Effects of therapeutic ultrasound on haematological dynamics and fibrinogen during the inflammatory phase after muscle injury in rats

Luis Ulisses Signori[1,2*], Andre de Oliveira Teixeira[1], Antônio Marcos Vargas da Silva[2], Silvio Teixeira da Costa[3], Thiago Dipp[4] and Rodrigo Della Méa Plentz[4]

[1]Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Avenida Itália, Km 8, 96201-900, Rio Grande, Rio Grande do Sul, Brazil.
[2]Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. [3]Centro de Educação Superior do Norte, Palmeira das Missões, Rio Grande do Sul, Brazil. [4]Universidade 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 investigated the effects of ultrasound therapy on haematological dynamics and plasma fibrinogen during the inflammatory phase of muscle injury. Forty-eight male Wistar rats were divided into control group (CG), continuous ultrasound treated group (CTU) and pulsed ultrasound (PTU). Animals were subjected to surgical incision. A transverse lesion was made in the biceps femoris muscle (50%). CTU (1.0 MHz) was applied at 12-hour intervals on the lesion, for three days, with $0.4 \ W \ cm^{-2}$ and three minutes of duration (six applications in the total). PTU was applied in the pulsed mode 20% (2 ms on/8 ms off), maintaining the other parameters. Fibrinogen, white and red blood cells were analyzed in the 24th, 48th and 72nd hour after the injury. PTU has reduced fibrinogen levels by 20% at the 24thh and by 30% at the 48thh ($p < 0.001$) and haemoglobin reduction at the 72nd hour ($p < 0.001$), which had already occurred during the 2nd collection in the other groups. CTU favoured erythrocyte reduction at the 48th h ($p = 0.003$). PTU presented an anti-inflammatory effect due to plasma fibrinogen reduction, and CTU favored haemorrhage due to the reduction of erythrocytes when applied in the first 72 hours after muscle injury.

Keywords: inflammation, ultrasound therapy, musculoskeletal system, rehabilitation, hematology.

Efeitos do ultrassom terapêutico na dinâmica hematológica e no fibrinogênio durante a fase inflamatória após lesão muscular em ratos

RESUMO. O objetivo desta pesquisa foi investigar os efeitos do ultrassom terapêutico na dinâmica hematológica e no fibrinogênio plasmático durante a fase inflamatória de lesão muscular. Quarenta e oito ratos Wistar foram divididos em grupo controle (CG), grupo tratado com ultrassom contínuo (CTU) e tratado com ultrassom pulsado (PTU). Os animais foram submetidos a uma incisão cirúrgica. A lesão transversal (50%) foi realizada no músculo bíceps femoral. O CTU (1,0 MHz) foi aplicado sobre a lesão em intervalos de 12h, por três dias, a $0,4 \ W \ cm^{-2}$ e duração de 3 min. (total de 6 aplicações). O PTU foi aplicado em modo de pulso de 20% (2 ms on/8 ms off) mantido os demais parâmetros. O fibrinogênio e as células sanguíneas brancas e vermelhas foram avaliados na 24ª, 48ª e 72ªh após a lesão. O PTU reduziu os níveis plasmáticos do fibrinogênio em 20% na 24ªh e 30% na 48ªh ($p < 0,001$), a hemoglobina apresentou uma redução na 72ªh ($p < 0,001$), sendo que nos outros grupos isso ocorreu na 2ª coleta. O CTU favorece a redução dos eritrócitos na 48ªh ($p = 0,003$). O PTU apresenta efeito anti-inflamatório pela redução plasmática do fibrinogênio e o CTU favorece a hemorragia pela redução dos eritrócitos quando aplicados nas primeiras 72h após lesão muscular.

Palavras-chave: inflamação, terapia por ultrassom, sistema musculoesquelético, reabilitação, hematologia.

Introduction

Therapeutic ultrasound, a form of acoustic energy, plays its effect on cells and tissues *via* both thermal and nonthermal mechanisms (GUFFEY; KNAUST, 1997). Low-intensity ultrasound (US) is used during physical therapy practice. Traditionally, this US varies in frequency (1 to 3 MHz), intensity and dosage (0.1 to 3 W cm^{-2}), application time and type (continuous and pulsed) of wave (BAKER et al., 2001; O'BRIEN JR, 2007). Baker et al. (2001)

claimed it is inadequate to assume that the thermal effects correspond to exposure to the continuous wave and the mechanical effects to the pulsed wave because these effects occur simultaneously. However, the thermal and/or mechanical therapeutic effects are optimized according to the type of wave (BAKER et al., 2001). They also depend on other parameters and on the interaction of these parameters with different biological tissues (BAKER et al., 2001; JOHNS, 2002; RANTANEN et al., 1999).

Muscle tissue injuries account for up to 55% of all lesions derived from the practice of physical and sporting activities. Although nonoperative treatment results in good functional outcomes in most cases, the consequences of failed treatment can be very dramatic, possibly postponing an athlete's return to sports for weeks or even months. Muscle repair and remodelling occur in four interrelated and time-dependent stages: degeneration, inflammation, regeneration and fibrosis (JÄRVINEN et al., 2005). Inflammation is the most important phase in the muscle remodelling process, when therapeutic interventions should limit the area affected by the hematoma and excessive inflammation (WORRELL, 1994). The functional damage is associated with spatial and temporal distribution of the inflammatory cells, as well as with the type and magnitude of the response (DOUGLAS et al., 2002). Clinically, first aid for muscle injuries follows the rest, ice, compression and elevation (RICE) principle. The objective of RICE is to stop the injury-induced bleeding into the muscle tissue and thereby minimize the extent of the injury (JÄRVINEN et al., 2007).

The first process that takes place as a reaction to injury is the prevention of local haemorrhage, for which fibrinogen is indispensable (LAURENS et al., 2006; MOSESSON, 2005). This is attained via platelet aggregation and via activation of the hemostasis cascade (MOSESSON, 2005). The binding of fibrinogen to hemostasis proteins and platelets as well as to several cell types, such as endothelial cells, smooth muscle cells, fibroblasts, leukocytes, and keratinocytes is indispensable during the wound repair process (JÄRVINEN et al., 2007). The resulting blood clot first contributes to stopping the bleeding and then functions as a provisional matrix for the wound healing that begins approximately 4 days after injury (LAURENS et al., 2006).

Therapeutic ultrasound is one of the most frequently used treatment for a variety of skeletal muscle injuries (WARDEN; MCMEEKEN, 2002). The US (intensity <1 W cm^{-2}) is commonly used to accelerate the tissue remodelling process after muscle injury (HILL et al., 2005), despite the scientific evidence obtained from animal studies (RANTANEN et al., 1999). This therapy is indicated because it decreases the size of the damaged area, and increases collagen deposition and elastic resistance (BYL et al., 1993). However, the biological mechanisms of its effects have yet to be fully understood (HILL et al., 2005). It is known that the damaged area becomes a source of physical and chemical signals which modify the haematological concentrations of the white blood cells (leukocytes) and red (erythrocytes) blood cells (JÄRVINEN et al., 2005; MERLY et al., 1999). Recent research data suggests that pulsed US within 24 hours on muscle injury promotes a reduction in total leukocytes as well as in segmented neutrophils and monocyte cells. These data suggest that pulsed US promotes inhibition of white blood cell proliferation (SIGNORI et al., 2011). On the other hand, continuous US promotes a reduction in erythrocytes and an increase in segmented neutrophils and eosinophils, favouring thus haemorrhage and increasing the inflammatory process (PLENTZ et al., 2008).

This study examined for 72h the interaction of the continuous and pulsed US effects on plasma fibrinogen levels and on the haematological dynamics of the different types of white cells (leukocytes) and red cells (erythrocytes) concentrations after muscle injury in rats.

Material and methods

Animals

Animal handling was performed according to the animal testing guide, and this study was approved by the Research Ethics Committee of the University of Cruz Alta (UNICRUZ/Rio Grande do Sul, State Brazil; process 002/2007). All the animals were maintained on a 12-hour dark/light cycle at 20 to 24ºC and relative humidity of approximately 50%. Food and water were given ad libitum during the entire experimental protocol. The animals' maturation time was 30 weeks. Forty-eight male mature Wistar rats (weighing 350 to 400 g) were used in this study. The rats were randomly distributed into the control group (submitted to the injury protocol and the therapeutic procedure with the ultrasound equipment turned off; CG = 16), pulsed therapeutic ultrasound group (PTU = 16) and continuous therapeutic ultrasound (CTU = 16), submitted to the injury protocol and the respective ultrasonic therapy. The groups were subjected to a surgical incision on the lateral aspect of the right hind limb according to the injury protocol.

Injury protocol

The animals were anesthetized with a combination of xylazine (7 mg kg^{-1}) and ketamine (70 mg kg^{-1}) administered intraperitoneally. A longitudinal surgical incision was made on the skin of the right hind limb to facilitate the subcutaneous tissue rupture and to provide easy access to the middle portion of the biceps femoris muscle. Its fibers were transversally incised in approximately 50% of the volume. Later, the skin lesion was surgically sutured. This muscle was chosen due to its easy access in rats and adequate distance from bone structures which could indirectly interfere with the therapeutic US stimulus (PLENTZ et al., 2008; SIGNORI et al., 2011).

Ultrasound treatment

After the surgery the rats were treated with USC and PTU, applied directly to the injured area. A commercially available ultrasound gel was used as a

coupling agent, and all animals were shaved prior to application of the ultrasound treatment. The ultrasound equipment was new, manufactured by *Biossistemas Equipamentos Eletrônicos Ltda.*, IBRAMED SONO PULSE brand, model 6763, and was calibrated beforehand. Calibration was based on IEC60601-2-5, which establishes the acceptable tolerance range (10%), and was performed at the beginning, in the middle and at the end of the study, assuring the linearity of the scale. The ultrasonic treatment was applied in the continuous and pulsed modes, with a 1 MHz frequency, 0.4 W cm^{-2}, for 3 min. (PLENTZ et al., 2008; SIGNORI et al., 2011). The PTU was applied at ¼ (20%; 2 ms on and 8 ms off) maintaining constant the other parameters (SIGNORI et al., 2011). The diameter of the headstock (n. TR3CCEO2) is 3 cm and the ERA (effective radiating area) is 5 cm^2. Circular movement of the treatment head was employed to avoid wave damage. The therapeutic procedures were performed after the lesion protocol and repeated at 12-hour intervals (for three days, six applications in the total). The animals from the CG were manipulated in the same way, but with the equipment switched off.

Haematological preparation and measures

Blood samples were collected by means of venipuncture of the right retro-orbital plexus, with the aid of a microhaematocrit capillary tube, previously heparinized, and conditioned in Eppendorf tubes with anticoagulants. The samples were collected twenty-four hours (24th), forty-eight hours (48th) and seventy-two hours (72th) after the injury.

A Neubauer chamber and a macrodilution technique were used to determine the number of leukocytes per millilitre (mL) of blood. For that, 20 µL of blood were diluted into 4 mL of Türk liquid and the number of leukocytes in the four wide angle squares was counted. This number was then multiplied by 50, and the results were given in µL. Before the cell count, the Neubauer chamber was placed inside an inverted Petri dish containing a moist cotton ball for five minutes to allow cell sedimentation. To observe the morphology and to perform the differential count of leukocytes, for which the examiners were blind, a smear of blood was made on a slide and received a Romanowsky stain. After being washed and dried at room temperature, the slide was examined under an optical microscope. One hundred cells were counted according to the Shilling zigzag technique, and the values were expressed in x10³ mm^{-3} (PLENTZ et al., 2008; SIGNORI et al., 2011).

A Neubauer chamber and a macrodilution technique were also used to determine the number of erythrocytes per millilitre (mL) of blood. Marcano liquid was used as diluent for the erythrocyte counting.

Four mL of the diluent to 20 µL of blood were used, and the erythrocytes in the five middle squares of the central square were counted. Then this number was multiplied by 10,000 and the values were expressed in x10⁵ mm^{-3}. In the haematocrit determination, the microhematocrit tube was filled with blood up to approximately 3/4 of its capacity and one of its ends was sealed with the aid of a Bunsen burner. Then the capillary tube was placed in a microcentrifuge for five minutes at 3000 rpm, and the reading was carried out on the appropriate card. Plasma fibrinogen levels were determined using a refractometer. The process consists in filling two haematocrit capillary tubes with whole blood and centrifuging them for five minutes. The plasma from one tube is transferred to the refractometer to determine total plasma protein (TPP). The second tube is heated to 57°C in a water bath for three minutes. The fibrinogen precipitated from the plasma is removed during a second centrifugation and transferred to the refractometer where the protein is quantified. The sample was centrifuged again for 5 min and a new PPT reading was taken from the refractometer; the difference between the two readings was multiplied by 1000 and the results of the plasma fibrinogen expressed as mg dL^{-1} (PRUDENTE et al., 2008). In order to quantify the haematological variables, two Neubauer chambers were counted and the average was calculated. When a difference of more than 10% was found between the two results, a recount was performed.

Statistical analyses

The data are presented as mean and standard error (SEM). A haematological comparison within each group at the three time periods (24, 48 and 72 hours post injury) and between the groups was undertaken by comparing the curves for the different times using a two-way analysis of variance with repeated measures for the two factors (group, time and interaction), followed by the Bonferroni *post hoc* test. A probability of less than 5% was considered to be statistically significant.

Results

Table 1 lists the effects of ultrasound on red blood cells concentration and other haematological variables. The haematocrit presented a reduction of approximately 10% ($p < 0.001$) between the 24th h and the 72nd h in all groups, while for the control group this reduction became evident only 48h after the muscle injury (Table 1). The erythrocytes had a reduction of approximately 8% ($p < 0.007$) on the 48^{th}h (in relation to the 24^{th}h) only for the group treated with CTU. Haemoglobin showed a reduction ($p < 0.001$) in the 2nd and 3rd collections both for the CTU and control groups, while

for PTU the reduction occurred only in the last collection, although with no differences among groups ($p < 0.966$) and interaction ($p < 0.513$). During the experiment, changes were not observed in the mean corpuscular volume (group: $p = 0.940$, time: $p = 0.230$ and interaction: $p = 0.283$), in the mean corpuscular haemoglobin concentration (time: $p = 0.184$, group: $p = 0.538$ and interaction: $p = 0.175$; data not shown) and in the total plasma proteins (Table 1).

Regarding the other groups, PTU has reduced the plasma fibrinogen values ($p = 0.013$) of approximately 30% at the 24th h and of 20% at the 48th h, while time ($p = 0.946$) and interaction ($p = 0.508$) differences were not registered for this variable (Figure 1).

The white blood cell data is shown in Table 2. The leukocytes presented a reduction in the PTU group ($p = 0.018$), mainly in the first 24 hours, which were the lowest values recorded during the experiment. However, this was not confirmed by the post hoc Bonferroni test ($p > 0.05$). The segmented neutrophil, the rod neutrophils (young), monocytes and lymphocytes had no modification during the study. Over time, the eosinophils increased in number

($p < 0.001$), and 72 hours after the experimental protocol these values had almost doubled in the three groups in relation to the first 24 hours, while in the CTU group this increase was also regostered in relation to 2nd blood collection. No difference was found in relation to the groups ($p = 0.535$) and their respective interactions ($p = 0.392$).
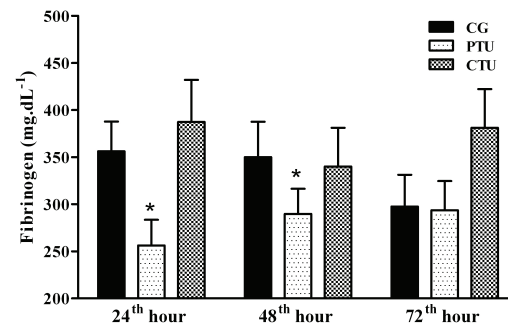
Figure 1. Plasma fibrinogen behaviour after the experimental protocol. Data are mean ± SEM. Values: mg dL^{-1} was used for comparison between the groups and 2-way ANOVA for repeated measures (Group $p = 0.013$; Time: $p = 0.946$; Interaction: $p = 0.508$; followed by *post hoc* Bonferroni test; *$p < 0.05$ variation between the groups.

Table 1. Effects of therapeutic ultrasound on red blood cells concentration and haematological variable 72 hours after muscle injury.

Haematological variable	Unit	Group n (16)	Collections 24th hour	Collections 48th hour	Collections 72th hour	ANOVA 2-way p-value Group	ANOVA 2-way p-value Time	ANOVA 2-way p-value Interaction
Haematocrit	%	CG	41.3 ±1.1	37.5 ±0.9#	37.8 ±0.8#			
		PTU	41.0 ±1.4	38.8 ±0.5	36.6 ±1.0#	0.979	<0.001	0.652
		CTU	40.3 ±1.4	37.8 ±1.0	37.69 ±1.0#			
Erythrocyte	x10^5 mm^{-3}	CG	8.2 ±0.3	7.7 ±1.2	7.9 ±1.3			
		PTU	8.1 ±0.2	7.8 ±1.5	7.7 ±1.0	0.951	0.003	0.298
		CTU	8.4 ±0.3	7.2 ±1.2#	7.9 ±1.1			
Haemoglobin	g dL^{-1}	CG	13.7 ±0.3	12.3 ±0.3#	12.5 ±0.3#			
		PTU	13.3 ±0.3	12.7 ±0.2	12.2 ±0.3#	0.966	<0.001	0.513
		CTU	13.4 ±0.4	12.5 ±0.3#	12.5 ±0.3#			
TPP	g dL^{-1}	CG	7.5 ±0.1	7.6 ±0.1	7.6 ±0.1			
		PTU	7.5 ±0.1	7.6 ±0.1	7.5 ±0.1	0.239	0.077	0.722
		CTU	7.6 ±0.1	7.8 ±0.1	7.7 ±0.1			

Data are mean ± SEM. CG: control group; continuous therapeutic ultrasound (CTU) and pulsed therapeutic ultrasound (PTU). TPP: Total plasma proteins; *p* evaluation of the comparisons between the groups using two-way ANOVA with repeated measures followed by the Bonferroni post hoc test. *$p < 0.05$ variation between the groups; #$p < 0.05$ variation over time *vs* 24th hour; +$p < 0.05$ variation over time *vs* 48th hour.

Table 2. Effects of therapeutic ultrasound on white blood cells concentration 72 hours after muscle injury.

Haematological variable	Unit	Group n (16)	Collections 24th hour	Collections 48th hour	Collections 72th hour	ANOVA 2-way p-value Group	ANOVA 2-way p-value Time	ANOVA 2-way p-value Interaction
Leukocyte	x10^3.mm^{-3}	CG	9693.6 ±482	10159.1 ±693	9463.1 ±827			
		PTU	7974.5 ±332	8797.8 ±393	8506.9 ±558	0.018	0.621	0.881
		CTU	10195.7 ±787	10231.0 ±735	10491.8 ±803			
Segmented Neutrophil	x10^{3}mm^{-3}	CG	5002.8 ±556	5192.6 ±793	4328.3 ±639			
		PTU	3592.6 ±519	3431.8 ±527	3512.3 ±463	0.141	0.368	0.768
		CTU	5475.6 ±899	4834.0 ±782	4660.3 ±791			
Young Neutrophil (Rod)	x10^{3}mm^{-3}	CG	264.0 ±89	152.8 ±76	209.4 ±53			
		PTU	172.0 ±33	202.1 ±67	131.7 ±27	0.832	0.818	0.436
		CTU	149.1 ±53	152.8 ±52	209.4 ±53			
Monocyte	x10^{3}mm^{-3}	CG	843.6 ±117	586.6 ±115	603.4 ±110			
		PTU	693.8 ±84	684.1 ±114	436.4 ±54	0.779	0.087	0.626
		CTU	779.1 ±112	801.0 ±142	660.6 ±91			
Eosinophil	x10^{3}mm^{-3}	CG	184.4 ±47	331.2 ±46	358.1 ±63#			
		PTU	132.7 ±27	244.2 ±50	356.5 ±74#	0.535	<0.001	0.392
		CTU	153.8 ±36	272.0 ±57	473.6 ±61#+			
Lymphocyte	x10^3.mm^{-3}	CG	3398.5 ±668	3896.4 ±366	3984.3 ±450			
		PTU	3383.2 ±460	4235.5 ±382	4069.4 ±330	0.399	0.504	0.967
		CTU	3638.0 ±663	4171.0 ±445	4487.8 ±411			

Data are mean ± SEM. CG: control group; continuous therapeutic ultrasound (CTU) and pulsed therapeutic ultrasound (PTU). p evaluation of the comparisons between the groups using two-way ANOVA with repeated measures followed by the Bonferroni post hoc test. *$p < 0.05$ variation between the groups; #$p < 0.05$ variation over time *vs* 24th hour; +$p < 0.05$ variation over time *vs* 48th hour.

Discussion

The original data from this study suggest that muscle injury treated with ultrasonic therapy in the pulsed form promotes a reduction in plasma fibrinogen in the first 24 and 48 hours after muscle injury. However, the continuous form of this therapy favours a reduction in erythrocytes at the 48th h.

Endothelial injury activates blood platelets and thus initiates the formation of a platelet plug, which is the primary hemostasis process that will stop the bleeding (FANG et al., 2005). Fibrinogen, by acting as a bridging molecule between the glycoprotein IIb/IIIa receptors on the platelet surface (KOENIG; ERNST, 2000), is essential for platelet aggregation. At the same time, the coagulation cascade is initiated and results in the conversion of soluble fibrinogen into a network of insoluble fibrin fibers (LAURENS et al., 2006). It represents the final substrate of the coagulation system and is converted into fibrin through generated thrombin (KOENIG; ERNST, 2000). This occurs in a dose-dependent manner: the higher the plasma fibrinogen levels, the greater the amount of fibrin produced (KOENIG; ERNST, 2000). Fibrinogen reduction found at the 24th and 48th hours after PTU application probably is related to changes in the signaling mechanisms, transduction and mobilization of this substance, and thus collaborating in the precocious recovery of the muscular lesion.

The liver is the primary source of plasma fibrinogen, but small amounts of fibrinogen can also be produced by lung epithelium where it may be locally incorporated into the provisional matrix (GUADIZ et al., 1997). This process is regulated by cytokines, such as IL-6, IL-1b, and glucocorticoids (NGUYEN; SIMPSON-HAIDARIS, 2000). Once the inflammatory reaction has begun it is intensified by satellite cells and necrotic muscle fiber tissue, which stimulate the local release of cytokines (IL-6, IL-1 and cellular growth factors (TNF, FGF, IGF) that amplify the cellular inflammatory response (JÄRVINEN et al., 2005).

Cell-culture studies have shown that PTU leads to an increase in the secretion of transforming growth factor-b1 (TGFb1) and a decrease in concentrations of IL-6 and tumour necrosis factor-α (TNFα) (LI et al., 2003), while also stimulating the production of angiogenic factors such as IL-8, fibroblast growth factor (bFGF) and the vascular endothelial growth factor (VEGF) in the culture medium (REHER et al., 1999), suggesting that these positive effects also occur in the myoregeneration. Other factors that may be considered are the serum creatine kinase (CK) levels and oxidative stress parameters, showing that PTU reduces serum CK levels, lipids (TBARS) and protein damage (carbonyl) on the first and third day after muscle injury in animals (FREITAS et al., 2007). These findings may be related to those found in this study.

The result of this sequence (endothelial lesion, cytokine action and fibrinogen activation) is the formation of a platelet-rich thrombus which provisionally acts as a buffer on the endothelial lesion (LEFKOVITS et al., 1995). This platelet-rich thrombus (white thrombus) is quickly infiltrated by fibrin and transformed into a fibrinous thrombus which captures (red thrombus) the erythrocytes (DAVIES, 1990). The reduction of erythrocytes concentration found at the 48th hour (compared to the 24th hour) after CTU use is possibly due to the greater imprisonment of these cells in a larger area of the muscular lesion. Results obtained by our research group demonstrated that the CTU led to a reduction of erythrocytes in the first hours after muscle injury (PLENTZ et al., 2008), probably owing thermal effects (JOHNS, 2002) inducing an additional local haemorrhage which may be repeated while the thrombus remains unstable (LAURENS et al., 2006). Besides, CTU did not present favourable microregeneration effects in the first five days of the application (ARAÚJO et al., 2003; MARKERT et al., 2005), additionally it increased the number of lymphocytes, favoured thrombosis (ARAÚJO et al., 2003) and decreased mechano-growth factor (MGF) messenger ribonucleic acid (mRNA) expression after blunt trauma (McBRIER et al., 2007).

The haematocrit and the haemoglobin (the main components of the erythrocytes) had a reduction throughout the study in relation to the first blood collection in all studied groups, and this is probably related to the successive blood collections, a fact observed in previous studies (PLENTZ et al., 2008; SIGNORI et al., 2011). However, for haemoglobin this alteration was not observed at 48 hours for the PTU group, suggesting that other mechanisms are involved in this therapy and in haemoglobin dynamics, deserving more advanced studies. The results of this study showed no alterations to white blood cells, but suggest a downward tendency in leukocytes in the PTU group in the first collection. This observation is in accordance with the results of other studies that evaluated CTU (PLENTZ et al., 2008) and PTU (SIGNORI et al., 2011) for 24 hours, respectively and demonstrated that CTU has produced an increase in the neutrophil concentration while PTU had reduced it.

The inflammatory response is dependent on two factors, namely the extent of actual physical damage and the degree of muscle vascularisation at the time of injury (SMITH et al., 2008). Immediately after the

musculoskeletal injury, exudates are formed in the space between the muscle fibers, where fibroblasts and macrophages are activated to produce additional chemotactic signals (growth factor, cytokines, and chemokines) for inflammatory cell circulation (JÄRVINEN et al., 2005) and satellite cell activation (MERLY et al., 1999). The injured myofibrils suffer necrosis and self-digestion (LILLE et al., 2001). The fast degeneration of these myofibrils activates the inflammation phase and contributes to tissue remodelling (MERLY et al., 1999). Studies have indicated that the treatment with PTU can promote satellite cell proliferation, increase the differentiation of muscle lineage cells (PIEDADE et al., 2008), achieve collagen supramolecular organization in the myoregeneration phase (RANTANEN et al., 1999) and reduced oxidative stress (FREITAS et al., 2007).

The functional damage is associated with the spatial and temporal distribution of the inflammatory cells, as well as with the type and magnitude of the response (DOUGLAS et al., 2002). In the damaged tissues, granulocyte and monocyte infiltration is regulated by chemoattractant factors, where thrombin cleaves fibrinogen to fibrin, which is degraded into other compounds that activate inflammation, such as fragments of collagen, elastin, fibronectin, enzymatically active thrombin and TGFb (LAURENS et al., 2006). Fibrinogen modulates the activity of monocytes and macrophages and therefore plays an important role in the transition rate between wound inflammation and tissue repair (FLICK et al., 2004). Thus, it is suggested that the anti-inflammatory effect of the PTU is not based only on leukocyte reduction (PLENTZ et al., 2008), but also on the reduction of their activity due to the smaller inflammatory stimulus of the fibrinogen found in our study.

A combination of factors, including the type of examined tissue and injury, and the US application (continuous or pulsed), intensity, and frequency of treatment, can explain the different results obtained in other studies (KARNES; BURTON, 2002). The limitations of the research concern the absence of platelet counting, histological and histochemical tissue analysis, as well as the quantification of the lesion area through ultrasonography.

In the present study, the modifications were characterized by increased haemorrhage in CTU application, reinforcing its contraindication, and an anti-inflammatory effect of PTU due to the plasma fibrinogen reduction. It is pointed out that further studies are needed for a better understanding of this interaction and to suggest the early clinical application of PTU during the inflammatory phase of muscle injury.

Conclusion

This experimental study evidenced for the first time that the use of continuous and pulsed ultrasound applied during the acute phase of muscle injury promotes alterations in the haematological dynamics with reduction in plasma fibrinogen.

Acknowledgements

References

ARAÚJO, M.; BAPTISTA-SILVA, J. C. C.; GOMES, P. O.; NOVO, N. F.; JULIANO, Y. Effects of low intensity ultrasound in the auricular vein of rabbits. **Acta Cirúrgica Brasileira**, v. 18, n. 1, p. 25-31, 2003.

BAKER, K. G.; ROBERTSON, V. J.; DUCK, F. A. A review of therapeutic ultrasound: biophysical effects. **Physical Therapy**, v. 81, n. 7, p. 1351-1358, 2001.

BYL, N. N.; MCKENZIE, A.; WONG, T.; WEST, J.; HUNT, T. K. Incisional wound healing: a controlled study of low and high dose ultrasound. **The Journal of Orthopaedic and Sports Physical Therapy**, v. 18, n. 5, p. 619-628, 1993.

DAVIES, M. J. A macro and micro view of coronary vascular insult in ischemic heart disease. **Circulation**, v. 82, n. 3, p. 38-46, 1990.

DOUGLAS, M. R.; MORRISON, K. E.; SALMON, M.; BUCKLEY, C. D. Why does inflammation persist: a dominant role for the stromal microenvironment? **Expert Reviews in Molecular Medicine**, v. 4, n. 25, p. 1-18, 2002.

FANG, J.; HODIVALA-DILKE, K.; JOHNSON, B. D.; DU, L. M.; HYNES, R. O.; WHITE, G. C.; WILCOX, D. A. Therapeutic expression of the platelet-specific integrin, alphaIIbbeta3, in a murine model for Glanzmann thrombasthenia. **Blood**, v. 106, n. 8, p. 2671-2679, 2005.

FLICK, M. J.; DU, X.; DEGEN, J. L. Fibrin(ogen)-alpha M beta 2 interactions regulate leukocyte function and innate immunity *in vivo*. **Experimental Biology and Medicine**, v. 229, n. 11, p. 1105-1110, 2004.

FREITAS, L. S.; FREITAS, T. P.; SILVEIRA, P. C.; ROCHA, L. G.; PINHO, R. A.; STRECK, E. L. Effect of therapeutic pulsed ultrasound on parameters of oxidative stress in skeletal muscle after injury. **Cell Biology International**, v. 31, n. 5, p. 482-488, 2007.

GUADIZ, G.; SPORN, L. A.; SIMPSON-HAIDARIS, P. J. Thrombin cleavage-independent deposition of fibrinogen in extracellular matrices. **Blood**, v. 90, n. 7, p. 2644-2653, 1997.

GUFFEY, J. S.; KNAUST, M. L. The use and efficacy of ultrasound. **Rehab Management**, v. 10, n. 6, p. 48-50, 1997.

HILL, G. E.; FENWICK, S.; MATTHEWS, B. J.; CHIVERS, R. A.; SOUTHGATE, J. The effect of low-

intensity pulsed ultrasound on repair of epithelial cell monolayers in vitro. **Ultrasound in Medicine and Biology**, v. 31, n. 12, p. 1701-1706, 2005.

JÄRVINEN, T. A.; JÄRVINEN, T. L.; KÄÄRIÄINEN, M.; AÄRIMAA, V.; VAITTINEN, S.; KALIMO, H.; JÄRVINEN, M. Muscle injuries: optimising recovery. **Best Practice and Research Clinical Rheumatology**, v. 21, n. 2, p. 317-331, 2007.

JÄRVINEN, T. A.; JÄRVINEN, T. L.; KÄÄRIÄINEN, M.; KALIMO, H.; JÄRVINEN, M. Muscle injuries: biology and treatment. **The American Journal of Sports Medicine**. v. 33, n. 5, p. 745-764, 2005.

JOHNS, L. D. Nonthermal effects of therapeutic ultrasound: The frequency resonance hypothesis. **Journal of Athletic Training**, v. 37, n. 3, p. 293-299, 2002

KARNES, J. L.; BURTON, H. W. Continuous therapeutic ultrasound accelerates repair of contraction-induced skeletal muscle damage in rats. **Archives of Physical Medicine and Rehabilitation**, v. 83, n. 1, p. 1-4, 2002.

KOENIG, W.; ERNST, E. Exercise and thrombosis. **Coronary Artery Disease**, v. 11, n. 2, p. 123-127, 2000.

LAURENS, N.; KOOLWIJK, P.; DE MAAT, M. P. Fibrin structure and wound healing. **Journal of Thrombosis and Haemostasis**, v. 4, n. 5, p. 932-939, 2006.

LEFKOVITS, J.; PLOW, E. F.; TOPOL, E. J. Platelet glycoprotein IIb/IIIa receptors in cardiovascular medicine. **The New England Journal of Medicine**, v. 332, n. 23, p, 1553-1559, 1995.

LI, J. K.; CHANG, W. H.; LIN, J. C.; RUAAN, R. C.; LIU, H. C.; SUN, J. S. Cytokine release from osteoblasts in response to ultrasound stimulation. **Biomaterials**, v. 24, n. 13, p. 2379-2385, 2003.

LILLE, S. T.; LEFLER, S. R.; MOWLAVI, A.; SUCHY, H.; BOYLE EM, J. R.; FARR, A. L.; SU, C.; FRANK, N.; MULLIGAN, D. C. Inhibition of the initial wave of NF-kappaB activity in rat muscle reduces ischemia/reperfusion injury. **Muscle and Nerve**, v. 24, n. 4, p. 534-541, 2001.

MARKERT, C. D.; MERRICK, M. A.; KIRBY, T. E.; DEVOR, S. T. Nonthermal ultrasound and exercise in skeletal muscle regeneration. **Archives of Physical Medicine and Rehabilitation**, v. 86, n. 7, p. 1304-1310, 2005.

McBRIER, N. M.; LEKAN, J. M.; DRUHAN, L. J.; DEVOR, S. T.; MERRICK, M. A. Therapeutic ultrasound decreases mechano-growth factor messenger ribonucleic acid expression after muscle contusion injury. **Archives of Physical Medicine and Rehabilitation**, v. 88, n. 7, p. 936-940, 2007.

MERLY, F.; LESCAUDRON, L.; ROUAUD, T.; CROSSIN, F.; GARDAHAUT, M. F. Macrophages enhance muscle satellite cell proliferation and delay their differentiation. **Muscle and Nerve**, v. 22, n. 6, p. 724-732, 1999.

MOSESSON, M. W. Fibrinogen and fibrin structure and functions. **Journal of Thrombosis and Haemostasis**, v. 3, n. 8, p. 1894-1904, 2005.

NGUYEN, M. D.; SIMPSON-HAIDARIS, P. J. Cell type-specific regulation of fibrinogen expression in lung epithelial cells by dexamethasone and interleukin-1beta. **American Journal of Respiratory Cell and Molecular Biology**, v. 22, n. 2, p. 209-217, 2000.

O'BRIEN JR., W. D. Ultrasound-biophysics mechanisms. **Progress in Biophysics and Molecular Biology**, v. 93, n. 1, p. 212-55, 2007.

PIEDADE, M. C. B.; GALHARDO, M. S.; BATTLEHNER, C. N.; FERREIRA, M. A.; CALDINI, E. G.; DE TOLEDO, O. M. S. Effect of ultrasound therapy on the repair of Gastrocnemius muscle injury in rats. **Ultrasonics**, v. 48, n. 5, p. 403-411, 2008.

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.

PRUDENTE, M. F. S.; LIMA, K. C.; CARVALHAES, M. S. Hematological, serum biochemical and serological profile of Felis domesticus with experimental *lagochilascariosis*. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 41, n. 5, p. 496-501, 2008.

RANTANEN, J.; THORSSON, O.; WOLLMER, P.; HURME, T.; KALIMO, H. Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury. **The American Journal of Sports Medicine**, v. 27, n. 1, p. 54-59, 1999.

REHER, P.; DOAN, N.; BRADNOCK, B.; MEGHJI, S.; HARRIS, M. Effect of ultrasound on the production of IL-8, basic FGF and VEGF. **Cytokine**, v. 11, n. 6, p. 416-423, 1999.

SIGNORI, L. U.; COSTA, S. T.; SAURIN NETO, A. F.; PIZOLOTTO, 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.

SMITH, C.; KRUGER, M. J.; SMITH, R. M.; MYBURGH, K. H. The Inflammatory response to skeletal muscle injury: illuminating complexities. **Sports Medicine**, v. 38, n. 11, p. 947-969, 2008.

WARDEN, S. J.; MCMEEKEN, J. M. Ultrasound usage and dosage in sports physiotherapy. **Ultrasound in Medicine and Biology**, v. 28, n. 8, p. 1075-1780, 2002.

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.