©Journal of Sports Science and Medicine (2006) **5,** 228-234
http://www.jssm.org

Research article

THREE INTERMITTENT SESSIONS OF CRYOTHERAPY REDUCE THE SECONDARY MUSCLE INJURY IN SKELETAL MUSCLE OF RAT

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Received: 26 January 2006 / Accepted: 28 March 2006 / Published (online): 01 June 2006

ABSTRACT
Although cryotherapy associated to compression is recommended as immediate treatment after muscle injury, the effect of intermittent sessions of these procedures in the area of secondary muscle injury is not established. This study examined the effect of three sessions of cryotherapy (30 min of ice pack each 2h) and muscle compression (sand pack) in the muscle-injured area. Twenty-four Wistar rats (312 ± 20g) were evaluated. In three groups, the middle belly of tibialis anterior (TA) muscle was injured by a frozen iron bar and received one of the following treatments: a) three sessions of cryotherapy; b) three sessions of compression; c) not treated. An uninjured group received sessions of cryotherapy. Frozen muscles were cross-sectioned (10 μm) and stained for the measurement of injured and uninjured muscle area. Injured muscles submitted to cryotherapy showed the smallest injured area (29.83 ± 6.6%), compared to compressed (39.2 ± 2.8%, p= 0.003) and untreated muscles (41.74 ± 4.0%, p = 0.0008). No difference was found between injured compressed and injured untreated muscles. In conclusion, three intermittent sessions of cryotherapy applied immediately after muscle damage was able to reduce the secondary muscle injury, while only the muscle compression did not provide the same effectiveness.

KEY WORDS: Tibialis anterior, hypothermia, damage.

INTRODUCTION

Cryotherapy is one of the least expensive and most used therapies recommended in the immediate treatment of the skeletal muscle injury. The major objective of the use of cryotherapy in the early follow-up of muscle injury is to minimize adverse effects related to the damage process, as pain, edema, haemorrhage and muscle spasm, but above all, reduce the area of secondary injury caused by ischemia induced by the primary injury (Knight, 1995; Knight and Londeree, 1980; Merrick et al., 1999, Jarvinen et al., 2005).

According to Knight (1995), the physiological responses to primary injury may lead to a secondary injury by means of enzymatic and hypoxic mechanisms that affect the peripheral cells of the primary injury. The secondary injury caused by post-trauma hypoxia is due to several factors such as bleeding of the injured vessels, hemostasis, decreased blood flow due to increased blood viscosity and increased extravascular pressure and swelling caused by injury of the cellular membrane, that may occlude small vessels further increasing the ischemic area (Fisher, 1990). Then, in the first hours after the primary injury there is an increase in the total area of injury, which is a consequence of the secondary injury (Knight 1995; Merrick et al., 1999).

The physiopathology of soft tissue injury is characterized by increased cell metabolism, bleeding, hyperemia, swelling, and recruiting of leukocytes (Olson and Stravino, 1972). These factors are the rational for the use of local cooling in the early treatment of soft tissue injuries including bruising, strains and luxation (Shelbourne and Wilckens, 1990; Jarvinen et al., 2005).

The physiological effects induced by the cooling of the skin include the reduction of the temperature, metabolism, circulation, pain, muscle spams and inflammatory process (Olson and Stravino, 1972; Kowal, 1983; Kellet, 1986).

Although several procedures have been reported for the use of ice (gel, spray, ice packs, immersion, etc), in clinics, hospitals and sports activities ice packs are the most used (Enwemeka et al., 2002).

Previous reports suggested that in acute skeletal muscle injuries, cryotherapy reduces the metabolic rate of hypoxic tissues, allowing better cell survival in this period and therefore reducing the area of secondary injury (Knight and Londeree, 1980; Knight et al., 1981; Merrick et al., 1993; Merrick et al., 1999).

An interesting report showed that an early treatment consisted of 5h of continuous cryotherapy application after crush lesion, slowed secondary injury in the triceps surae muscle of rat (Merrick et al., 1999). Nevertheless, is not common the continuous application of cryotherapy for hours in humans because the risk of frozen injuries (Knight, 1995). The intermittent use of cryotherapy, as for example 30 minutes of ice pack each one or two hours has been recommended to be used after muscle injury in humans because is therapeutically effective and safe (Knight 1995; Jarvinen et al, 2005).

Although sessions of cryotherapy associated to compression is recommended and used as immediate treatment during the first 72 hours after muscle injury (Knight, 1995), the effect of intermittent sessions of this treatment in the area of secondary muscle injury is not well established. Also, it is not well established which number of sessions, applied immediately after injury are effective to reduce the secondary muscle injury. In addition, it is important to consider that for several subjects are difficult or impossible to apply intermittent sessions of cryotherapy for long periods, as for example during the night when they sleep.

Several aspects difficult the studies of the muscle injury in humans, as for example, the variability of injured muscles, differences in the extension of the injured area and in the local of injury. Also, models of human muscle injury could have serious ethical restrictions.

Then, as studies about the skeletal muscles of the mammals using animal models revealed similarities during the regeneration process, they have been used to evaluate the effect of different kind of treatments after muscle damage (Jarvinen, 2005). Also, animal models were used to evaluate the effect of cryotherapy and hypothermia in the skeletal muscle injury (Knight, 1995; Merrick, et al. 1999; for review see Jarvinen et al., 2005) and provided important information about the use of cryotherapy after injury in the soft tissues.

The studies using animal muscles are necessary in this case because it permits to induce a similar area of injury in the same skeletal muscle of a large number of animals, which permits a scientific comparison among them. Then, in the present study the skeletal muscle of rat was injured to evaluate the effect of sessions of cryotherapy in the extension of the secondary injury. Specifically, it was examined the effect of three intermittent sessions of cryotherapy (ice pack) and muscle compression (sand pack), applied immediately after muscle damage, in the area of secondary injury of the rat skeletal muscle.

METHODS

Animals care

Twenty-four Wistar rats (312 ± 20g) were randomly divided into four groups of six animals each one. They were housed in plastic cages in a room with controlled environmental conditions and had free access to water and standard food. This study was conducted in accordance with the University approval for the care and use of laboratory animals, which is accordance to the policy statement with respect to the Declaration of Helsink. The animals were anaesthetized by an intraperitoneal injection of xylazine (12 mg/kg) and ketamine (95 mg/kg) during the induction of tibialis anterior (TA) muscle injury, application of cryotherapy and compression on the muscle, and for the muscle remotion. Afterwards, they were euthanised by an overdose of the anaesthetic.

Induced muscle injury

To induce muscle injury on the middle belly of the right TA, the skin around the muscle was trichotomized and cleaned. Then, a transversal cut (about 1 cm) of the skin over the muscle middle belly was carried out, exposing the TA muscle. Afterwards, TA middle belly was injured by freezing (cryoinjury), which is a common procedure used to induce muscle injury (Miyabara et al., 2006). A rectangular iron bar (40 x 20 mm^2) was frozen in fluid nitrogen and then kept for 10s on the muscle

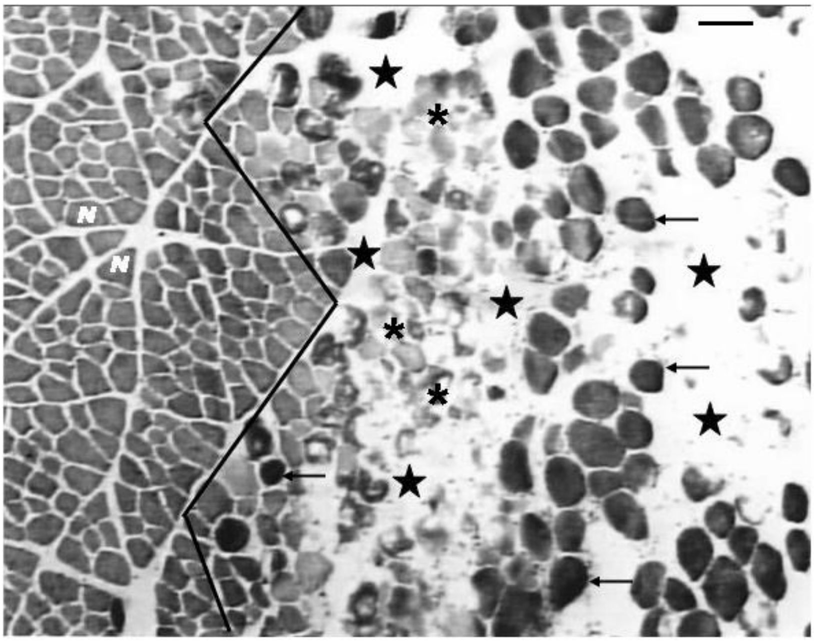

Figure 1. Cross-section of tibialis anterior (TA) muscle middle belly, 4½ hours after induced injury. Note two different muscle regions separated by a black continuous line: one composed by normal muscle fibres (N, left side of the line), and the other one composed by injured fibres (right side of the line). Hypercontracted fibres (arrows), disrupted fibres (asterisk) and large clear areas among the muscle fibres (stars) characterize the injured area, indicating edema and muscle fibres disruption. Bar: 40µm. Toluidine blue stain.

belly. The same procedure was repeated two consecutive times with a time interval of 30s. Finally, the skin was sutured and cleaned with iodine alcohol. This injury procedure was previously tested in our laboratory and it was chosen because produce a similar area of primary muscle injury in the superficial middle belly of TA muscle of rats.

Cryotherapy

Immediately after the induced muscle injury, the animals were maintained in horizontal position on a plastic table and the ankle of right paw was fixed by tape for the exposition of TA muscle skin. The sessions of cryotherapy (3 sessions of 30 minutes applied each 2 hours) consisted of the application of a plastic pack filled with crushed ice, fixed by tape directly on the skin of the right TA muscle. The ice pack covered all the extension of the TA muscle. As the ice pack also produces a muscle compression, which could affect the extension of the secondary muscle injury, an additional group of animals was evaluated using the same experimental conditions, but the ice pack was replaced by a sand pack with the same weight used in the ice pack (30g).

Animal groups

The middle belly of the right TA muscle of three groups of animals (n = 18) was injured and each group was submitted to one of the following procedures: a) three sessions of cryotherapy (ice pack, n = 6), as previously described; b) three sessions of compression (sand pack, n = 6); c) not treated (n = 6). One group of animals was not injured, but also received three sessions of cryotherapy (n = 6). This group was included to evaluate possible presence of muscle injury induced by the sessions of muscle cooling.

Immediately after the last cryotherapy or compression sessions, e.g., 4 ½ hours after induced muscle injury, both right (injured side) and left (uninjured side) TA muscles of all animals group were carefully dissected, avoiding mechanical injuries and removed. Afterwards, they were individually weighed (Denver Instruments Company, Model 100a, USA) and frozen in isopentane, previously frozen in liquid nitrogen and stored at -80°C (Forma Scientific, USA).

Muscle injury area

Histological serial muscle cross-sections were obtained (one section of 10 µm each 100 µm) in cryostat (Microm HM 505E, Germany), along of the TA muscles middle belly. Afterwards, Toluidine Blue stained the sections for morphological evaluation by light microscopy (Axiolab, Carl Zeiss, Germany).

The signs of TA muscle injury were characterized by disrupted and hypercontracted muscle fibres and large clear areas between the fibres (Figure 1), as well as by the presence of tissue

Table 1. Body and tibialis anterior (TA) muscle weight of Wistar rats. Data are means (±SD).

Groups	Body weight (g)	Injured TA (g)	Contra lateral TA (g)
Injury + Cryotherapy	328 (13)	.70 (.07)	.66 (.05)
Injury + Compression	324 (12)	.69 (.05)	.67 (.08)
Injury	293 (19)	.55 (.03) †	.51 (.03)
Cryotherapy	302 (17)	.52 (.04)	.53 (.03)

† p = 0.004 compared with Contra lateral TA.

infiltration with polimorphonuclears cells and swelling, as previously reported (Minamoto et al., 1999; Salvini et al., 2001). After that, one histological cross-section of each TA muscle located in the central region of muscle injury was choose to measure the cross-sectional area of both injured and uninjured area of the muscle, using the light microscope and a software for morphometry (Axiovision 3.0.6 SP4, Carl Zeiss, Germany). For this, pictures of the cross-section were obtained by light microscopy to reconstruct the total muscle cross-section, which permitted to identify and measure the injury and uninjured areas of the musc1e. Since the primary injury was standardized for all damaged muscles, possible differences in the final area of injury were considered as a consequence of different extensions in the secondary muscle injury. A double-blind procedure was used for both the selection of the muscle cross-section and the measurements of the injured and uninjured areas of the muscles.

Statistic analysis

Student paired t-test was used for the comparison between TA muscles of the same animals group. ANOVA and Duncan tests were used for the comparisons among groups. Significance level considered was 5%.

RESULTS

Muscle weight

Only the injured, but not treated group of animals increased the TA muscle weight, when compared to contra lateral one (0.55 ± 0.03g vs 0.51 ± 0.03g; p = 0.004), Table 1. Contrarily, there was not muscle weight difference between injured TA muscles submitted to cryotherapy or isolate compression, in comparison to the contra lateral ones. Also, normal TA muscle treated with cryotherapy did not present muscle weight difference compared to its contralateral one (Table 1).

Muscle injury area

Injured TA muscles submitted to cryotherapy presented the smallest injured area (29.8 ± 6.62%), compared to the group submitted only to the muscle compression (39.2 ± 2.88%; p = 0.003) and injured not treated muscles (41.7 ± 4.03%; p = 0.0008), Table 2. However, there was not difference in the injured area between the muscles treated only with compression and the injured but not treated muscles (p > 0.05, Table 2). Normal TA muscles submitted to cryotherapy did not present signs of muscle injury (Table 2).

DISCUSSION

The results of this study showed that three intermittent sessions of cryotherapy, associated to muscle compression and applied immediately after primary muscle injury, was effective to avoid the gain of muscle weight and to reduce the area of secondary injury. Contrarily, although sessions consisted only of muscle compression were effective to avoid the increase of muscle weight, they were not able to avoid a significant increase in the area of secondary muscle injury, which was similar to the injured, but not treated muscles.

The increased muscle weight observed in the injured, but not treated TA muscles indicate the presence of an acute inflammatory process. As previously described, the mechanism of injury causes swelling and bleeding in the damaged muscle, resulting in increased muscle weight (Crisco

Table 2. Injured and uninjured cross-sectional area of the tibialis anterior (TA) muscle middle belly. Data are means (±SD).

	TOTAL AREA	INJURED AREA		UNINJURED AREA	
	(mm^2)	(mm^2)	(%)	(mm^2)	(%)
Injury + Cryotherapy	39.1 (6.0)	11.5 (2.2)	29.8 (6.6) †	25.6 (7.4)	70.2 (6.6)
Injury + Compression	42.3 (1.9)	16.6 (1.2)	39.2 (2.9)	25.7 (1.9)	60.8 (2.9)
Injury	33.2 (1.4)	13.9 (1.6)	41.7 (4.0)	19.3 (1.3)	58.2 (4.0)
Cryotherapy	29.6 (3.6)	0	0	29.6 (3.6)	100

† p = 0.0008 compared with the Injury group and p = 0.003 compared with Injury + Compression group.

et al., 1994; Jarvinen, 1976). The absence of muscle weight gain in the injured muscles submitted to a similar compression (ice pack and sand pack), indicate that the compression itself was effective to reduce the local blood flow and edema.

However, considering that only cyotherapy treatment caused a significant reduction in the area of secondary muscle injury, the results of this study suggest that only the reduction of local blood flow and edema were not able to avoid the increase of secondary injury. Some studies have suggested that the use of cryotherapy in acute injuries has beneficial effects, more likely due to metabolism reduction than to circulatory changes (Hocutt, 1982; Knight, 1985; 1995). The results of the present study confirm this statement.

According to Merrick et al. (1999) the combination of cryotherapy and compression slow the development of secondary injury, and cryotherapy acts at a tissue level in the treatment of skeletal muscle injuries.

It is know that compression over an ice pack produces greater decrease in superficial and deep temperatures during application of the ice pack than the ice pack alone. This temperature decrease may result from blood flow reduction due to compression (Merrick et al., 1993; Thorsson et al., 1987).

Previous reports on microcirculatory dynamics after contusion and the immediate application of cryotherapy suggest that cryotherapy does not change the arteriolar diameter but it increases the venular diameter, which could explain the increase observed in the reabsorption of swelling as well as in leukocyte endothelial reduction (Menth-Chiar et al., 1999; Smith et al., 1991).

Recent study examined the effect of local tissue cooling on induced rat muscle contusion in the microvascular hemodynamics and leukocites behaviour using real-time intravital microscopy, suggested that local tissue cooling, similar to cryotherapy, improves edema and inflammatory reaction, and may be useful for reducing inflammatory response without inhibiting blood flow after muscle contusion (Lee et al, 2005). It was also reported that cryotherapy reduces the microvascular permeability by the reduction of the number of rolling and adherent leukocytes, and this association suggests that the reduction in edema in injured skeletal muscles following cryotherapy may be due to a reduction in leukocyte-endothelial interactions (Deal et al., 2002).

Recently, an interesting report found by means of magnetic resonance image that cooling attenuates the perfusion elevation and prevents the edema formation in skeletal muscle when used immediately after exercise (Yanagisawa et al., 2004). Eston and Peters (1999) also described that cold water immersion may reduce the amount of post-exercise damage after strenuous eccentric activity in humans. In the present study, cryotherapy treatment was effective to reduced both edema and secondary injury in muscles previously injured.

A few studies have investigated the effect of cryotherapy in muscle injuries, reporting that decreased tissue temperature causes a decrease in metabolism and in the demand of cellular oxygen, therefore minimizing the injury by secondary hypoxia (Merrick et al., 1999).

To our knowledge the present study provides new information about the effect of a small number of sessions of cryotherapy and compression used immediately after induced muscle injury. Although the results of this study were obtained in the rat skeletal muscle, they are interesting for both rehabilitation and sport activities because they showed the beneficial effect of the immediate use of cryotherapy in the muscle injury area evaluated under scientific experimental conditions. Considering the similarities among the muscles of mammals, the results of this study provide new information about the role of cryotherapy in the muscle injury and contribute to support the clinical recommendation of the immediate use of cryotherapy after muscle injury.

Despite this kind of study is difficult to be developed in humans, future investigations in the human skeletal muscles are necessary for comparison. In addition, complementary studies will also be important to evaluate the area of secondary injury at different periods after the protocol of cryotherapy used here.

CONCLUSION

In conclusion, three intermittent sessions of cryotherapy (30 minutes each 2 hours), applied immediately after induced muscle injury, were effective to avoid the increase of muscle weight and to reduce the area of secondary muscle injury. Only the use of muscle compression did not provide the same effectiveness in the secondary area of injury.

ACKNOWLEDGEMENTS

This study received financial support of Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Grant: 2001/00904-0), SP, Brasil. Elaine P. Rainero was fellow of FAPESP (Grant: 01/009505-6). The authors are grateful to Dr. Jorge Oishi for his help with statistical analysis and to Tereza F. Piassi for her technical assistance.

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KEY POINTS

- Three sessions of cryotherapy (30 min each 2 hours) applied immediately after muscle damage reduce the secondary muscle injury.
- Sessions of compression applied after muscle damage are not able to reduce the secondary muscle injury.

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