Histopathological analysis of gangliosides use in peripheral nerve regeneration after axonotmesis in rats[1]

Análise histopatológica da regeneração nervosa periférica com uso de gangliosídeos após axonotmese em ratos

Camila Maria Beder Ribeiro[I], Belmiro Cavalcanti do Egito Vasconcelos[II], Joaquim Celestino da Silva Neto[III], Valdemiro Amaro da Silva Júnior[IV], Nancy Gurgel Figueiredo[V]

[I] Master, Department of Oral and Maxillofacial Surgery, Federal University of Pernambuco (UFPE). Fellow PhD degree, Program in Oral Diagnosis Department, Oral Pathology and Oral Semiology Sections, Piracicaba Dental School, State University of Campinas (UNICAMP), São Paulo, Brazil.
[II] PhD, Full Professor, Department of Oral and Maxillofacial Surgery. Director of the PhD Program in Oral and Maxillofacial Surgery, University of Pernambuco, FOP, Camaragibe, Brazil.
[III] PhD, Full Professor, Department of Morphological Sciences, University of Pernambuco, ICB / UPE, Recife, Brazil.
[IV] PhD, Full Professor, Department of Animal Morphology and Physiology, Federal Rural University of Pernambuco, UFRPE, Recife, Brazil.
[V] Dentist, University of Pernambuco, FOP, Camaragibe, Brazil.

ABSTRACT

Purpose: To analyze the action of gangliosides in peripheral nerve regeneration in the sciatic nerve of the rat. **Methods:** The sample was composed of 96 male Wistar rats. The animals were anaesthetized and, after identification of the anaesthesic plane, an incision was made in the posterior region of the thigh, followed by skin and muscle divulsion. The right sciatic nerve was isolated and compressed for 2 minutes. Continuous suture of the skin was performed. The animals were randomly divided into two groups: the experimental group (EG), which received subcutaneous injection of gangliosides, and the control group (CG), which received saline solution (0.9%) to mimic the effects of drug administration. **Results:** No differences were observed between the experimental and control groups evaluated on the eighth day of observation. At 15 and 30 days the EG showed an decrease in Schwann cell activity and an apparent improvement in fibre organization; at 60 days, there was a slight presence of Schwann cells in the endoneural space and the fibres were organized, indicating nerve regeneration. At 15 and 30 days, the level of cell reaction in the CG had diminished, but there were many cells with cytoplasm in activity and in mitosis; at 60 days, hyperplastic Schwann cells and mitotic activity were again observed, as well as nerve regeneration, but to a lesser extent than in the EG. **Conclusion:** The administration of exogenous gangliosides seems to improve nerve regeneration.
Key words: Gangliosides. Nerve Regeneration. Sciatic Nerve. Rats.

RESUMO

Objetivo: Avaliar os efeitos de gangliosídeos na regeneração nervosa periférica em nervo isquiático de ratos após axonotmese. **Métodos:** Foram utilizados 96 ratos machos albinos (Wistar). Os animais foram anestesiados e após constatação do plano anestésico, foi realizada incisão na face posterior da coxa direita do animal. Em seguida, foi realizada a dissecção cirúrgica da pele e do músculo e divulsão dos músculos. O nervo isquiático direito foi isolado e sofreu compressão por 2 minutos. Efetuou-se a sutura contínua da pele. Os animais foram distribuídos aleatoriamente em 2 grupos: experimental (GE) que receberam gangliosídeos pela via sub-cutânea e controle (GC) que receberam soro fisiológico 0,9% com a finalidade de mimetizar os efeitos de administração da droga de estudo. A análise histopatológica foi realizada em 8, 15, 30 e 60 dias. **Resultados:** Não se evidenciaram diferenças significantes entre os grupos controle e experimental avaliados com 8 dias. Nos grupos experimentais de 15 e 30 dias observou-se uma diminuição da atividade das células de Schwann e aparente melhora na organização das fibras nervosas; com 60 dias havia discreta presença células de Schwann no espaço endoneural e as fibras nervosas estavam organizadas sinalizando a regeneração nervosa. Nos grupos controles de 15 e 30 dias o padrão de reação celular diminuiu, entretanto havia muitas células com citoplasmas em atividade e mistose; com 60 dias observou-se ainda a presença de hiperplasia de células de Schwann, atividade mitótica ainda presente e regeneração nervosa presente, porém em menor grau comparando-se com aquele visto no grupo experimental. **Conclusão:** A administração de gangliosídeos exógenos parece incrementar a regeneração nervosa.
Descritores: Gangliosídeos. Regeneração Nervosa. Nervo Isquiático. Ratos.

[1] Research performed at Department of Oral and Maxillofacial Surgery, FOP, Department of Morphological Sciences, ICB, UFPE and Department of Animal Morphology and Physiology, Federal Rural University of Pernambuco (UFRPE), Brazil.

Introduction

Peripheral nerve damage is the most common surgical and traumatic complication in human beings or animals. Such damage may be caused by traumatic, neoplastic or iatrogenic agents. The consequences of a nerve fibre injury depend on its nature, site and intensity[1].

Crushed nerve injury deliberately induced by compression (axonotmesis) is a good methodological design for research on nerve regeneration. Since there is preservation of the crushed nerve, the axons use their own endoneural tubes to migrate towards the distal fragment, thereby enhancing regeneration with little intervention by extraneous factors. The basal laminae of the endoneural tubes resist the trauma, but some of the tubes may rupture, exposing their content, with consequent loss of material and functional alterations. Animal experiments represent quite an important study model as a basis for nerve regeneration, and the sciatic nerve of the rat is the established experimental model in the investigation of peripheral nerve regeneration[2,3,4,5,6,7,8,9]. The choice of this experimental model was further justified by the fact that the sciatic nerve of a rat has a large diameter and is relatively easy to access[10,11,12,13].

Axonotmesis is characterized by blocked nerve conduction and takes place in two phases: the first involves disintegration of the axon and the breakage of the myelin sheath (axonal or Wallerian degeneration) with disruption of the axoplasmatic flow and distal nerve conduction. The second phase, in response to the lesion, is the regeneration process of the segment between the axon and its terminal organ, which is influenced by the condition of the endoneuro. This type of lesion may be caused by heavy bruising, compression of the nerve, or even an excessive stretching, manifesting itself in a complete sensory motor paralysis. Functional nerve repair begins from two to six weeks after surgery[9,14]. Nerve regeneration takes place from the point where the damage was caused, so that the axons proliferate towards the effector organ[15], taking as their guide the Hanken-Bunger bands, composed of Schwann cells. When there is the preservation of the endoneural tubes, the growth cone may easily reach the target organ. However, complete sections not treated with the aid of sutures and located far from the target organ tend to get lost on the way[16].

Compression of peripheral nerves (axonotmesis) may result in neuropathic pain or the sensory deficit syndrome. The acute response is expressed by edema and inflammation. In the first stage blood changes occur in the nerve barrier, resulting in subperineural or endoneural edema. This induces changes in the nerve's connective tissue with a resultant widening of the perineuro and endoneuro, followed by changes originating in the nerve fibre (demyelination). Wallerian degeneration then becomes apparent. The response to injury is inflammation of the nerve, confirmed by the occurrence of neuroinflammatory events, represented by endoneural infiltration of granulocytes and immune cells (CD-4, CD-8 and T lymphocytes) at the site of the nerve injury[17].

Regeneration takes place at a defined pace, and the signs of its occurrence may be expected from the time of the trauma. The time required for axonal regeneration involves a number of considerations including the following: a) it takes approximately two weeks for the regenerating axons to reach the distal nerve at the site of the lesion or suture; b) once on the distal segment, regenerating axons grow at a rate of 1 mm a day; c) there is a further period of weeks or months from the arrival of the nerve fibres at their targets to the maturation of the axons and their receptors, when maximum function is finally achieved[17].

The development of drugs with neuropathic and neurotrophic characteristics that mimic the neuronal growth factor represents a landmark in the drug treatment of nerve injury, for such nerve growth factors present in embryogenesis steer nerve growth in the direction of the target organs[18].

There are reports in the literature of the use of exogenous substances (gangliosides) to enhance peripheral nerve regeneration in the lower animals[8,9,18,19,20,21]. Such substances are involved in the process of development, differentiation and nerve regeneration[22].

In view of the above, the aim of this paper was to evaluate, by means of histopathologic analysis, the effect of exogenous gangliosides on nerve regeneration in the sciatic nerve of rats submitted to axonotmesis.

Methods

This was an experimental laboratory study in which 96 young adult male albino mice were used (Rattus norvegicus of the Muridae family, Wistar laboratory variety), aged 3 months and body weight ranging from 250 to 350 g at the time of surgery, obtained from the animal colony of the Federal Rural University of Pernambuco (UFRPE). The animals were submitted to a quarantine period and confinement for adaptation to the experimental conditions, when they were germ-freed against endo- and ectoparasites (Mebendazol and Ivermectina). Next, they were conditioned in plastic cages, carpeted with Pinus, and given purified water from the public water supply and solid ration (Labina® - Purina Nutrimentos), with a high *ad libitum* protein content (20-27%). They were then kept at the bioterium of the UFRPE at a temperature of 23ºC ± 2ºC in a light-darkness cycle of 12 h at a relative humidity of 70% +/- 5%.

The animals were weighed and submitted to general anaesthesia. The anaesthetics used were Ketamine (Dopalen - Vertbrands® / 60-90 mg/kg) and Xilazine (Anasedan - Vertbrands® / 4-8 mg/kg) in a 1:1 solution in a dose of 0.1 mg/100g intramuscularly in the left thigh. The animals were considered anaesthetized when there was no longer any plantar response after digital stimulation by the operator. The surgery was performed in the following standardized sequence:

1. A linear incision of approximately 15 mm was made using a #15 blade (tegumentary and subcutaneous planes) on the dorsal surface of the animal's right thigh.

2. A blunt dissection was performed using 12-cm Metzembaum curved scissors to separate the tegumentary and subcutaneous planes from the lateral and medial surfaces of the thigh.

3. A blunt dissection was performed using a 12-cm haemostatic tweezer between the femoral biceps and semiten-

dinous muscles to view the sciatic nerve.

4. The right sciatic nerve was isolated and subjected to compression for 2 minutes with a straight 12-cm haemostatic tweezer. The specific site of compression was the intermediate region of the sciatic nerve in its course down the thigh before bifurcating into the tibial and common fibular nerves. The strength used for compression was standardized at the second locking position of the haemostatic tweezer (Figure 1).

5. A cutaneous linear suture of the tegumentary and subcutaneous planes was performed using 5-0 nylon.

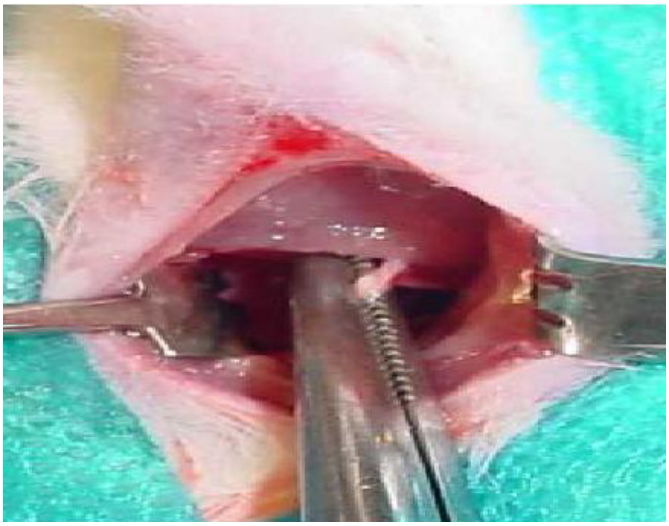

FIGURE 1 – Damage to the sciatic nerve by standard compression

The animals used in this research were randomly distributed in 2 groups – an experimental group (EG) and a control group (CG), with 48 animals each, divided into 8 subgroups (EG1, EG2, EG3, EG4; CG1, CG2, CG3 and CG4), with 12 animals each, in accordance with the evaluation periods, and were assessed postoperatively at 8, 15, 30 and 60 days.

In the animals of the experimental group a ganglioside (Sinaxial®-TRB Pharma) was used. The drug was administered in a dose of 2 mg (0.2 mL) for the attack dose in the first 5 postoperative days and 1 mg (0.1 mL) for the therapeutic dose in the 5 subsequent days, based on the mean body mass of the animals, as recommended by the manufacturer, for the 10 days following the operative act, likewise in accordance with the manufacturer's recommendations. The animals in subgroups CG1, 2, 3 and 4 were subcutaneously injected with 0.2 mL of saline solution (Physiologic Serum® - Darrow) in their backs for five days following surgery and with 0.1 mL of the same physiologic solution for the subsequent five days, with the aim of mimicking the volumetric aspects of the injected fluid and the psychological aspects of the animals in the experimental subgroups.

For the histopathologic analysis, the surgical specimens from the sciatic nerve were removed with minimum trauma approximately 10 mm from the site of the trauma and were fixed in 4% glutaraldehyde, following the sacrifice of the animals. Next, following immersion in historesin, sections were performed and stained with haematoxilin and eosin (HE), which allowed the observation of the hyperplasia of the Schwann cells, Wallerian degeneration, myelin sheath degeneration and formation, endoneural space, nerve regeneration and myelin sheath density postoperatively at 8, 15, 30 and 60 days.

For the analysis of data the statistical mean and standard deviation (techniques of descriptive statistics) were obtained using the Student-t tests with equal or unequal variances. The verification of the hypothesis of equality of variances was conducted by means of the F test for the specific purpose (technique of inferential statistics). The level of significance used in the results of the statistical tests was 5%. The data were typed on the Excel spreadsheet and the software used for obtaining of statistical calculations was the SAS (Statistical Analysis System), version 8.

The present study was submitted to the Ethics in Research Committee of the University of Pernambuco and approved (120/04) on June 15th, 2004, being in conformity with good research practices on animals and the Universal Declaration of Animal Rights[23].

Results

Descriptive histopathology result

Histopathologically, hyperplasia of the Schwann cells and Wallerian degeneration were observed in both groups at 8 days. At 15 and 30 days, in the control group the pattern of cell reaction had decreased, but there were many cells with cytoplasm in activity and cell mitosis; at 60 days, hyperplasia of the Schwann cells, mitotic activity and nerve regeneration were also observed (Figure 2).

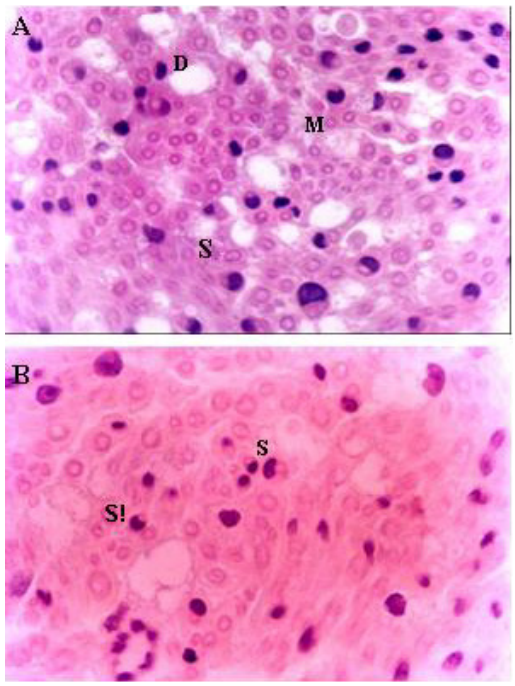

FIGURE 2 – Photomicrograph of the sciatic nerve of the rat in cross-section in the control group (original magnification 100X.). **A:** D – Wallerian Degeneration / M – Macrophage / S – Schwann Cell / S! – Schwann Cell forming myelin sheath. **B:** S – Schwann Cell / S! – Schwann Cell forming myelin sheath

At 15 and 30 days, a decrease in the activity of the Schwann cells was observed in the experimental group, as well as an apparent improvement in the organization of the nerve fibres; at 60 days, there were few Schwann cells in the endoneural space, and the nerve fibres were organized, signaling nerve regeneration (Figure 3).

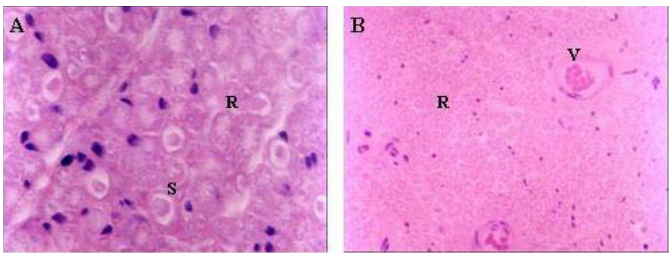

FIGURE 3 – Photomicrograph of the sciatic nerve of the rat in cross-section in the experimental group (original magnification 3A-100X, 3B-40X.). **A:** R – Nerve Regeneration / S – Schwann Cell. **B:** S – R – Nerve Regeneration / S – Schwann Cell./ V – Vessel.

Categorized histopathology result

Tables 1 to 4 show the results of the vacuolization in Schwann cells and macrophages, hyperplasia of Schwann cells, Wallerian degeneration, formation of a myelin sheath and occurrence of nerve regeneration according to group in the evaluations at 8, 15, 30 and 60 days.

In Table 1 it can be seen that in the evaluation at 8 days 25% of the animals in the experimental group showed moderate vacuolization, while the remaining 75% possessed intense vacuolization. In the control group, moderate vacuolization was not observed, although 50% of the animals exhibited intense vacuolization and the other 50% very intense vacuolization.

At the 15 day evaluation, two thirds (66.7%) of the animals in the experimental group exhibited slight vacuolization, while 25% and 8.3% showed moderate and intense vacuolization, respectively. In the control group the corresponding figures were 36.4%, 36.4% and 27.3%, respectively (Table 2).

At 30 days, in the experimental group 54.6% of the animals exhibited slight vacuolization and 45.4% intense vacuolization. In the control group 36% exhibited slight vacuolization, 54.6% moderate vacuolization and 9.1% intense vacuolization. At 60 days, all the animals in the experimental group presented slight vacuolization in the control group; the vacuolization was considered slight in 20% of the animals, moderate in 40% and intense in 40%. At different times of evaluation significant differences were found between the two groups at a level of significance of 5.0% ($p < 0.05$).

TABLE 1 – Evaluation of the vacuolization of Schwann cells and macrophages in control and experimental groups at 8, 15, 30 and 60 days

Time (in days)	Vacuolization	Group						p-Value
		Control		Experimental		Total		
		N	%	N	%	n	%	
□ 8	Moderate	-	-	3	25.0	3	12.5	$p^{(1)} = 0.0071$*
	Intense	6	50.0	9	75.0	15	62.5	
	Very intense	6	50.0	-	-	6	25.0	
TOTAL		12	100.0	12	100.0	24	100.0	
□ 15	Slight	-	-	8	66.7	8	34.8	$p^{(1)} = 0.0017$*
	Moderate	4	36.4	3	25.0	7	30.1	
	Intense	4	36.4	1	8.3	5	21.7	
	Very intense	3	27.3	-	-	3	13.0	
TOTAL (2)		11	100.0	12	100.0	23	100.0	
(2) – For one animal this information was not available.								
□ 30	Slight	4	36.0	6	54.6	10	45.4	$p^{(1)} = 0.0149$*
	Moderate	6	54.6	-	-	6	27.3	
	Intense	1	9.1	5	45.4	6	27.3	
TOTAL (2)		11	100.0	11	100.0	22	100.0	
(2) – For two animals this information was not available.								
□ 60	Slight	2	20.0	12	100.0	14	63.6	$p^{(1)} = 0.0001$*
	Moderate	4	40.0	-	-	4	18.2	
	Intense	4	40.0	-	-	4	18.2	
TOTAL (2)		10	100.0	12	100.0	22	100.0	
(2) – For two animals this information was not available.								

(*) – Significant difference at 5.0% level.

(1) – Using Fisher's Exact test.

In relation to the hyperplasia of the Schwann cells it can be seen in Table 2 that at 8 days 16.7% of the animals in the experimental group had slight hyperplasia and 66.7% exhibited intense hyperplasia. In the control group the hyperplasia of these cells ranged from intense (33.3%) to very intense (66.7%).

At the 15 day evaluation, most of the animals in the experimental group had slight hyperplasia (83.3%), while 8.3% had moderate and 8.3% intense hyperplasia. In the control group moderate hyperplasia was observed in 36.4% of the animals, intense hyperplasia in 27.3% and very intense hyperplasia in 36.4% (Table 3). At 30 days, in the experimental group 81.8% of the cases were classified as having slight hyperplasia and the remaining 18.2% moderate hyperplasia. In the control group, only 9.1% of the animals had slight hyperplasia. On the other hand, 72.7% and 18.2% of the animals had moderate and intense hyperplasia, respectively (Table 2).

At 60 days, in the experimental group slight and moderate hyperplasia was observed in 91.7% and 8.3% of the animals, respectively. In the control group, on the other hand, 40% of the animals had slight hyperplasia and 60% moderate hyperplasia. At the different times of evaluation significant differences were found between the two groups, with a significance level of 5.0%, in relation to the categories of hyperplasia of the Schwann cells ($p < 0.05$) (Table 2).

TABLE 2 – Evaluation of hyperplasia of Schwann cells in control and experimental groups at 8, 15, 30 and 60 days

Time (in days)	Hyperplasia of the Schwann cells	Group Control N	%	Experimental n	%	Total n	%	p-Value
□ 8	Slight	-	-	2	16.7	2	8.3	$p^{(1)} = 0.0014$*
	Moderate	-	-	2	16.7	2	8.3	
	Intense	4	33.3	8	66.7	12	50.0	
	Very intense	8	66.7	-	-	8	33.3	
TOTAL		12	100.0	12	100.0	24	100.0	
□ 15	Slight	-	-	10	83.3	10	43.5	$p^{(1)} < 0.0001$*
	Moderate	4	36.4	1	8.3	5	21.7	
	Intense	3	27.3	1	8.3	4	17.4	
	Very intense	4	36.4	-	-	4	17.4	
TOTAL (2)		11	100.0	12	100.0	23	100.0	
(2) – For one animal this information was not available.								
□ 30	Slight	1	9.1	9	81.8	10	45.6	$p^{(1)} = 0.0032$*
	Moderate	8	72.7	2	18.2	10	45.6	
	Intense	2	18.2	-	-	2	9.1	
TOTAL (2)		11	100.0	11	100.0	22	100.0	
(2) – For two animals this information was not available.								
□ 60	Slight	4	40.0	11	91.7	15	68.2	$p^{(1)} = 0.0201$*
	Moderate	6	60.0	1	8.3	7	31.8	
TOTAL (2)		10	100.0	12	100.0	22	100.0	
(2) – For two animals this information was not available.								

(*) – Significant difference at 5.0% level

(1) – Using Fisher's Exact test

Table 3 shows the results in relation to Wallerian degeneration. At 8 days, both experimental and control groups showed intense degeneration. At 15 and 30 days, however, they exhibited moderate and slight degeneration, respectively. At the 60 day evaluation all the animals in the control group showed a slight Wallerian degeneration, while in the experimental group such degeneration was absent and a significant difference was found for this evaluation ($p < 0.05$). Comparative statistical tests were not applied in the evaluations at 8, 15 and 30 days because all the cases in both groups were classified in a single category.

TABLE 3 – Evaluation of Wallerian degeneration in control and experimental groups at 8, 15, 30 and 60 days

Time (in days)	Wallerian degeneration	Group Control n	%	Experimental N	%	Total n	%	p Value
□ 8	Intense	12	100.0	12	100.0	24	100.0	**
TOTAL		12	100.0	12	100.0	24	100.0	
□ 15	Moderate	11	100.0	12	100.0	23	100.0	**
TOTAL (2)		11	100.0	12	100.0	23	100.0	
(2) – For one animal this information was not available.								
□ 30	Slight	11	100.0	11	100.0	22	100.0	**
TOTAL (2)		11	100.0	11	100.0	22	100.0	
(2) – For two animals this information was not available.								
□ 60	Absent	-	-	12	100.0	12	54.6	$p^{(1)} < 0.0001$*
	Slight	10	100.0	-	-	10	45.4	
TOTAL (2)		10	100.0	12	100.0	22	100.0	
(2) – For two animals this information was not available.								

(*) – Significant difference at 5.0% level

(1) – Using Fisher's Exact Test

Concerning the formation of the myelin sheath, it was seen that at 8 days neither group showed evidence of restructuring of this component of the nervous fibre. In the evaluation at 15 days, 100% of the animals the control group had only a weakly present myelin sheath. In the experimental group, the myelin sheath was only weakly present in 33.3%, while it was moderately present in 41.7% and strongly present in 25% (Table 4).

TABLE 4 – Evaluation of myelin sheath formation in control and experimental groups at 8, 15, 30 and 60 days

Time (in days)	Myelin sheath formation	Group Control n	%	Experimental n	%	Total n	%	p Value
□ 8	Absent	12	100.0	12	100.0	24	100.0	**
TOTAL		12	100.0	12	100.0	24	100.0	
□ 15	Weak presence	11	100.0	4	33.3	15	65.2	$p^{(1)} = 0.0013$*
	Moderate presence	-	-	5	41.7	5	21.7	
	Strong presence	-	-	3	25.0	3	13.0	
TOTAL (2)		11	100.0	12	100.0	23	100.0	
(2) – For one animal this information was not available.								
□ 30	Weak presence	11	100.0	-	-	11	50.0	$p^{(1)} < 0.0001$*
	Strong presence	-	-	11	100.0	11	50.0	
TOTAL (2)		11	100.	11	100.0	22	100.0	
(2) – For two animals this information was not available.								
□ 60	Weak presence	6	60.0	-	-	6	27.3	$p^{(1)} < 0.0001$*
	Moderate presence	4	40.0	-	-	4	18.2	
	Strong presence	-	-	12	100.0	12	54.5	
TOTAL (2)		10	100.0	12	100.0	22	100.0	
(2) – For two animals this information was not available.								

(*) – Significant difference at 5.0% level

(1) – Using Fisher's Exact test

At 30 days, the animals in the control group had 100% of their fibres with the myelin sheath only weakly present, while in the experimental group it was strongly present in the nerve fibres of 100% of the animals (Table 4). At 60 days, in 100% of the animals in the experimental group the myelin sheath was strongly present. In the control group it was only weakly present in 60% of the animals and moderately present in 40%. Significant differences were recorded between the two groups at the 15, 30 and 60 day evaluations ($p < 0.05$).

Nerve regeneration was shown to be absent in the two groups at the 8 and 15 day evaluations. At 30 days, it was found that the animals in the control group displayed no nerve regeneration. On the other hand, 100% of the animals in the experimental group showed moderate regeneration. At 60 days, in the control group 100% of the animals showed moderate nerve regeneration, while in the experimental group intense nerve regeneration was found in 100%. Significant differences were observed ($p < 0.05$) between the groups at the 30 and 60 days evaluations (Table 4).

Discussion

The experimental model adopted in this study was based on an intentional injury to a mixed peripheral nerve for the purpose of causing the total disruption of nerve conduction towards the effector organ. The choice of the sciatic nerve of mice not only met the specified requirements, but was also found to be well established in the literature consulted[2,4,5,6,7,8]. The choice of this experimental model was also favoured by the diameter of the sciatic nerve and by its relatively easy surgical access on the posterior surface of the thigh of the animal in question[10,11]. In our study, after specific technical training, the surgical technique for producing the nerve injury was shown to be feasible and was compatible with the duration of the general anaesthesia.

Peripheral nerve compression represents an experiment delineated with the intention of minimizing interference from local agents. This is corroborated by the fact that it maintains the integrity of most of the basal laminae of the endoneural tubes, thus isolating the content of the tubes in relation to the adjacent tissues, thereby minimizing the effect of biological barriers, which might jeopardize the natural course of axonal growth towards the target organs[6]. The choice of the methodology was also based on studies[12,13] which reported that the most frequent lesions in sciatic nerves of humans are due to neurapraxia or axonotmesis.

Tos *et al.*[16] described the proliferation of Schwann cells, the presence of active macrophages and the fragmentation of the distal segment of the axon, together with the myelin sheath, as peculiar characteristics of nerve injury. Our studies corroborate such physiologic phenomena, for the descriptive histopathology findings at 8 days for both experimental and control groups demonstrated the occurrence of a cellular pattern with the presence of macrophages and Schwann cells with active cytoplasms (vacuolization), a cellular pattern that had receded at the 15, 30 and 60 day evaluations. Tos *et al.*[16] also reported that Schwann cells produce a series of molecules with neurotrophic activity, such as growth factors derived from glia and the brain (NGF and BDNF), and especially growth factors associated with the extracellular matrix, such as the proteoglican-laminine complex. In our study, there was a significant difference between the groups for the proliferation of Schwann cells at 8 days and for regeneration at 30 and 60 days, which suggests neurotrophic activity on the part of the Schwann cells.

Our results corroborate those of Langone and Silva[19] in that we found significant differences between the experimental and control groups, which was indicative of the action of the gangliosides as a drug able to stimulate axonal growth and the maturation of nerve fibres in regeneration. However, Langone and Silva[19] studied the local action of the GM1, GD1a, GD1b and GT1b gangliosides, while our study was based on their systemic action through the subcutaneous route of administration. On the basis of our set of results, there is evidence that the route of administration did not interfere with the combined action of the gangliosides studied.

Other studies[9,20,21] have suggested that the action of the ganglioside GM1 produces a slight increase in peripheral nerve regeneration. Our results were based on the use of a combination of the gangliosides GM1, GD1a, GD1b and GT1b, but we obtained significant findings for several parameters, such as Schwann cells, macrophages, myelin sheath, regeneration, myelin density and ischiatic functional index.

Our results suggest that the action of the gangliosides was proliferative for Schwann cells at 8 days and inhibitory for inflammatory cells at 8 and 15 days. These results are in partial disagreement with other studies[9, 18], in which the experiments covered periods of more than 45 days. At 60 days, our results were no longer significant for Schwann cells or macrophages; however, we found a larger quantity of mature nerve fibres in the experimental group than in the control group which, for its part, contained a greater density of myelin in the process of formation. In our opinion, factors such as the evaluation period, study of the nerve longitudinally rather than in cross-section and the immersion of the nerve segment in paraffin instead of historesin, may have affected the results of the abovementioned authors.

On the basis of the results obtained and the literature consulted[9,18,19,20,21], we suggest that further studies be conduced with a view to deepening our understanding of the action of the gangliosides in peripheral nerve regeneration, bearing in mind the gaps in our knowledge that still exist regarding this question. To this end, we suggest an investigation of the action of gangliosides over periods of less than 8 days and greater than 60 days, a comparison of the use of Gm1 with the combination of GM1, GD1a, GD1b and GT1b, haematological studies for the characterization of a possible modulating action of the immune system, pharmacological studies with the aim of observing a possible modulating action of inflammation, and a toxicological study for the purpose of establishing safe doses for time periods greater than 15 days.

Conclusion

The administration of exogenous gangliosides seems to increase nerve regeneration, for it stimulated the proliferation of the Schwann cells and reduced the presence of inflammatory ones, thereby promoting nerve regeneration after axonotmesis.

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Correspondence:
Prof. Dr. Belmiro Cavalcanti do Egito Vasconcelos
Av. Gal. Newton Cavalcanti, 1650
54753-220 Camaragibe – PE Brazil
Phone: (55 81)3458-2867
belmiro@pq.cnpq.br

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