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Original article

Evaluation of grip strength in normal and obese Wistar rats submitted to swimming with overload after median nerve compression☆

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ABSTRACT

Objective: To verify the functionality through muscle grip strength in animals with obesity induced by monosodium glutamate (MSG) and in control animals, which suffered compression of the right median nerve, and treated with swimming with overload.
Methods: During the first five days of life, neonatal Wistar rats received subcutaneous injections of MSG. The control group received a hypertonic saline solution. Forty-eight rats were divided into six groups: G1 (control); G2 (control + injury); G3 (control + injury + swimming); G4 (obese); G5 (obese + injury); and G6 (obese + injury + swimming). The animals in groups G2, G3, G5 and G6 were submitted to compression of the median nerve and G3 and G6 groups were treated, after injury, with swimming exercise with load for three weeks. The swimming exercise had a progressive duration, according to the week, of 20, 30 and 40 min. Muscle strength was assessed using a grip strength meter preoperatively and on the 3rd, 7th, 14th and 21st days after surgery. The results were expressed and analyzed using descriptive and inferential statistics.
Results: When the grip strength was compared among assessments regardless of group, in the second assessment the animals exhibited lower grip strength. G1 and G4 groups had greater grip strength, compared to G2, G3, G4 and G6.
Conclusion: The swimming exercise with overload has not been effective in promoting improvement in muscle grip strength after compression injury of the right median nerve in control and in obese-MSG rats.

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Avaliação da força de preensão em ratos Wistar, normais e obesos, submetidos à natação com sobrecarga após compressão do nervo mediano

Palavras-chave:
Força muscular
Compressão nervosa
Obesidade
Natação

RESUMO

Objetivo: Verificar a funcionalidade por meio da força muscular de preensão em animais com obesidade induzida por glutamato monossódico (MSG) e animais controle, que sofreram compressão do nervo mediano direito, tendo como tratamento a natação com carga.
Métodos: Ratos Wistar neonatos durante os primeiros cinco dias de vida receberam injeções subcutâneas de MSG. O grupo controle recebeu solução salina hiperosmótica. Quarenta e oito ratos foram divididos em seis grupos: G1(controle); G2 (controle com lesão); G3 (controle com lesão + natação); G4 (obesos); G5 (obesos com lesão); G6 (obesos com lesão + natação). Os animais dos grupos G2, G3, G5 e G6 foram submetidos à compressão do nervo mediano e os dos grupos G3 e G6 foram tratados, após a lesão, com exercício de natação com carga durante três semanas. A natação teve duração progressiva conforme as semanas, de 20, 30 e 40 minutos. A força muscular foi avaliada por meio de um medidor de força de preensão no pré-operatório, no terceiro, sétimo, 14° e 21° dia pós-operatório. Os resultados foram expressos e analisados por estatística descritiva e inferencial.
Resultados: Quando comparada a força de preensão entre as avaliações, indiferentemente de grupos, na segunda avaliação os animais apresentaram menor força de preensão. Os grupos G1 e G4 apresentaram força de preensão maior, em comparação com os grupos G2, G3, G4 e G6.
Conclusão: O exercício de natação com sobrecarga não foi eficaz em promover melhoria na força muscular de preensão após lesão de compressão do nervo mediano direito em ratos controle e obesos-MSG.

Introduction

Peripheral nerve lesions are commonly encountered in the clinical practice of physiotherapy, especially traumatic injuries such as crushing, compression or stretching, resulting in functional impairment, caused by the interruption in the proper transmission of nerve impulse.[1,2] The interruption of the nerve supply leads to a decreased muscle activity, causing muscular atrophy, and the main effect of this atrophy is the reduction of the area and diameter of the muscle fiber and consequent reduction in its strength.[1] Axons of injured peripheral nerves have the capacity to regenerate; however, this process is slow and the functional recovery is usually not complete.[3] Studies involving disorders in peripheral nerves and obesity can be found in the literature;[4,5] however, the approach of conservative treatment for peripheral nerve injury in obese subjects is still scarce.

Physical therapy seeks to repair the consequences of peripheral nerve injury, restoring functionality to the individual. The treatment can be performed by various therapeutic approaches, such as passive and active cinesiotherapy, electrotherapy, functional skills training, specific proprioceptive neuromuscular facilitation techniques and therapeutic exercise.

Animal studies demonstrate the efficacy of exercise on peripheral nerve regeneration.[6,7] The exercise practice promotes recovery of contractile and metabolic properties of muscle after denervation,[8] helps removing degenerated myelin and subsequent synthesis,[9] aids in axonal diameter recovery[10] and axonal sprouting, favors the regeneration of injured nerves and functional recovery[11] and also increases the expression of nerve growth factors such as BDNF and NGF, stimulating the growth and development of new cells.[12] The physiological effects of exercise in the aquatic environment provide benefits to the cardiovascular, skeletal, muscular and nervous systems, increasing the tissue repair process.[13] However, Oliveira et al.,[10] despite observing improvements in axonal diameter, report that the swimming practice did not affect the maturation of regenerated nerve fibers or their functionality, and when associated with electrical stimulation, delayed functional recovery. These findings disagree with what was observed by Teodori et al.,[7] who observed a significant effect of swimming exercise, with acceleration of nerve regeneration in post-axonotmesis of sciatic nerves of rats.

One way to evaluate the functionality of the individual is by the measurement of muscle strength, which enables a functional diagnosis by an evaluation of improvement or worsening during treatment, and as a predictive or prognostic measure.[14] In this context, the aim of this study was to assess the muscle grip strength in MSG-obese and in control animals, which suffered compression of the right median nerve and underwent swimming with load.

Materials and methods

Characterization of the study and sample

This is an experimental research approved by the Ethics Committee on Animal Experimentation and Practical

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Neonatal Wistar rats during the first five days of age received subcutaneous injections of monosodium glutamate (MSG) in a concentration of 4 g/kg body weight/day, forming the obese group. The control group received a hyperosmotic saline solution at a concentration of 1.25 g/kg body weight/day.[15] The animals were kept in a light/dark photoperiod of 12 h and at a temperature of 23 ± 2 °C, with food and water ad libitum.

At 68 days of life, 48 rats were divided into six experimental groups: G1 (control); G2 (control + injury); G3 (control + injury + swimming); G4 (MSG); G5 (MSG + injury); and G6 (MSG + injury + swimming). At 73 ± 4 days of life, the animals in groups G2, G3, G5 and G6 underwent surgery for median nerve compression.

Nerve compression

The compression of the right median nerve was based on the model presented by Chen et al.,[16] with nerve tie-down using 4.0 chromic catgut in 4 points, with an approximate distance of 1 mm in the median nerve, proximal to the elbow. To perform the surgical procedure for compression of the median nerve, the animals were anesthetized with ketamine hydrochloride solution (50 mg/kg) and xylazine (10 mg/kg).

Swimming

Five days prior to the surgery, the animals were adapted and trained in a gradual manner to swim, wherein in the first three days the rats swam for 15 min with an overload of 5% of body weight; in the following two days, they swam 20 min with an overload of 10% of body weight. The swimming exercise was held in an oval tank made of strong plastic material (200 l capacity, 60 cm deep) and containing water maintained at a controlled temperature of 32 ± 1 °C. The treatment began on the third day postoperatively; the exercises were performed once a day, five times a week, totaling 15 days of swimming. The animals were weighed every day for weight control and adjustment of their loads for the swimming exercise. In the first week, G3 and G6 groups started with 20 min of exercise; during the second week, 30 min; and in the third week, 40 min. In all practices, the animals supported a load of 10% of body weight. The other groups of animals were placed in water for 1 min.

Muscle strength

For muscle strength assessment, one grip strength meter described by Bertelli and Mira[17] was used. This assessment is a useful tool for analyzing the recovery of median nerve lesions, through the function of the flexor digitorum muscle. To perform the evaluation, the animal was pulled by the tail with increasing force. The rat could seize a grid attached to a force transducer, till the animal lost its grip. The anterior left limb was temporarily immobilized by wrapping with tape. Five days prior to surgery, the animals were adapted and trained on the equipment. The first evaluation (AV1) was performed before the compression of the median nerve, to obtain baseline values, i.e., preoperatively, followed by a second assessment on the 3rd postoperative day (AV2). The other assessments were carried out at the end of each week of treatment, i.e., the 7th (AV3), 14th (AV4) and 21st (AV5) day, with the aim to observe the evolution of the lesion and the type of treatment used. In each evaluation the test was repeated three times, and the mean value of repetitions was used.

Statistical analysis

The results were expressed and analyzed using descriptive and inferential statistics. To compare groups and times, one-way ANOVA with Tukey post-test was used, with a significance level of 5%.

Results

Both G1 and G4 showed no intragroup variations ($P > 0.05$), which was expected, since in these groups injuries were not inflicted. For the other groups, the values of AV1 were superior to the other assessments ($P < 0.05$), whereas G2 and G6 showed significant increases in AV4, when compared to AV2 ($P < 0.05$); the same was observed for G2, G3 and G6, when comparing AV5 versus AV2 ($P < 0.05$) (Table 1).

As for the comparison among groups, in AV1 (the pre-injury time) there were no significant differences ($P > 0.05$). However, from AV2 to AV5, differences between G1 versus G2, G3, G5 and G6 ($P < 0.05$) groups were observed, occurring the same with respect to G4 (Table 1), i.e., the values found revealed that the lesion reduced the grip strength, when comparing control groups (G1 and G4) with those which only underwent injury (G2 and G5), or those which underwent injury associated with swimming (G3 and G6). No significant differences were noted among groups of obese animals compared to eutrophic animals.

Discussion

Peripheral nerve injuries are responsible for high morbidity and functional loss, requiring therapeutic interventions[18] to assist in the morphological and functional tissue repair, hence the importance of controlled experiments to evaluate the effectiveness of therapies. Most of the experimental studies in rats are carried out on models of sciatic nerve injury/compression. However, the most common injuries in humans occur in the upper limbs.[19] Thus, in the present study, we sought to find a model that could produce a compressive lesion in the median nerve. The nerve compression model used was presented by Bennett and Xie[20] for compressing the sciatic nerve; subsequently, the procedure was modified for the median nerve by Chen et al.,[16] which causes, besides hypernociception, muscle activity dysfunction. These changes begin from the 2nd day postoperatively (PO), reaching its maximum around the 10th to 14th PO day, disappearing after the 2nd month. Thus, in the present study, we began the treatment with swimming exercise in the 3rd PO, during which the changes arising from nerve compression had already been established. It was realized that the aquatic exercise could be important in recovering from paresis, both by decreasing the

Table 1 – Values found for handgrip of Wistar rats (mean and standard deviation), in grams, for different time points (AV1–AV5) in the different groups (G1–G6).

	AV1	AV2	AV3	AV4	AV5
G1	265.8 ± 106.3	330.9 ± 87.6	327.8 ± 99.6	303.7 ± 124.3	332.1 ± 121.3
G2	284.6 ± 60.2	32.1 ± 19.6[a,b,c]	83.1 ± 48.2[a,b,c]	95.4 ± 26.7[a,b,c,d]	103.5 ± 21.1[a,b,c,d]
G3	329.8 ± 113.9	39.5 ± 26.1[a,b,c]	79.6 ± 31.3[a,b,c]	93.3 ± 25.5[a,b,c]	124.0 ± 46.9[a,b,c,d]
G4	212.9 ± 55.3	270.5 ± 54.7	277.8 ± 79.6	268.3 ± 88.8	294.3 ± 127.6
G5	247.5 ± 72.9	34.0 ± 27.8[a,b,c]	62.0 ± 49.3[a,b,c]	74.5 ± 18.9[a,b,c]	83.7 ± 33.5[a,b,c]
G6	226.6 ± 49.2	42.7 ± 35.3[a,b,c]	81.1 ± 26.1[a,b,c]	109.4 ± 42.4[a,b,c,d]	107.3 ± 57.5[a,b,c,d]

[a] Significant difference when comparing with G1.
[b] Significant difference when comparing with G4.
[c] Significant difference when comparing with AV1, within the same group.
[d] Significant difference when comparing with AV2, within the same group.

protein degradation[21] and by functioning as a possible analgesia mediated by endogenous opioids,[22] which could reduce the immobility of the limb due to pain.

When a peripheral nerve suffers compression (inducing local ischemia), some electrophysiological nerve conduction change occurs,[23] leading to muscle weakness.[24,25] The results show that, at the first assessment (when the nerve compression had not yet been performed), the animals showed a significantly higher strength compared with the other results – which is consistent with normal standards. A reduction in muscle strength may be related to the hypernociception generated by nerve compression, producing muscle inhibition.[26] Silva et al.[27] warned that, after the surgical compression of the median nerve, a painful condition settles down, lasting at least until the 8th postoperative day, not decreasing in its intensity.

The results also revealed that there was no increase in grip strength in injured/swimming-treated animals versus injured/sedentary animals, suggesting that the swimming exercise was not efficient to produce an increase in muscle strength, after the injury by compression of the median nerve. In a study by Possamai, Siepko and Andrew,[28] 40 Wistar rats were functional and histologically evaluated, being divided into four groups according to the day the treatment would begin after an axonotmesis-type sciatic nerve injury. The animals were submitted to freestyle swimming for 30 min/day. The results show that there was no interference of physical exercise on peripheral nerve regeneration. Additionally, the treated groups showed no histological changes compared to sedentary rats. Accordingly, Oliveira et al.[10] noted that daily swimming exercise for 30 min, 5 times a week for 22 days, was ineffective with respect to nerve recovery in rats subjected to axonotmesis; when this was combined with electrical stimulation, the functional recovery was delayed. On the other hand, Teodori et al.,[7] evaluating functional and morphological characteristics of rats with sciatic axonotmesis, found that 30 min/day of swimming for two weeks accelerated nerve regeneration. Thus, a disagreement regarding the effects of exercise on aquatic recovery from nerve injury becomes evident.

This divergence is also found in the case of exercise outside the aquatic environment. Sobral et al.[29] performed histomorphometric and functional analyses to evaluate the influence of exercise on a treadmill, applied in early and late stages of sciatic nerve regeneration in rats following axonotmesis. The authors concluded that the treadmill exercise protocol applied to the immediate and late phases did not influence axonal sprouting, degree of maturation of the regenerated fibers, nor the functionality of the reinnervated muscles. Conversely, Seo et al.[11] reported that 30 min of walking on a treadmill between the 3rd and 14th day post-injury and with a speed of 18 m/min, played an important role in axonal regeneration. Ilha et al.[6] reported that, after two weeks of compression of the sciatic nerve in rats, the animals performed exercises during five weeks, namely: progressive treadmill exercises (about 9 m/min) in the first week and, in the remaining four weeks, 60 min/day. These authors noted improvement in nerve regeneration. However, animals that performed climbing-stair exercises (training against resistance) with body overload, with or without swimming, exhibited a delayed functional recovery.

The obesity model used in this study was the neonatal administration of MSG. Animals exposed to this substance undergo a neural reorganization that is reflected in a new metabolic structure, which predisposes to obesity in adulthood,[30] and that also causes changes in the animal by application of MSG, such as a reduction in lean body mass.[31] These animals have lower levels of growth hormone (GH), hence lower body weight and length, but with increased fat deposition.[15] In the results found at the end of the experiment, despite the MSG animals have presented lower means for grip strength, there were no significant differences in control and obese-MSG animals (independent of having been injured/treated, or not) with swimming – a fact that may be related to the nociception due to injury.

Because of the discrepancy in the literature regarding the optimal exercise time and about what is the better stage to start the practice, it is believed that swimming has been ineffective with respect to increasing grip strength as a result of some of these parameters, considering that the exercises were implemented in the immediate phase after injury, with progressive duration along the weeks. Additionally, an excessive load may have been generated in animals which swam, by virtue of the load of 10% of their body weight during the treatment – a factor identified as an important cause of delay in nerve regeneration.[32] We wish to emphasize, as a limitation of the present study, the absence of correlations with morphological findings of the median nerve and flexor carpi radialis muscle. This issue is suggested as a topic for future studies. Additionally, we also postponed the onset

of physical exercise, with different protocols (i.e., with the addition of different overloads).

Conclusion

The swimming exercise with overload has not been effective in promoting improvement in muscle grip strength after compression injury of the right median nerve in control and obese-MSG rats.

Conflicts of interest

The authors declare no conflicts of interest.

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