study was made by means of variance analysis (AVOVA) with repeated measurements, having as factors the SFIs for the groups with time. Whenever statistically significant differences were found, we proceeded with Tukey's multiple comparisons. Values of $p < 0.05$ were regarded as statistically significant[8,9].
Neural histomorphometry
At study completion, the animals were sacrificed and a 10-mm segment of the right sciatic nerve was removed for histological analysis.
Nerve fixation was made in 2% glutaraldehyde for 1 hour. After that period, the piece was post-fixated into 2% osmium tetroxide for 2 hours and kept sunk in 1% uranyl for at least 6 hours. When this process was finished, the nerve segment was dehydrated into increasing acetone concentration and included into Araldite®. The slides were 1 μm thick and were stained with 1% methylene blue and Azur II.
The slides were double-blinded evaluated, with a different

slide analysis team from the team responsible for nerve collection. The histological analysis team received the material only with assignment numbers, being unaware of which group the sample belonged to.
The morphometric study was performed by means of a stereologic method (point-counting) blinded to which group the analyzed slide belonged to. A coherent system of 36 points created by the intersection of 12 perpendicular lines was adjusted to a 21" Gradiente® screen. The image was captured by an analogical video camera Sony® connected to an optical microscope Zeiss Axioplan® and transmitted to the monitor screen. Six representative non-coincident fields of each nerve were randomly selected. The following measurements were made: myelinic fibers percentage, myelinic fibers density, Wallerian-degenerating fibers percentage, and Wallerian-degenerating fibers density (Figures 3 and 4).

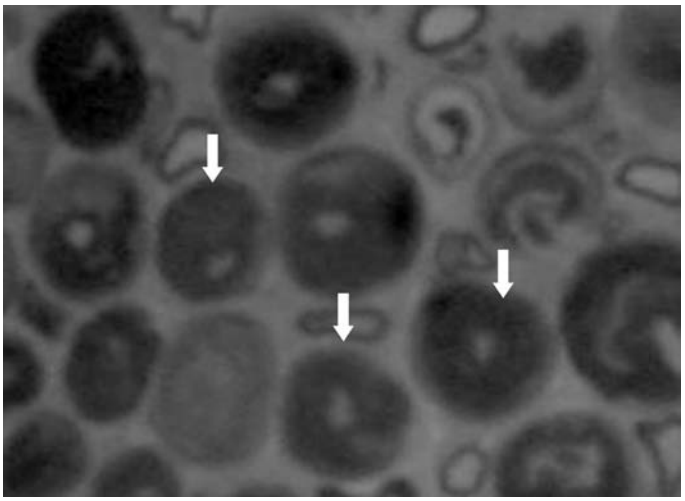


Figure 3 - *Histological slide of the sciatic nerve with uncountable Wallerian-degenerating fibers (GROUP A).*

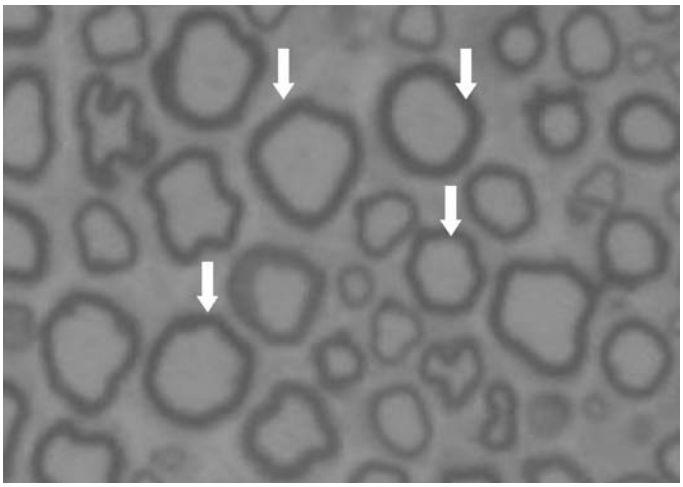


Figure 4 - *Histological slide with strong concentration of myelinic fibers (GROUP C).*

Nerves presenting longitudinally sectioned axonal fibers were excluded from the first analysis. The cases initially excluded from analysis were re-included, and, then, a new slide was made.
We used ANOVA for statistical analysis. P values <0.05 were regarded as statistically significant[8,9]

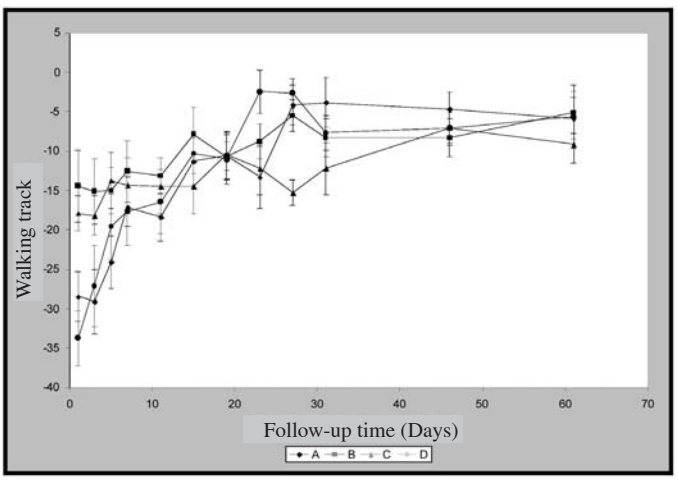
RESULTS

Walking Track

During follow-up, 1 death occurred in the groups A, B and D, and 2 deaths in the group C.
Animals' footprint analysis conducted by means of the SFI showed significant motor functional recovery as a function of time (Table 1). The average value for preoperative SFI was –10.47 ± 7.46. Control group (group C) did not present statistically significant difference compared to preoperative SFI values (Graph 1).

TIME (days)	A		B		C		D	
	SFI	SD	SFI	SD	SFI	SD	SFI	SD
1	-28.43	3.10	-14.42	4.59	-17.88	2.26	-23.69	3.46
3	-29.09	4.12	-15.09	4.13	-18.17	2.50	-17.11	5.12
5	-24.09	3.34	-14.99	2.91	-13.74	3,53	-19.54	4.11
7	-17.14	2.47	-12.54	3.89	-14.34	3.53	-17.60	4.33
11	-18.35	3.00	-13.11	2.21	-14.44	3.55	-16.41	4.09
15	-11.30	3.05	-7.86	3.41	-14.44	3.55	-10.33	2.51
19	-10.60	3.00	-10.66	1.77	-10.48	3.05	-11.03	3.18
23	-13.30	3.90	-8.74	2.18	-12.20	3.25	-2.46	2.77
27	-4.12	2,54	-5.46	2.00	-15.26	1.64	-2.64	1.92
31	-3.83	3.14	-8.28	2.46	-12.20	3.25	-7.67	2.17
46	-4.65	2.13	-8.28	2.46	-7.03	2.20	-7.06	1.93
61	-5.85	2.59	-5.10	2.69	-9.14	2.34	-5.59	4.02

Table 1 - *Mean sciatic function index for each group per day analyzed (A: intraneural hematoma; B: longitudinal epineurotomy; C: Control; D: internal neurolysis).*



Graph 1 - *SFI evolution curve with time (A: intraneural hematoma; B: longitudinal epineurotomy; C: Control; D: internal neurolysis).*

The group with intraneural hematoma (A) presented functional deficit of 28% at baseline (SFI= -28.43±9.8), with sciatic function recovery on the 5th day.
The immediate drainage of this hematoma by longitudinal epineurotomy (group B) determined the return of normal sciatic function since day 1 (SFI= -14.42±13.76). Comparing this group with group A, a statistically significant difference was found only for day 1 (p 0.03).
By approaching the hematoma by interfascicular neurolysis (group D), we found an initial SFI of -23.69±10.95 and return of normal sciatic function as early as day 3. No statistically significant difference was found between this group and groups A and B.

Histomorphometry

The analysis of myelinic fibers percentage and density showed a statistically significant difference between group A and groups B and D, with a higher number being found for these two latter groups.
Regarding Wallerian-degenerating fibers' percentage and density, a statistical difference exists between group A (hematoma) and the other groups. There was no post-hematoma statistical difference between both kinds of treatment employed (B and D) and the CONTROL group.

DISCUSSION

Hematoma formation is favored by the rich vascularization of the peripheral nerve. Through vascular branches originating from mesoneurium, two major vascular plexi are formed: an external plexus predominantly formed by vessels longitudinally arranged at the epineurium and an internal plexus, located between perineurium's lamellas. In variable distances, the vessels present at the perineurium send branches at an oblique angle, which nourish the endoneurium[10,11].
The rupture of the external plexus associated to epineurium integrity can develop an intraneural hypertension situation. In this condition, according to Lundborg[12], intraneural venous pressure and protein exudates are raised, which can be revertible, provided this raise is transitory. However, if such edema remains for long periods, it may determine fibroblasts invasion and, in the long term, an epineural constrictive scar.
The blood-nervous barrier rupture of intrafascicular vessels or internal plexus can cause an endoneural edema[12,13]. In this situation, due to the oblique arrangement of the vessels nourishing the endoneurium, a valve mechanism can occur, closing these vessels and causing more damages to the intrafascicular blood flow. Maintaining an endoneural hypertension picture can trigger hypoxia and, subsequently, tissue necrosis, thus configuring a "miniature compartment syndrome"[14].
Ischemia resulting from the increased intraneural pressure compromises the Na/K pumps and the axoplasmatic transportation system, which depend on ATP to adequately play their role. These systems' integrity is paramount as it allows for nervous impulse to be conducted and the transfer of neurotransmitters and other peptides produced in the cellular body up to axonal ends[10,15].
The presence of blood within intrafascicular space can also lead to an increased number of fibroblasts at the endoneurium, resulting in a considerable intraneural fibrosis degree. Fibrosis is extremely deleterious to peripheral nerve's physiology, leading to a mechanical reduction of the axoplasmatic transport by constriction, and creating an inappropriate exchange interface between capillaries and the axon. Axonal regeneration itself is compromised by this cicatricial barrier[15].
Considering all these mechanisms potentially involved on the physiopathology of the intraneural hematoma, we developed this experimental model comparing, both functionally and histologically, the evolution of surgical treatment against the conservative approach. Clinically, some authors have been described peripheral neuropathy by intraneural hematoma, with decompressive surgical approach by means of internal neurolysis[16,17,18].
Described by Babcock in 1907 apud Terris et al.[19], indications for internal neurolysis was not fully established yet. According to some authors, potential indications for its use in chronic peripheral compressive syndromes would be situations with motor or sensitive deficit and persistent irritation with pain and paresthesia unresolved within a reasonable period of time appropriate clinical and postural treatment[20,21].
In chronic compressive syndromes, a mechanical nerve restraint occurs as a result of intra- and extra-neural fibrosis. Early protein exudation, secondary to ischemia, can course with fibroblasts proliferation, precluding nutrients exchange between neural tissue and microvascular system[20].
In these cases, microsurgical internal neurolysis determines an additional improvement of histological, morphological and electrophysiologic parameters when compared to nervous decompression alone[20].
Nevertheless, internal neurolysis is not a harmless procedure, and may represent, according to Rydevik et al.[21], additional trauma to nervous structure. Intraneural dissection may introduce fibrosis into all nerve layers leading to a functional damage to nervous fiber. Some authors suggest that such procedure would be indicated in those situations where a scar resulting from surgical procedure is less significant than the fibrosis present in chronic compressive syndromes[21,22].
Internal neurolysis can lead to intraneural vascular plexus destruction, although preserving perfusion by endoneural capillaries[21,23]. The risk of causing an injury is directly proportional to the extension of fascicles manipulation[23].
In this experimental model, producing an intraneural hematoma (group A) by means of autogenous blood injection led to damages to nervous function. Initially, it presented a SFI of –28.43, which evolved with normalization of the functional parameters within 5 days. The histomorphometric evaluation identified a lower myelinic fibers percentage and density compared to control and treated groups (B and D), suggesting an ischemic compressive effect of the hematoma. Accordingly, in group A, a higher percentage and density of Wallerian-degenerating fibers was found when compared to the other groups, which also suggests neural damage.
These data corroborate the hypotheses previously mentioned in literature in which situations leading to intraneural pressure increase and to fascicular compression promote changes on nervous conduction and neural ischemia. The mechanism involved on the transitory functional damage would be ischemia, which would lead to damages on axons' energy production, precluding the proper functioning of axoplasmatic transport systems and ATP-dependent Na/K pumps.
Sciatic function recovery in this group is potentially associated to intraneural hematoma re-absorption, thus losing early compressive effect and re-establishing an appropriate blood perfusion.

Draining the hematoma by means of longitudinal epineurotomy (group B) determined a faster return of the normal sciatic function, with a SFI consistent to preoperative values, as early as the first day. The comparison between this group and group A showed a statistically significant difference up to day 5.

The approach with interfascicular neurolysis determined a subtle gain in functional recovery (SFI -23.69), with SFI normalized on the 3rd day. In this study, no statistically significant difference was found between both surgical approaches: epineurotomy and interfascicular neurolysis.

The histomorphometric parameters presented a significant improvement with surgical treatment. Thus, we notice a higher percentage and density of myelinic fibers, and a lower percentage and density of Wallerian-degenerating fibers in surgical groups, suggesting a reversion of the compressive neuropathy and neural ischemia.

However, in a real clinical situation, it would not be possible to perform a surgical procedure in such a short time after an injury. Thus, new experimental models intended to determine the maximum time spam in which a surgical intervention would cause an impact to functional recovery and reversion of the histological parameters associated to neural compression and ischemia.

CONCLUSIONS

The presence of isolated intraneural hematoma causes a significant functional deficit with sciatic function recovery within 5 days.

The immediate drainage of this hematoma with epineurotomy determines a faster recovery, with normal sciatic function as early as the first day.

Histomorphometric parameters point out to an improvement of ischemic parameters after both kinds of surgical intervention applied in this study. There is no difference between both kinds of approaches used in this study.

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