A NEW ADJUSTABLE PINCH DESIGNED FOR PRODUCING CRUSH NERVE INJURIES IN THE SCIATIC NERVE OF RATS

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ABSTRACT

Objective: A new adjustable pinch has been developed for producing a crush injury, with a previously known load of 5 kg, on a 5 mm-long segment of the nerve. Methods: Stainless steel was the material selected for building the pinch due its durability and possibility of sterilization with anti-septic substances, which are often corrosive. The crushing load of the pinch is adjustable by increasing or decreasing the tension of the spring by means of a screw used for calibration, which is performed by a load cell. Result: This pinch has been used in a few experimental investigations and was shown to be as efficient as both the universal testing machine and the dead weight machine, previously used. Conclusion: The developed pinch has the advantages of being portable and user-friendly. In addition, the pinch is cheap and allows for the standardization of the applied load.

Keywords: *Nerve crush. Sciatic nerve. Rats.*

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INTRODUCTION

Much of the accrued knowledge on physiology, pathology, degeneration, treatment and regeneration of peripheral nerves have resulted from experimental studies on small animals[1-5], particularly on rats, whose spontaneous regeneration speed favors short-term studies. The sciatic nerve of rats is a reliable model for studying different kinds of injuries and treatment methods, with crushing injuries being one of the preferred types, because it causes rupture of nervous fibers without rupturing most of the nerve's supporting structures[6,7], enabling an easier regeneration after injury.[8-13]

Although experimental crushing injuries on rats' sciatic nerve with different purposes and objectives are well disseminated, there is no definitive standard concerning almost all parameters associated to the mechanism of injury, starting from the instrument or device employed. Described mechanisms range from applying external compression with a tourniquete[14], to a direct nerve approach and the application of some kind of crushing instrument, such as microsurgical or watchmaker pinches[8] or even sutures with surgical wires[15] and some types of machines.[7,9,16]

In a series of previous studies, the authors hereof used a universal assay machine[9,10,12] or a dead-load machine[17], always with predetermined and controlled loads throughout the time established for producing it. Despite of the reliability of the results achieved with both machines, its use itself is relatively difficult, because an anesthetized animal with exposed sciatic nerve must be taken there and adapted on the accessories enabling its use in order to manipulate such a small anatomical structure. The universal assay machine has the additional disadvantage of gradually reducing the applied load, with crushed nerve accommodation, requiring continuous adjustments in order to keep it at the planned levels. The dead-load machine solves this problem[17], but it is a robust instrument.

After using both kinds of machines, the authors of the present study envisaged the possibility of obtaining the same results with a much simpler and user-friendly device, which is an adjustable crush-maker pinch for *in loco* use, without the need of transferring and adapting the animals as happened with previously employed machines. The pinch was designed and built with a mechanism activated by an adjustable tension spring, its calibration being made with a load cell.

MATERIALS AND METHODS

Design details

The material selected for the pinch was stainless steel due to its endurance and potential to be sterilized by antiseptic solutions, usually corrosive. The pinch is equipped with two extended S-shape folded shafts connected by a mechanism of reverse hinge, i.e., when handling and calibrating ends are pressed, it opens, and, when the ends are released, it closes (Figure 1). The connection hinge is displaced closer to the pinch's working end (15 mm), with the handling end being longer (30 mm) than the working one. (Figure 2) Its crushing load is adjustable by increasing or reducing tension of the spring activating it, by means of a calibration adjustment screw, made with a load cell.

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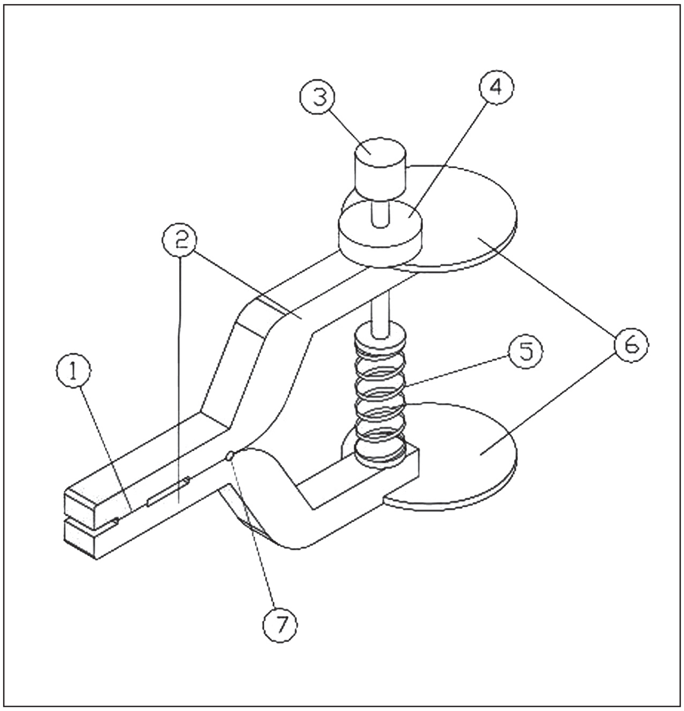

Figure 1 – *Schematic illustration of the crushing pinch: Injury base (1); pinch shaft (2); calibration screw (3); locker washer (4); tension spring; (5); retraction base (6); hinge pin (7).*

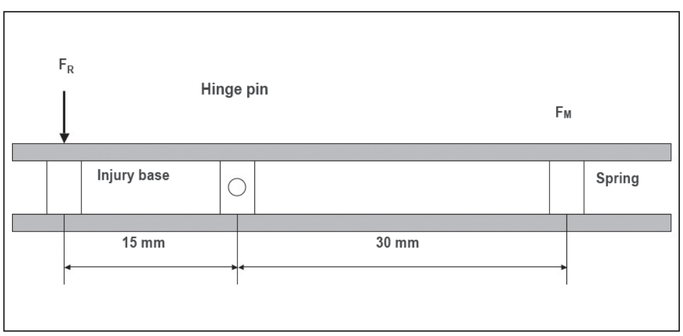

Figure 2 – *Scheme of the crushing pinch. Resulting force (Fr), spring force (Fm).*

Calibration and activation

For consisting of a first order lever system, or an interfixed lever, the resulting force (F_R) at the working end is a function of the spring calibration force (F_M), applied on the handling end, according to the formula below:

$$F_R \times 15 = F_M \times 30$$

Since the spring calibration force is known (F_M), the resultant force is calculated by the following equation:

$F_R = \dfrac{F_M \times 30}{15}$, or $F_R = 2 \times F_M$, resulting that the resulting force on the working end is twice the calibration force applied on adjustment and handling ends.

Therefore, for calibrating a crushing load of 5 kg, a 2.5 kg force was applied on the spring by simply activating the calibration screw, which was locked with a washer, thus keeping a stable load.

The pinch is calibrated on the universal assay machine with a 50 kgf capacity (Kratos®) and a digital display (Kratos®, model IKE-01).

Experimental use of the pinch

The pinch was first used in an investigation on the reproducibility of the functional analysis method[13] known as Sciatic Functional Index (SFI), assessed on footprints of 20 Wistar rats' rear paws. The SFI was measured by four investigators before and after a crushing injury was produced on the sciatic nerve, with a 5 kg load (Figure 3), at weekly intervals, until the eighth postoperative week. The gross analysis of the nerve crushed with the pinch showed a similar aspect as injuries produced by assay and dead-load machines. Similarly, the SFI analysis showed that the degree of functional compromise was consistent with that found in similar injuries produced with those machines, for the same injury load, according to previously conducted studies.

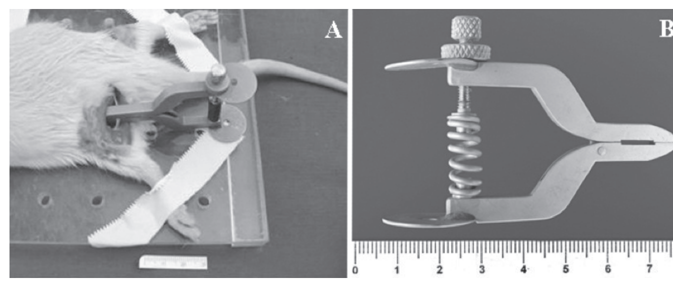

Figure 3 – *Rat's sciatic nerve crushing using the crushing pinch (A). Crushing pinch (B).*

DISCUSSION

The phenomena involved on the regeneration of peripheral nerves can be studied from several experimental models, but the crushing injury model presents the advantage of not involving the variables introduced on cutting + suture injuries. In fact, in the controlled crushing injury, at least in part, some structures of the nerve remain intact, which enables an easier regeneration, and no suturing is required, since it requires previous training with microsurgical techniques, in addition to appropriate and usually expensive instruments and materials.

It is undeniable the fact that crushing produces structural changes on the nerve, which vary according to the severity of the applied strength. Dahlin and Rydevik[18] showed structural and functional injuries in peripheral nerve compression, emphasizing that intraneural vessels (*vasa nervorum*) are occluded when strong pressures are applied, causing focal ischemia. Furthermore, structural changes occur on nervous fibers, with disorganization of the neural envelopes, including the Schwann sheath, leading to nervous function impairment. They also showed a two-way axonal transport shutdown at pressures equal or above 200 mmHg applied for 8 hours. In a recent study in our group (data pending publication), we found that the application of increasing crushing loads (500 g, 1,000 g, 5,000 g, 10,000 g and 15,000 g) starts to produce an axonotmesis-type injury as early as with the initial load, and the injury becomes more severe, according to the classification by Sunderland, with heavier loads, without reaching, however, a neurotmesis degree.[17]

Specialized literature is abundant in publications about the use of crushing injuries to study different aspects of peripheral nerves regeneration and treatment.[1,2,6,8,9,10,12] However, no consensus exist regarding the optimal mechanism for producing these injuries, which makes the reproduction and comparison of the proposed methods difficult. Many mechanisms have been suggested, but none has prevailed over the others. Chen et al.[7] introduced the use of assay or other kinds of machines, where load can be adjusted and kept for the necessary time to cause an injury, which, started to be controlled. Our group used the crushing injury model with an assay machine in a number of investigations.[9,10,12,13] Nevertheless, after some years using it, it became clear that its use is considerably cumbersome, because an animal with exposed sciatic nerve must be transferred to the machine and there adapted to accessories that, alone, cause some damage to the nerve; in addition, the pressure applied by the machine is not steady, being reduced with nerve accommodation after some minutes, requiring continuous adjustments. More recently, a dead-load machine was introduced, solving this problem, but its use is still cumbersome.[17] On the other hand, user-friendly surgical pinches, largely employed for the same purposes[8], do not enable quantification and standardization of pressures applied on the nerve.

From these observations emerged the idea of building an adjustable pinch that could apply the exactly desired pressure, which is the object of our study. This pinch was designed to be simple, both in terms of design and of manufacturing, and, most of all, to be user-friendly. It allows for calibration, which is then done on a universal assay machine, with the most diversified loads, ranging from few grams to several kilograms, including the maximum load employed in our previous investigations - 15 kg. The results achieved with this pinch were consistent to those obtained with assay and dead-load machines, particularly concerning sciatic nerve function assessment.[13]

CONCLUSION

This crushing pinch idealized, built and tested in an experimental study was shown to be much more user-friendly and practical than the assay and dead-load machines. Due to its easy calibration method with any intended load, its use should be disseminated, keeping in mind that its use depends on the availability of an assay machine to calibrate it.

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