



Temperature of cutaneous and subcutaneous tissue during the application of aerosols in rats

Andre de Oliveira Teixeira¹, Cassio Noronha Martins¹, Antônio Marcos Vargas da Silva², Alexandro Marques Tozetti³, Rodrigo Della Múa Plentz⁴ and Luis Ulisses Signori^{1,2*}

¹Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália, Km 8, 96201-900, Rio Grande, Rio Grande do Sul, Brazil.

²Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. ³Universidade do Vale do Rio dos Sinos, São Leopoldo, Rio Grande do Sul, Brazil. ⁴Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil.

*Author for correspondence: E-mail: l.signori@hotmail.com

ABSTRACT. This study compared the thermal changes of cutaneous and subcutaneous tissues exposed to different aerosols. Thirty-six adults Wistar rats were arranged in two treatment groups, one exposed to methyl salicylate (GSM; n = 9 skin and n = 9 subcutaneous) and the other exposed to diclofenac diethylammonium (GDD; n = 9 skin and n = 9 subcutaneous) aerosols. Five jets were applied for one-second through an apparatus to reduce spray dispersion (3 cm diameter) in the lateral left thigh of the animals. Temperatures were measured every minute (min.) during 30 min., with a digital thermometer. In the skin tissue the sensor was positioned manually, in the subcutaneous tissue it was surgically inserted through the rear face and positioned in the lateral thigh. The skin temperature has homogeneously reduced in both groups. In the subcutaneous tissue the GDD has induced hypothermia from the 2nd to 20th min., the lowest temperature was recorded on the 7th min. ($-3.6 \pm 0.2^\circ\text{C}$ in relation to basal). Lowering the temperature by GSM took place from the 1st to 21st min, and the lowest temperature occurred on the 1st min ($-9.7 \pm 0.5^\circ\text{C}$ in relation to basal). In the three initial minutes the GSM had temperatures 25, 10 and 5% lower than the GDD. Aerosols have induced hypothermia in the tissues, while the GSM has decreased faster and reached lower values of temperature shown in the subcutaneous tissue.

Keywords: aerosols, inflammation, physical therapy, induced hypothermia, cryotherapy.

Comportamento térmico dos tecidos cutâneo e subcutâneo durante a aplicação de diferentes tipos de aerossóis em ratos

RESUMO. A proposta do estudo foi comparar as alterações térmicas dos tecidos cutâneo e subcutâneo submetidos à aplicação de diferentes aerossóis. Trinta e seis ratos adultos da raça Wistar foram divididos em dois grupos submetidos à aplicação de aerossóis contendo salicilato de metila (GSM; n = 9 cutâneo e n = 9 subcutâneo) e diclofenaco dietilamônio (GDD; n = 9 cutâneo e n = 9 subcutâneo). Cinco jatos de duração de um segundo foram aplicados com um redutor de dispersão (diâmetro: 3 cm) na face lateral da coxa esquerda dos animais. As temperaturas foram avaliadas a cada minuto durante 30 min., por meio de termômetro digital. No tecido cutâneo, o sensor foi posicionado manualmente, para o tecido subcutâneo este foi inserido cirurgicamente por meio da face posterior e posicionado na face lateral da coxa. A temperatura cutânea foi homogeneamente reduzida em ambos os grupos. No tecido subcutâneo, o GDD induziu a hipotermia do 2º ao 20º min., a menor temperatura ($-3,6 \pm 0,2^\circ\text{C}$ em relação ao basal) foi registrada no 7º min. A redução da temperatura pelo GSM ocorreu do primeiro ao 21º min., sendo que a menor temperatura ocorreu no primeiro min ($-9,7 \pm 0,5^\circ\text{C}$ em relação ao basal). Nos 3 min. iniciais, o GSM apresentou temperaturas 25, 10 e 5% menores que o GDD. Os aerossóis induziram a hipotermia nos tecidos estudados, sendo que o GSM reduziu mais rapidamente e atingiu menores valores de temperatura evidenciados no tecido subcutâneo.

Palavras-chaves: aerossóis, inflamação, fisioterapia, hipotermia induzida, crioterapia.

Introduction

Musculoskeletal injuries are common in the practice of physical activity and/or sports (JARVINEN et al., 2005), and after the tissue damage the inflammatory response takes place (WORRELL, 1994), where dead or damaged cells

release their intracellular contents into the surrounding area, resulting in a series of physical and chemical reactions which aims to repair the damaged tissue (BALBINO et al., 2005). The treatment in the inflammatory phase seeks to reduce secondary damages, reducing the excessive

inflammatory reaction, since the primary injury is irreversible (OLIVEIRA et al., 2006).

In order to minimize the inflammatory response from musculoskeletal injuries during and / or immediately after sports activities are commonly used different types of aerosols or sprays, which are colloidal dispersions of liquids or solids in gases (BRUNTON et al., 2006). This therapeutic resource is based on a set of physical, chemical and pharmacological effects, which favor the repair of lesions (HALKOVICH et al., 1981; JONES, 2001).

Physical effects of this therapeutic resource are based on the reduction of skin temperature from the rapid evaporation of colloids that act as a counter-irritant (GREEN, 1991), inhibiting thus pain impulses (ALGAFLY; GEORGE, 2007), working as an analgesic / or local anesthetic (REIS; HOLUBKOV, 1997), reducing muscle spasms and promoting early mobilization (HALKOVICH et al., 1981). The hypothermia also induces vasoconstriction, decreases the blood flow and metabolism, which reduces enzymatic reactions (BURKE et al., 2000), the formation of free radicals and inflammatory mediators (CARVALHO et al., 2010). All these factors mitigate tissue damage and excessive inflammatory response (HALKOVICH et al., 1981).

These aerosols are commercially combined with non-steroidal anti-inflammatories, widely used and purchased without prescription (HAWKEY et al., 2000). Among them are found the diclofenac and salicylates (JONES, 2001), which given their wide availability and ease application at the site of musculoskeletal injuries favor the use by sportsmen (HAWKEY et al., 2000; JONES, 2001). This resource as a form of transport through the cutaneous and subcutaneous tissues for the delivery of the active substance has been investigated in vitro (DJORDJEVIC et al., 2005). Nevertheless, the cutaneous absorption of the drug is small and half the amount absorbed is spread through the circulatory system and metabolized by the liver (BIOFENAC, 2006), greatly reducing the bioavailability of the drug at the site of injury (HAWKEY et al., 2000).

The mechanisms by which aerosols play their therapeutic benefits have been clinically little tested, lacking scientific evidences and may occur an extrapolation of the thermal and pharmacological therapeutic effects (NEWTON, 1985). Aerosols are widely used to treat musculoskeletal injuries, however temperature changes have not been investigated in the main products commercially available in Brazil. The present study aimed at

verifying thermal changes of cutaneous and subcutaneous tissues caused by aerosols containing diclofenac diethylammonium and methyl salicylate applied directly on the skin of laboratory animals.

Material and methods

Laboratory animals

This research consisted of a controlled experimental study, approved by the Research Ethics committee of the University of Cruz Alta (CEP/Unicruz protocol 070/08) and developed at the animal house of this institution. Trials were performed from 8 to 12h, using 36 adults *Wistar albicans* rats, kept for 12 hours in light/dark cycle, with temperature between 20 and 24°C and relative humidity of approximately 50%. The animals' maturation time was 30 weeks, with water and food "ad libitum", and animals weighed between 200 and 300 g. Animals were not killed by the end of the experiment.

Animals were randomly assigned into two groups (even numbers GDD and odd numbers GSM): group subjected to the application of aerosol containing diclofenac diethylammonium (GDD: 9 animals for the cutaneous tissue, and 9 animals for the subcutaneous), and group subjected to the aerosol with methyl salicylate (GSM: 9 animals for the cutaneous tissue, and 9 animals for the subcutaneous). The basal temperature was used as control, with the animal being the control thereof. Before collecting the data, a pilot experiment was carried out to develop the instrumentation of the protocol to be used, with the other procedures based on this trial.

Temperature evaluation

Animals were shaved (on the site of study and of surgical access), weighed (initial body weight) and anesthetized through an intraperitoneal injection of a solution composed of Xylazine Hydrochloride (7 mg kg⁻¹) and ketamine (70 mg kg⁻¹) for the sensor placement and application of the protocol. For the subcutaneous collection, it was performed a cutaneous incision of approximately one centimeter on the middle portion of the biceps femoris muscle of the left hind limb of each rat. Then it was introduced the sensor on the posterior direction, being located on the outer thigh. This region was chosen given its easy access (PLENTZ et al., 2008; SIGNORI et al., 2011). The sensor was inserted between the skin and muscle, approximately 2cm from the cut. In the cutaneous tissue the sensor was attached with Micropore (JANWANTANAKUL, 2009). The temperature was evaluated by a digital

thermometer (Icel, TD750), which has a K thermocouple sensor with resolution of 1°C, accurate of 0 - 500°C ($\pm 1^\circ\text{C}$). This type of sensor was used in previous (JANWANTANAKUL, 2009; LEVENTHAL et al., 2008). The equipment had the quality control approved by INMETRO.

After placed the sensor in respective tissues, three temperature measurements were taken (1 min-interval), and the mean values of these measurements were registered as the basal value (0'). Thereupon, aerosols were applied and tissue temperature was evaluated every minute ('), for 30 minutes (Figure 1).

Specification of aerosols

The specifications of the aerosols are described on the vials and package inserts of the products studied.

GDD (diclofenac diethylammonium): net weight 60 g. Each g contains: diclofenac diethylammonium (equivalent to 10 mg diclofenac potassium) 11 mg. Excipients: methyl salicylate, menthol, triclosan, propylene glycol, isopropyl alcohol and deodorized mixture of butane and propane – values not specified in the package insert (BIOFENAC, 2006).

GSM (methyl salicylate): net weight 44 g content 60 mL⁻¹. Each mL of the product contains 0.0333 mL methyl salicylate, 0.0333 g camphor, 0.0083 g menthol, 0.0833 mL turpentine, vehicle* q.s. 1.0000 mL. *mustard oil, lavender oil, ethyl alcohol and propellant (GELOL, 2009).

Application of aerosols

The aerosols were applied with five jets of one second each, kept at a distance of 30 cm from the applied area. Throughout the collection, the drug was no longer sprayed. In order to limit the spread of the jet beyond the area of data collection

it was used a reducer jet dispersion (CROSS et al., 1997) made of paper, which was discarded after the experiments. This had 3 cm diameter and was placed on the area with the sensor. The rest of the animal body was covered with fabric. Vials of the different types of aerosols were simultaneously used in the experiments, once they had the purpose to maintain constant the pressure used in usual applications.

Quantification of aerosol sprayed

The local amount sprayed was estimated by means of weighing the vials (without the lid) before and after the application of five jets of each aerosol, as described in the application of aerosols, respecting the period of 30 seconds between each measurement. This procedure was performed in the same place of the experiments. To weigh the vials it was used a high precision balance (Sartorius MC1 Research RC 250s). Data of each application were expressed by the mean value of the variation delta of the weights ($\Delta P = P1 - P2$) and by the summation of five jets (Σ), expressed in milligram (mg).

Statistical analysis

Data are expressed as mean and standard error. Data with two measurements were compared by a Student's t-test for dependent samples, with the presentation of the difference between means and their respective confidence intervals of 95% (CI_{95%}). For the comparison of thermal changes between groups it was used a two-way ANOVA (group and time) for repeated measures followed by a Bonferroni post hoc test. Differences were considered significant when $p < 0.05$.

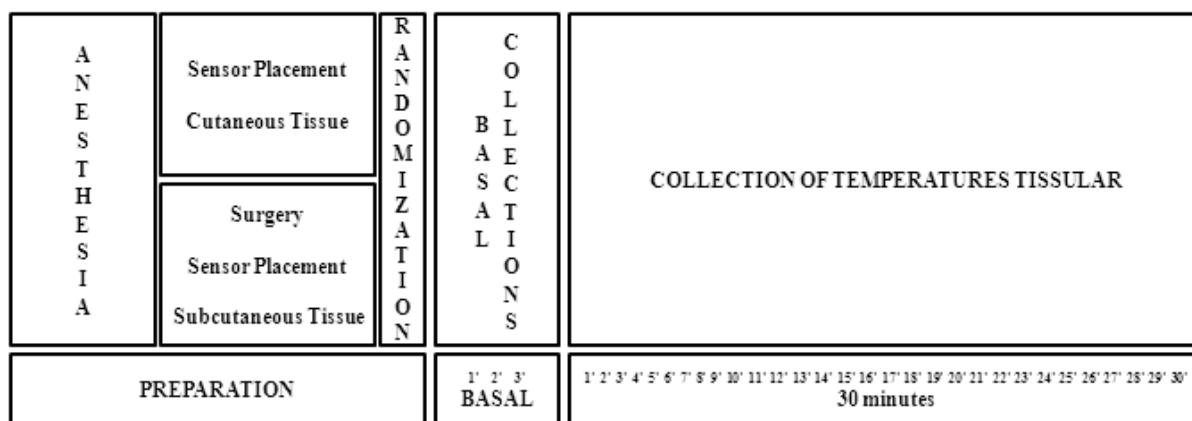


Figure 1. Experimental design.

Results

Amount of aerosol sprayed

The weight of opened vials of aerosols without lids was 87,050 mg for the GDD and 75,725 mg for the GSM. However no difference was found in the mean weight sprayed of each vial of aerosol (-628 ± 40 vs -582 ± 68 mg; $p = 0.623$) in each procedure (Figure 2), with the differences between mean values of 0.460 mg ($CI_{95\%}$ between -0.194 and 0.286 mg). The total of product taken from the vials after the five jets was 3.140 mg for the diclofenac diethylammonium and 2.910 mg for the methyl salicylate.

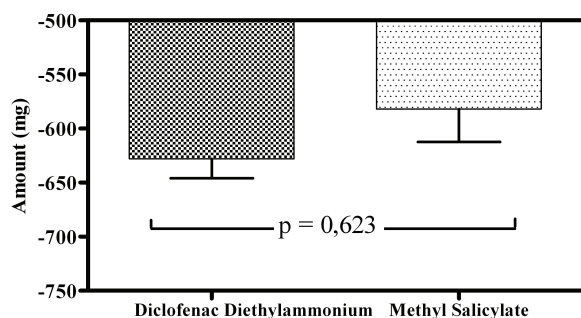


Figure 2. Mean difference in the weight of the vials after applications. Data were compared by the Student's t-test for dependent samples.

Cutaneous tissue temperature

Basal cutaneous temperature ($0'$) were similar for both groups (GDD: 29.1 ± 0.2 vs GSM: $28.9 \pm 0.3^\circ\text{C}$; $p = 0.490$) of aerosols studied (Figure 3). Aerosols have reduced the basal temperatures from the first minute of application, which have not returned to these values throughout the experiment ($p < 0.05$). The GDD had its lowest value on the 25th minute and the GSM on the first minute of the experiment, with reduction of 1.6 and 2.3°C , respectively, in relation to the basal temperature. No difference ($p = 0.892$) was detected for cutaneous temperatures between groups during the experiment.

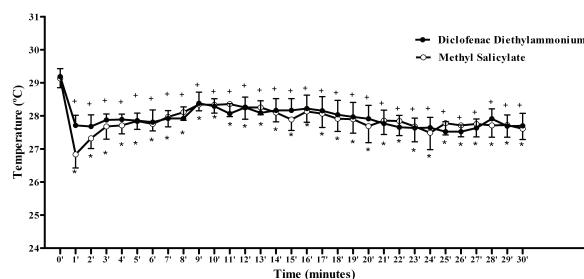


Figure 3. Variation in the cutaneous tissue temperature. Two-way ANOVA for repeated measures (Group: $p = 0.892$; Time: $p < 0.001$) followed by Bonferroni post hoc test. * $p < 0.05$ differences relative to basal temperature ($0'$) for the methyl salicylate, + $p < 0.05$ differences relative to basal temperature for the diclofenac diethylammonium.

Subcutaneous tissue temperature

Basal temperatures ($0'$) of the subcutaneous tissue (GDD: 30.6 ± 0.4 vs GSM: $30.7 \pm 0.4^\circ\text{C}$; $p = 0.704$) were similar in both groups (Figure 4). The group subjected to diclofenac diethylammonium has reduced the temperature, compared to the basal, from the second minute of application of the aerosol, and remained low up to the twenty minute ($p < 0.05$). In this group the lowest subcutaneous temperature was 27°C (± 0.2), 12% lower than the basal, after seven minutes of application. The hypothermia induced by the methyl salicylate started on the first minute and lasted up to the twenty-first minute ($p < 0.05$). The greatest reduction in temperature was observed in the first minute after the application, reaching 23°C (± 0.5), 25% lower than the basal temperature.

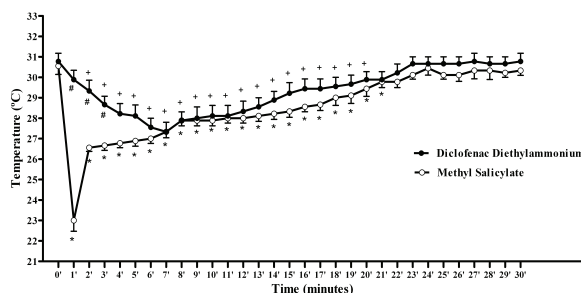


Figure 4. Variation in the subcutaneous tissue temperature. Two-way ANOVA for repeated measures (Group: $p < 0.001$; Time: $p < 0.001$) followed by Bonferroni post hoc test. * $p < 0.05$ differences relative to basal temperature ($0'$) for the methyl salicylate, + $p < 0.05$ differences relative to basal temperature for the diclofenac diethylammonium; # $p < 0.05$ differences between groups.

The reduction in the subcutaneous temperature occurred for both aerosols, but the animals subjected to aerosol containing methyl salicylate has presented a more evident reduction in temperature than those subjected to diclofenac diethylammonium, this difference was observed only in the first three minutes being respectively 25% (GDD: 29.9 ± 0.4 vs GSM: $23 \pm 0.5^\circ\text{C}$; $p < 0.05$), 10% (GDD: 29.3 ± 0.5 vs GSM: $26.5 \pm 0.1^\circ\text{C}$; $p < 0.05$) and 5% (GDD: 28.6 ± 0.4 vs GSM: $26.6 \pm 0.2^\circ\text{C}$; $p < 0.05$) lower, remaining similar in the other times studied for both groups (Figure 4).

Discussion

Our main finding was that aerosols lead to hypothermia of cutaneous and subcutaneous tissues, but the spray with methyl salicylate had an earlier and more pronounced temperature reduction in the subcutaneous tissue compared with the diclofenac diethylammonium.

The hypothermia induced by aerosols derives from the heat loss by convection, in which the sprayed liquid droplets are heated on contact with the skin, increasing volume and reducing density (GARCIA, 2005). This phenomenon makes the sprayed liquids lighter than the air causing their expansion (GARCIA, 2005) leading to rapid evaporation and removing the heat from the skin during this process (KNIGHT, 1995; NEWTON, 1985). Our results have indicated that different types of aerosols promote this effect especially at a subcutaneous level with only a slight reaction in the skin temperature. Some factors can influence this response, such as the position of the sensor in the skin, the ambient temperature, and vascularization of the tissue. In the subcutaneous tissue, the sensor was surgically placed below the skin, minimizing thus the influence of the ambient.

The reduction in cutaneous temperature was observed throughout the period of data collection in both groups, showing the effectiveness in inducing the hypothermia in the studied tissue. However, it is worth mentioning that the temperature has not returned to basal values by the end of the experiment. In this case, the anesthesia may cause an additive effect to aerosols, once this impairs the body's ability to keep the homeostatic control, reducing the temperature in 0.5 - 1.5°C in the first thirty minutes (SESSLER, 2008). Studies on the human cutaneous tissue (DYKSTRA et al., 2009; KANLAYANAPHOTPORN; JANWANTANAKUL, 2005) have shown that in the first minute of hypothermia induction using different physical media by conduction (ice, crushed ice, cold water and cold water with alcohol), the temperature is reduced in approximately 5 - 7°C. These studies suggest that the decrease in temperature promoted by physical media by conduction is more effective than aerosols analyzed in our study, and that the area and temperature of the conducting and/or convection media interfere with this effect.

The decrease in the metabolic rate by hypothermia is of 5 to 8% for each degree Celsius (1°C) of reduction in central temperature, and this metabolic rate can be proportional to the reduction in local temperature (LANIER, 1995). The results of the present study for the subcutaneous tissue represented a reduction of around 7°C for the GSM and 4°C for the GDD, which suggest a considerable metabolic effect on this tissue, especially in the first minutes after the application, described in literature (GARCIA, 2005; NEWTON, 1985) as brief and intense at the beginning of its application.

The induced hypothermia leads to a progressive reduction in tissue metabolism (DEAL et al., 2002), reduction in enzymatic reactions (ESTON; PETERS, 1999), decrease in oxygen demand (JANATA; HOLZER, 2009), vasoconstriction with reduction in blood flow in the injured area (LEE et al., 2005) and posttraumatic microvascular dysfunction (SCHASER et al., 2007), which decrease the size of the hematoma and edema (DEAL et al., 2002). Moreover it reduced the formation of free radicals, inflammatory mediators (CARVALHO et al., 2010), decreasing thus leukocyte-endothelial interactions which leads to less attraction and attachment of these cells in the injured area (DEAL et al., 2002). The reduction in the tissue temperature attenuates excessive inflammatory reaction (WORRELL, 1994) and secondary damage (DEAL et al., 2002), with these occurring simultaneously with the analgesic effect (ALGAFLY; GEORGE, 2007), which favor the recovery of the injured tissue. Possibly these effects are independent from the induction of hypothermia and from the type of tissue studied, and in the case of aerosols examined herein these results can be only given by the vehicle (sprayed liquid) used.

Aerosols commercially available are combined with drugs, aiming to add anti-inflammatory, pharmacological and thermal (hypothermia) effects in this therapeutic resource (HAWKEY et al., 2000). In our study, we sought to standardize the dose of the aerosol sprayed on the site by using a reducer (3 mm diameter) and by the way of application in order to minimize possible differences between the amounts of drugs. Effects of drugs contained in the aerosols present limitations, since the hypothermia induces the reduction in systemic clearance of the cytochrome P450, where the decrease of 1°C reduces between 7 and 22% the rate of metabolism of drugs, changing thus the peak effect of the drug (TORTORICI et al., 2007).

The diclofenac diethylammonium is only 6% absorbed by the skin, and after reaching the bloodstream, 50% of this is metabolized in the liver, reducing its bioavailability to the injured tissue (BIOFENAC, 2006). This suggests that aerosols have limited pharmacological effects, with the thermal changes representing their major therapeutic benefits. However approximately 96% of methyl salicylate is metabolized in the dermis and subcutaneous tissues, through hydrolysis reactions forming salicylate (CHANK, 1996), stimulating the perception of cold and associating to the analgesic effects (GREEN, 1989, 1991), such perceptions are also observed by the induction of hypothermia by other physical media (ALGAFLY; GEORGE, 2007).

This higher bioavailability of methyl salicylate in studied tissues can partially explain its better effects on the reduction of the subcutaneous temperature.

Among the limitations of the study, stands out it was not measured the concentration of the drugs on the blood. Another fact to be considered is that aerosols commercially available present different types of drugs and solvents in their compositions, which present isolate actions, and their respective interactions deserve further studies, which was not our purpose. Future studies should elucidate whether aerosols commercially available promote histological and histochemical changes, besides evaluating their potential effects in changing outcomes such as complications and prognosis of musculoskeletal injuries.

Conclusion

In summary, aerosols induce the hypothermia, more evident in the subcutaneous tissue, and their effect occurs especially in the first minutes after application. These results suggested that the use of this therapeutic resource can favor the treatment of musculoskeletal injuries, and the methyl salicylate was more effective given the more pronounced and immediate reduction in the subcutaneous temperature in rats.

References

- ALGAFLY, A. A.; GEORGE, K. P. The effect of cryotherapy on nerve conduction velocity, pain threshold and pain tolerance. **British Journal of Sports Medicine**, v. 41, n. 6, p. 365-369, 2007.
- BALBINO, C. A.; PEREIRA, L. M.; CURI, R. Mecanismos envolvidos na cicatrização: uma revisão. **Revista Brasileira de Ciências Farmacêuticas**, v. 41, n. 1, p. 27-51, 2005.
- BIOFENAC. **Diclofenaco dietilamônio**. Aerossol. Bula de Remédio. Guarulhos: Aché Laboratórios Farmacêuticos S.A., 2006. Available from: <<http://www.ache.com.br/Downloads/LeafletText/100/bu%20diclofenaco%20dietilam.pdf>>. Access on: Mar. 14, 2014.
- BRUNTON, L. L.; LAZO, J. S.; PARKER, K. L. Goodman & Gilman. **As bases farmacológicas da terapêutica**. 11th ed. Rio de Janeiro: McGraw-Hill, 2006.
- BURKE, D. G.; MACNEIL, S. A.; HOLT, L. E.; MACKINNON, N. C.; RASMUSSEN, R. L. The effect of hot or cold water immersion on isometric strength training. **Journal of Strength and Conditioning Research**, v. 14, n. 1, p. 21-25, 2000.
- CARVALHO, N.; PUNTEL, G.; CORREA, P.; GUBERT, P.; AMARAL, G.; MORAIS, J.; ROYES L.; ROCHA, J.; SOARES, F. Protective effects of therapeutic cold and heat against the oxidative damage induced by a muscle strain injury in rats. **Journal of Sports Sciences**, v. 28, n. 9, p. 923-935, 2010.
- CHANK, T. Y. Potential dangers from topical preparations containing methyl salicylate. **Human and Experimental Toxicology**, v. 15, n. 9, p. 747-750, 1996.
- CROSS, S. E.; ANDERSON, C.; THOMPSON, M. J.; ROBERTS, M. S. Is there tissue penetration after application of topical salicylate formulation? **The Lancet**, v. 350, n. 9078, p. 350-636, 1997.
- DEAL, D. N.; TIPTON, J.; ROSENCRANCE, E.; CURL, W. W.; SMITH, T. L. Ice reduce edema. A study of microvascular permeability in rats. **The Journal of Bone and Joint Surgery**, v. 84, n. 9, p. 1573-1578, 2002.
- DJORDJEVIC, L.; PRIMORAC, M.; STUPAR, M. *In vitro* release of diclofenac diethylamine from caprylocaproyl macrogolglycerides based microemulsions. **International Journal of Pharmaceutics**, v. 296, n. 1-2, p. 73-79, 2005.
- DYKSTRA, J. H.; HILL, H. M.; MILLER, M. G.; CHEATHAM, C. C.; MICHAEL, T. J.; BAKER, R. J. Comparisons of cubed ice, crushed ice, and wetted ice on intramuscular and surface temperature changes. **Journal Athletic Training**, v. 44, n. 2, p. 136-141, 2009.
- ESTON, R.; PETERS, D. Effects of cold water immersion on the symptoms of exercise-induced muscle damage. **Journal of Sports Sciences**, v. 17, n. 3, p. 231-238, 1999.
- GARCIA, E. A. C. **Biofísica**. 2. ed. São Paulo: Sarvier, 2005.
- GELOL. **Salicilato de metila**. Bula de remédio. Aerossol. Barueri: Hypermarcas S.A. 2009. Available from: <<http://www.bulasonline.com.br/gelolaerossol.php?codigo=gelolaerossol>>. Access on: Mar. 14, 2014.
- GREEN, B. G. Interactions between chemical and thermal cutaneous stimuli: inhibition (counterirritation) and integration. **Somatosensory and Motor Research**, v. 8, n. 4, p. 301-312, 1991.
- GREEN, B. G.; FLAMMER, L. J. Methyl salicylate as a cutaneous stimulus: a psychophysical analysis. **Somatosensory and Motor Research**, v. 6, n. 3, p. 253-274, 1989.
- HALKOVICH, L. R.; PERSONIUS, W. J.; CLAMANN, H. P.; NEWTON, R. A. Effect of Fluori-Methane® Spray on Passive Hip Flexion. **Physical Therapy**, v. 61, n. 2, p. 185-189, 1981.
- HAWKEY, C. J.; CULLEN, D. J. E.; PEARSON, G.; HOLMES, S.; DOHERTY, M.; WILSON, J. V.; GARRUD, P.; GARNER, S.; MAYNARD, A.; LOGAN, R. F. A. Pharmacoevidence of non-steroidal anti-inflammatory drug use in Nottingham general practices. **Alimentary Pharmacology and Therapeutics**, v. 14, n. 2, p. 177-85, 2000.
- JANATA, A.; HOLZER, M. Hypothermia after cardiac arrest. **Progress in Cardiovascular Diseases**, v. 52, n. 2, p. 168-179, 2009.
- JANWANTANAKUL, P. The effect of quantity of ice and size of contact area on ice pack/skin interface temperature. **Physiotherapy**, v. 95, n. 2, p. 120-125, 2009.
- JARVINEN, T. A.; JARVINEN, T. L.; KAARIAINEN, M.; KALIMO, H.; JARVINEN, M. Muscle injuries:

- biology and treatment. **The American Journal of Sports Medicine**, v. 33, n. 5, p. 745-764, 2005.
- JONES, R. Nonsteroidal anti-inflammatory drug prescribing: past, present, and future. **The American Journal of Medicine**, v. 110, n. 1, p. 4-7, 2001.
- KANLAYANAPHOTPORN, R.; JANWANTANAKUL, P. Comparison of Skin Surface Temperature During the Application of Various Cryotherapy Modalities. **Archives of Physical Medicine and Rehabilitation**, v. 86, n. 7, p. 1411-1415, 2005.
- KNIGHT, K. L. **Cryotherapy in Sports Injury Management**. Champaign: Human Kinetics 1-300, 1995.
- LANIER, W. L. Cerebral metabolic rate and hypothermia: their relationship with ischemic neurologic injury. **Journal of Neurosurgical Anesthesiology**, v. 7, n. 3, p. 216-221, 1995.
- LEE, H.; NATSUI, H.; AKIMOTO, T.; YANAGI, K.; OHSHIMA, N.; KONO, I. Effects of cryotherapy after contusion using real time intravital microscopy. **Medicine & Science in Sports and Exercise**, v. 37, n. 7, p. 1093-1098, 2005.
- LEVENTHAL, L. C.; BIANCHI, R. C.; OLIVEIRA, S. M. J. V. Ensaio clínico comparando três modalidades de crioterapia em mulheres não grávidas. **Revista da Escola de Enfermagem**, v. 44, n. 2, p. 339-345, 2010.
- NEWTON, R. A. Effects of vapocoolants on passive hip flexion in healthy subjects. **Physical Therapy**, v. 65, n. 7, p. 1034-1036, 1985.
- OLIVEIRA, N. M. L.; RAINERO, E. P.; SALVINI, T. F. Three intermittent sessions of cryotherapy reduce the secondary muscle injury in skeletal muscle of rat. **Journal of Sports Science and Medicine**, v. 5, n. 2, p. 228-234, 2006.
- PLENTZ, R. D. M.; STOFFEL, P. B.; KOLLING, G. J.; COSTA, S. T.; BECK, C.; SIGNORI, L. U. Hematological changes produced by 1MHz continuous ultrasound, applied during the acute phase of iatrogenic muscle injury in rats. **Revista Brasileira de Fisioterapia**, v. 12, n. 6, p. 495-501, 2008.
- REIS, E. C.; HOLUBKOV, E. Vapocoolant spray is equally effective as EMLA cream in reducing immunization pain in school-aged children. **Pediatrics**, v. 100, n. 6, p. 1-6, 1997.
- SCHASER, K. D.; DISCH, A. C.; STOVER, J. F.; LAUFFER, A.; BAIL, H. J.; MITTLMEIER, T. Prolonged superficial local cryotherapy attenuates microcirculatory impairment, regional inflammation, and muscle necrosis after closed soft tissue injury in rats. **The American Journal of Sports Medicine**, v. 35, n. 1, p. 93-102, 2007.
- SESSLER, D. I. Temperature monitoring and perioperative thermoregulation. **Anesthesiology**, v. 109, n. 2, p. 318-338, 2008.
- SIGNORI, L. U.; COSTA, S. T.; SAURIN NETO, A. F.; PIZZOLOTTO, R. M.; BECK, C.; SBRUZZI, G.; SILVA, A. M. V.; PLENTZ, R. D. M. Haematological effect of pulsed ultrasound in acute muscular inflammation in rats. **Physiotherapy**, v. 97, n. 2, p. 163-169, 2011.
- TORTORICI, M. A.; KOCHANNEK, P. M.; POLOYAC, S. M. Effects of hypothermia on drug disposition, metabolism and response: a focus of hypothermia-mediated alterations on the cytochrome P450 enzyme system. **Critical Care Medicine**, v. 35, n. 9, p. 2196-2204, 2007.
- WORRELL, T. W. Factors associated with hamstring injuries. An approach to treatment and preventative measures. **Sports Medicine**, v. 17, n. 5, p. 338-345, 1994.

Received on July 2, 2011.

Accepted on November 1, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.