



Creation of a contusion injury method for skeletal muscle in rats with differing impacts¹

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Abstract

Purpose: To realize a morphological examination of the musculoskeletal tissue, assessing the effect of a contusion method for the production in rat gastrocnemius, comparing the inflammatory responses generated by different impacts.

Methods: For the analysis of a contusion method, twelve female Wistar rats were distributed into four groups. The lesion was generated by 324 g of mass that was dropped from different predetermined heights for each group (30, 45, 60 and 70 cm).

Results: In the analysis of musculoskeletal tissue, the response to injury varied according to the mass of the height drop onto the muscle. Only the group that was injured from 70 cm responded with uniform and severe inflammation, whereas the groups 30, 45 and 60 cm showed inflammation in some regions of the tissue with mild and moderate infiltrates.

Conclusion: The method with the 324-gram mass dropped from a 70-cm height onto the gastrocnemius muscle of rats seems to be the most suitable for the production of muscle injury in these animals after 72 hours, showing an important inflammatory infiltrate.

Key words: Inflammation. Muscle, Skeletal. Contusions. Rats.

■ Introduction

Mechanical injuries are the most common type of skeletal muscle injuries, and are classified into one of the following areas: strain, laceration and contusion¹. Muscular injuries have broad implications among professional athletes, standing out as approximately 30% of injuries sustained in sports that are mostly caused by mechanical trauma². The lesions generated by shear loads are the most frequent and can result in contusion, sprains or lacerations. The variation occurs according to the mechanism of trauma, occurring most often in the lower limbs^{3,4}.

Muscle injury accounts for approximately 90% of sports injuries, and occurs when a muscle is damaged by a significant compressive force generating high energy impact, which rapidly compresses local tissues, such as a direct knock. That is a common mechanism in contact sports^{1,5}. In order to replicate the musculoskeletal injuries resulting from trauma, especially to then test the effect of a drug, some production models of animal lesion are proposed in the literature. However, the severity of the lesion generated by these devices can vary in each study due to the mechanism used to produce the trauma⁶.

The injury of production models seek to simulate the mechanism that occurs in practice, particularly in contact sports⁷. Some models using a falling mass onto the animal muscle have been reported in the literature, in which a density slides to directly contact the animal's muscle - usually being the gastrocnemius the most widely used muscle to these assessments⁸⁻¹².

This technique replicates the original injury mechanism, often seen in clinical practice and that has also been used in studies to assess acute inflammatory lesion and trials testing drugs and treatments in this type of lesion^{13,14}. However, there is still no consensus in the

literature about the mechanism of injury, and different studies show a relative discrepancy about the fillers used and the heights mass drop onto the limbs. Thus, inflammatory responses developed in animals vary depending on the impact of the force generated in the muscles⁶.

The aim of this study was to develop a method for the production of acute muscular injury in rats with different height mass drop. It is expected that the results can be later employed to create a baseline in future studies in which therapeutic modalities of treatments for injured muscle may be compared.

■ Methods

Experimental protocols and procedures were approved by the Ethics Committee of the Universidade Federal do Vale do São Francisco under the number 0006/110414.

Experiments were performed on twelve female adult Wistar rats (200.0 to 300.0 g). The animals were randomly housed in appropriate cages at $22 \pm 2^\circ\text{C}$ on a 12-hour light-dark cycle with free access to food and water. The animals were acclimatized for four days before beginning the experiments. Rats were allocated into four groups of three rats each, and in each group, the right gastrocnemius muscles were traumatized once and the left limb was used as control.

Equipment for muscle contusion

The 20 x 12 cm base of the contusion device was made of iron. A $\frac{3}{4}$ PVC tube (135 cm of height and 8 mm of diameter) was used to drive the mass of 324 g that was used to contuse the leg of the animal (Figure 1). A 60-cm fixed metal rod was used to support the tube. Four different heights (30, 45, 60 and 70 cm) were made on the tube, and in each height a small opening was made to facilitate the sliding of the iron rod.

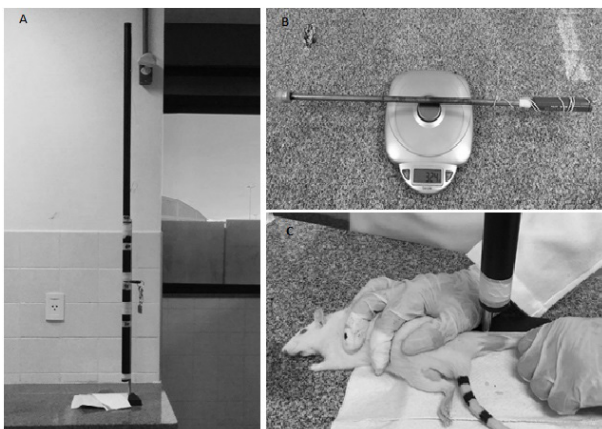


Figure 1 - Muscle contusion device. **A**, device with pre-set times to drop the mass. **B**, the 324-gram cylindrical weight used to cause injury. **C**, positioning of the animal for the injury.

The iron rod inside the tube was fixed at a certain point attached by a key. Immediately after the withdrawal of the key, the rod slid until it reached the right hind limb. Thus, we could get four drops of the same mass, with varying kinetic energy of the drops. Therefore, the kinetic energies of 0.970 J, 1.455 J, 1.940 J and 2.2634 J were obtained through the heights of 30, 45, 60 and 70 cm, respectively.

Contusion model

Before being injured, the animals were weighed and anaesthetized. The ketamine and xylazine (80.0 and 10.0 mg/kg body weight, respectively) were administered through intraperitoneal (i.p.) anesthesia. The hind limbs were shaved and cleaned with alcohol; the measurement of its diameter was made with the aid of a digital caliper, and the circumference of the medial region of the muscle was evaluated through perimetry with the aid of tape (Cescorf®). All measurements were performed prior to the injury and 72 h after injury, before euthanasia.

The mid-belly region of the gastrocnemius was determined through palpation and marked with a permanent marker. Each animal was positioned prone in the injury device with the hind limb fully

extended on the leg holder and clamped into position. That ensured an impact directly over the mid-belly region of the gastrocnemius muscle. A metal mass fell through a guide tube causing the injury. The mass (324 g) was dropped once from a height of 30, 45, 60 or 70 cm depending on the group, in the mid-belly region of the gastrocnemius muscles. Left gastrocnemius (LG) muscles were not injured and they were considered as control muscles.

Histological analysis

After 72 h of the injury, the rats were anaesthetized and sacrificed. The paw tissues were removed, fixed in 10% formalin in PBS, embedded in paraffin, and cut into 4- μ m-thick sections. The sections were stained by hematoxylin-eosin for histological analysis. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 10 \times objective. The inflammatory response was analyzed qualitatively according to the presence of inflammatory cells. Then, the lesion was classified as “mild”, “moderate” and “severe”. Histological analysis was performed by a specialist and based on the literature¹².

Statistical analysis

Descriptive statistics was used to determine the mean and standard deviation of diameter and circumference of muscles before and after the injury. A 95% confidence interval was used. SPSS for Windows, 15.0 version, was used for data analysis in this study.

■ Results

Macroscopic analysis of the gastrocnemius muscle after injury

The macroscopic aspect of the gastrocnemius muscle showed a slight change in the color of the injured muscle

of the animals only in groups of 60 and 70 cm. The descriptive values of diameter and circumference measurements of the lower

limbs measured before and 72 hours after injury and measurements between the injured and control limbs are show in Table 1.

Table 1 - Values of diameter and circumference measurements of the lower limbs of animals before and 72 hours after injury.

Group	Diameter (mm)		Circumference (cm)	
	Before	After	Before	After
G1 (35 cm)	0.487 ± 0.290 (CI 95%: 0.413 - 0.560)	0.403 ± 0.008 (CI 95%: 0.383 - 0.424)	5.26 ± 0.23 (CI 95%: 4.69 – 5.84)	4.93 ± 0.29 (CI 95%: 4.64 – 5.22)
G2 (40 cm)	0.466 ± 0.61 (CI 95%: 0.314 - 0.617)	0.433 ± 0.013 (CI 95%: 0.400 -0.466)	5.03 ± 0.37 (CI 95%: 4.09 – 5.97)	4.56 ± 0.05 (CI 95%: 4.42 – 4.71)
G3 (60 cm)	0.452 ± 0.213 (CI 95%: 0.398 - 0.505)	0.446 ± 0.006 (CI 95%: 0.430 - 0.463)	5.13 ± 0.15 (CI 95%: 4.75 – 5.51)	5.16 ± 0.35 (CI 95%: 4.24 – 6.03)
G4 (70 cm)	0.468 ± 0.301 (CI 95%: 0.393 -0.543)	0.441 ± 0.017 (CI 95%: 0.398 - 0.485)	5.00 ± 0.20 (CI 95%: 4.50 – 5.49)	5.03 ± 0.25 (CI 95%: 4.40 – 5.65)

CI: Confidence Interval

Microscopic analysis of the gastrocnemius muscle submitted to injury

The microscopic analysis of control muscles showed normal morphology and signs

of inflammation were not observed. Figure 2 illustrates the aspect of the tissues 72 h after injury from the impact of a 324-gram mass from different heights (30, 45, 60 and 70 cm).

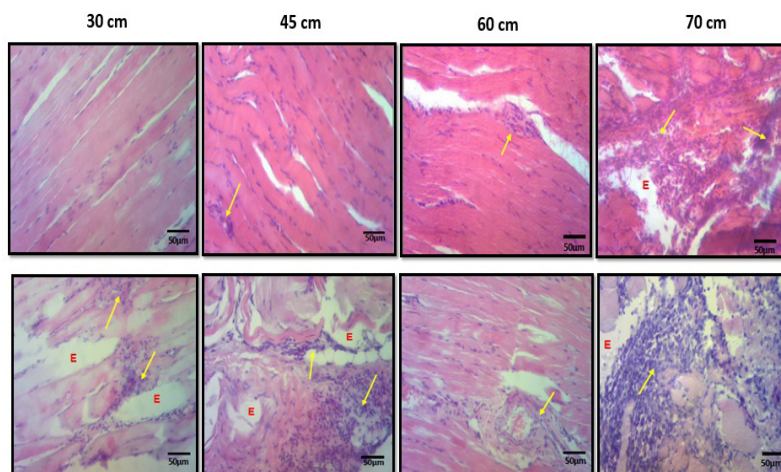


Figure 2 - H&E Histological aspect of the injured gastrocnemius muscles of rat paws from different impacts. Regions of inflammatory infiltrate and degeneration of muscle fibers are indicated by arrows and the edema is indicated by letter E.

The impact of the 324-gram mass from 30-cm height onto the right hind limb showed no uniformity in its histological analysis. In some regions, inflammatory signals were not identified, while other areas of the same tissue showed mild signs of inflammation with

edema and mild inflammatory infiltrate with undifferentiated spindle cells.

Similarly, the animals in both the 45-cm and 60-cm groups also responded to the lesion in different ways, with the presence of non-uniform inflammatory infiltrate and regions of

tissue without apparent injury, and others with moderate inflammatory signals like increased cellularity and interstitial inflammatory cells adhered to the muscular fibers.

The histological aspect of the injured muscle of rats that received a contusion from a 70-cm height was different from the others. The inflammatory response had uniform appearance with inflammatory infiltrate signals in all regions of the tissue. There was the presence of fibroblasts between mononuclear and polymorphonuclear cells, and more intense lesions with inflammatory cells in almost all regions of the muscle could be observed, as well as edema and necrosis of the muscle fibers.

■ Discussion

Many models have been used to replicate skeletal muscle injury associated with trauma in animal models to evaluate the effect of pharmacological tests as physiotherapeutic procedures, such as cryotherapy, massage, phonophoresis and others^{12,15,16}. However, these studies have a discrepancy regarding the method, device, impact and level of severity⁶. The purpose of our study was to develop a new contusion device that would allow different degrees of injury to be generated in rats and determine the best protocol to be applied with security by histological analysis of muscle tissue.

The histological analysis of the musculoskeletal tissue of the animals of each group demonstrated that the greater the height of the mass, the stronger was the muscle inflammatory response. When the drop height was increased to 30, 45 and 60 cm the injury was mild to moderate, with regions where no inflammatory cells were identified. The 70-cm height is likely to have showed another level of severity (moderate to severe). On the other hand, unlike McBrier *et al.*⁷, there was no bone fracture cases of the tibia or fibula.

McBrier *et al.*⁷, in their study, also assessed changes in muscle response to injury

caused by different heights, though injury severity was determined using magnetic resonance imaging. When a 267-gram weight was dropped from a height between 40 and 50 cm, it produced one level of injury (mild to moderate), and when the drop height was increased to 60 cm or higher, another level of severity (moderate to severe) was sustained. The 70-cm height or greater resulted in fractures of the tibia and fibula. In our study, we used similar heights and more weight, but there was no fracture.

Although an analysis with magnetic resonance imaging is valid for the injury classification, the majority of studies uses similar methods to prove the efficacy of drugs with anti-inflammatory potential. The histological analysis is more used in the qualitative or quantitative comparisons of tissue to compare the results of the methods tested^{12,15,17}.

Abreu *et al.*¹² caused a muscle injury and demonstrated the anti-inflammatory effect of the *Lychnophora pinaster* gel after acute injury in the gastrocnemius muscle of rats. The injury was caused by a 30-cm-high drop of a 300-gram mass onto the lower limbs of rats. In this study, the similar methodology application showed a tissue with little infiltration areas and other regions without signs of inflammation. Minamoto *et al.*¹⁸ analyzed the soleus muscle of rats after light bruising and recurring using a mass of 204 g loose to 16 cm in the posterior region of the lower limbs of 32 rats, and through histological analysis, abundant signs were found of acute damage-injured muscle, and major changes in tissue morphology. In another study, Minamoto and Salvini¹⁹ compared the morphology of the injury in the tibialis anterior generated by a single and periodic impact. However, the same researchers chose another method, they used other variables in the production of the injury, with a loose weight of 200 g at 36 cm.

In order to verify the effects of the therapeutic ultrasound on the mechanical

properties of the rat muscle after injury, another study induced mechanical trauma by a 200-gram weight at a 30-cm height. However, the authors did not analyze the histological tissue response²⁰. This discrepancy between the methods employed for the production of injury in rats bore variations of height and kinetic energy used from those found in the literature⁷. Other studies also seek the development and validation of methods for producing muscle injury in rabbits²¹.

The method of choice in studies that use the injury model in animals should be safe when the new substance or treatment is tested. In studies whereby this injury is caused even using the same animal model, with the same characteristics, age and lack of training, the tissue response presents a variable way. Although all studies use models of control, the award of the effect of any type of treatment may be linked to the non-response of the tissue, rather than the effect of the treatment tested.

The mechanical trauma induced on the musculature in order to produce muscle injury in rats is applied in several studies where these methods are used to test anti-inflammatory treatments. However, even the muscle where the impact caused the damage by 30, 45 and 60 cm regions without consistent signs of inflammatory response were identified. Thus, studies that analyze the action of drugs in the inflammatory process caused by mechanical trauma must choose more carefully methods of choice, and offer a way to study treatment interventions and determine their efficacy based on injury severity.

Our study had a limitation as we did not evaluate the contusion in other times plus 72h, and the response of the cells on tissue injury can be different in other hours after injury. Therefore, we suggest that the method of the drop height be increased to 70 cm and that it be the first choice for studies that evaluate the effect of anti-inflammatory treatments because this protocol presented a uniform lesion with

intense inflammatory infiltrate. Further studies need to be done to confirm these findings and to study the mechanisms of action.

■ Conclusion

All protocols adopted stimulated muscle inflammatory response. The method with the 324-g mass dropped from the 70-cm height onto the gastrocnemius muscle of rats seems to be the most suitable for the production of muscle injury in these animals for 72 hours, showing an important inflammatory infiltrate.

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