Osteoarthritis and Cartilage 24 (2016) 169–177

Osteoarthritis and Cartilage

Aerobic exercise training and low-level laser therapy modulate inflammatory response and degenerative process in an experimental model of knee osteoarthritis in rats

L. Assis † * [a], L.P. Milares † [a], T. Almeida †, C. Tim ‡, A. Magri †, K.R. Fernandes †, C. Medalha †, A.C. Muniz Renno †

† *Department of Bioscience, Federal University of São Paulo, Santos, SP, Brazil*
‡ *Department of Physiotherapy, Federal University of São Carlos, São Carlos, SP, Brazil*

ARTICLE INFO

Article history:
Received 24 March 2015
Accepted 27 July 2015

Keywords:
Low-level laser therapy
Articular cartilage
Knee osteoarthritis
Rehabilitation

SUMMARY

Objective: The aim of this study was to evaluate the effects of an aerobic exercise training and low-level laser therapy (LLLT) (associated or not) on degenerative modifications and inflammatory mediators on the articular cartilage using an experimental model of knee OA.
Material and methods: Fifty male Wistar rats were randomly divided into five groups: control group (CG); knee OA control group (OAC); OA plus exercise training group (OAT); OA plus LLLT group (OAL); OA plus exercise training associated with LLLT group (OATL). The exercise training (treadmill; 16 m/min; 50 min/day) and the laser irradiation (two points-medial and lateral side of the left joint; 24 sessions) started 4 weeks after the surgery, 3 days/week for 8 weeks.
Results: The results showed that all treated groups showed (irradiated or not) a better pattern of tissue organization, with less fibrillation and irregularities along the articular surface and chondrocytes organization, a lower degenerative process measured by OARSI score and higher thickness values. Additionally, all treated group showed a reduced expression in IL-1β, caspase-3 and MMP-13 compared to OAC. Moreover, a lower caspase-3 expression was observed in OATL compared to OAL and OAT.
Conclusion: These results suggest that exercise training and LLLT were effective in preventing cartilage degeneration and modulating inflammatory process induced by knee OA.

Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the loss of articular cartilage, bone alterations and joint inflammation[1]. The knee is the most frequently joint affected in OA and it is a major cause of pain and functional disability among older adults[2,3]. This disease is characterized by progressive degeneration of the extracellular matrix of articular cartilage, subchondral bone remodeling and inflammation of periarticular tissues, culminating in chronic pain and reduction in functional capacity of the patient[4,5]. The exact mechanism of cartilage degradation in OA is still unclear but it involves a complex interplay of genetic, environmental, metabolic and biochemical factors, including the overexpression of degrading enzymes are overexpressed, resulting in loss of collagen and proteoglycans of the matrix[4–6].

OA treatment is based mainly in the use of analgesics, non-steroidal anti-inflammatory drugs and COX-2 inhibitors[2]. More recently, more innovative therapeutic approaches have been developed, aiming to avoid and to reduce joint damage and, consequently, the minimization of the functional restrictions in affected joint is of extreme clinical importance[7]. Among the innumerable range of therapeutic interventions, the positive effects of physical exercise programs on cartilage metabolism can be highlighted[8,9].

Physical exercise focusing on muscle strengthening or on aerobic activity, is an effective, low-cost and accessible therapeutic modality and might play a crucial role in the prevention and treatment of OA[4,10,11]. It is well established that regular physical

* Address correspondence and reprint requests to: L. Assis, Department of Bioscience, Federal University of São Paulo, R. Silva Jardim, 136, Vila Mathias, Santos, Sao Paulo, 11015-020, Brazil. Tel: 55-13-38783823.
E-mail address: livinha_fisio@yahoo.com.br (L. Assis).
[a] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.joca.2015.07.020
1063-4584/

exercise can improve function and reduce pain and muscle fatigue[10–13]. Moreover, several researchers have shown that regular low/moderate-intensity exercise can decrease plasmatic and articular inflammatory markers, attenuating cartilage proteolysis process and subsequent knee OA progression[14–16]. According to Gomes *et al.*[16], aerobic exercise (walking training, three times/week for 12 weeks) was effective in decreasing the levels of soluble receptor for tumor necrosis factor (sTNFR2) from plasma, which has been associated with reduced inflammation in articular cartilage damage and improved physical function in OA patients[16]. Moreover, Samut *et al.*[17], found that aerobic exercise (walking training, 3 days/week for 6 weeks) decreased pain and increased functional capacity and muscle strength in patients with knee OA.

Additionally, low-level laser therapy (LLLT) has been considered a promising alternative to many medical conditions, mainly due to its stimulatory effect on tissue healing, ability of modulating the inflammatory process and positive effects on pain relief[2]. A wide range of works have showed LLLT is effective in treating OA in both experimental models and clinical studies[18–21]. Anti-inflammatory and chondroprotective effects and inhibition of cartilage degradation after laser-irradiation treatment were observed in several experimental model of OA in rats[19,22,23]. Furthermore, clinical trials have been demonstrated that LLLT has the ability to reduce pain and knee swelling, increasing the functional activity in knee OA patients[24,25].

Despite the positive effects of the exercise training and LLLT on OA treatment demonstrated by many authors, there is a lack of information about the interaction of both therapies on knee OA progress. In this context, it was hypothesized that the association of exercise training and LLLT may modulate the joint inflammatory process and favor cartilage metabolism in an experimental model of OA in the knee of rats, preventing the progression of the degenerative process and constituting a more suitable and effective treatment to be used. It is expected that both treatments were able of modulating the degenerative modifications induced by OA and decreasing expression of pro-inflammatory mediators expression, such as IL-1β, caspase-3 and MMP-13. Thus, the aim of this study was to determinate the effectiveness of an aerobic exercise training associated to LLLT (associated or not) on degenerative modifications and inflammatory mediators on the articular cartilage of rats in a model of knee OA. For this purpose, morphological characteristics and the expression of inflammatory markers of the articular cartilage (IL-1β, Caspase-3 and MMP-13) were evaluated.

Materials and methods

Experimental groups and knee OA induction

This study was conducted according to the Guiding Principles for the Use of Laboratory Animals and it was approved by the Animal Care Committee guidelines at Federal University of São Paulo (protocol 814715/2013). In this investigation, 50 adult male Wistar rats (*Rattus norvegicus*), weighing ±150 g, 8 weeks old were used. Animals were purchased and placed in plastic cages with sawdust bedding, with three to four animals per cage and were allowed to move freely in the cages and had free access to commercial food and water. The room had 12 h dark/light cycle and a controlled temperature (24±2°C).

The experimental animals were randomly distributed into five groups ($n = 10$ per group): control group − animals with no interventions (CG); OA animals without treatment (OAC); OA animals submitted to aerobic exercise training (OAT); OA animals submitted to LLLT treatment (OAL); OA submitted to aerobic exercise training and LLLT treatment (OATL).

The knee OA induction was performed as previously described by Galois *et al.*[14]. Rats received intra-peritoneal anesthesia with 40 mg/kg ketamine (Dopalen; Vetbrands; São Paulo; Brazil) and 20 mg/kg xylazine (Anasedan; Vetbrands; São Paulo; Brazil). The skin around the left knee was shaved and cleaned and then, a lateral para-patellar incision (about 1 cm) of the skin was carried out. The patella was then dislocated laterally to provide access to the joint space and anterior cruciate ligament (ACL) was isolated and transected in the flexed knee. The Lachman testing confirmed complete transection of the ligament. The incision was sutured and antiseptically treated. All these surgery were conducted in the light phase. Further surgery, the animals were housed in single plastic cages and were given appropriate postoperative care.

Treatments

The treatments (aerobic exercise training, LLLT and aerobic exercise training plus LLLT) were performed four weeks after knee OA induction. Moreover, all sessions were done in the afternoon after 4 p.m.

Aerobic exercise training

For aerobic exercise training program, a motorized treadmill (EP 131, Insight®, SP, Ribeirão Preto, Brazil) with eight individual lanes and no inclination was used. One week before the exercise training program, all rats were familiarized to the procedures (10 min/day) to decrease their stress regarding the new environment. The moderate-intensity aerobic exercise training was performed for 8 weeks, 3 days/week, running speed of 16 m/min and duration of 50 min/day[9].

LLLT protocol

A gallium-aluminum-arsenide (GaAlAs) diode laser (Photon Laser II, DMC® equipment Ltda, SP, São Carlos, Brazil), was used in the following parameters: continuous irradiation mode, 808 nm wavelength, 50 mW power output, 28 s irradiation time, 0.028 cm^2 spot area, dose 50 J/cm^2, irradiance 1.7 W/cm^2, 1.4 J total energy per point/section. Laser irradiation was applied 3 days/week, at two points on left knee joint (medial and lateral side of the joint), through the punctual contact technique, for 24 sessions (8 weeks). The optical fiber was positioned perpendicularly to the skin. For the animals of OATL, laser irradiation was performed immediately after the exercise protocol[7].

Twelve weeks after the surgery, all animals were euthanized individually by carbon dioxide asphyxia and left knee joints of each animal were removed for analysis.

Evaluations

Histological analysis

For histological analysis, the specimens were fixated in 10% buffered formalin for 24 h, followed by decalcified in 10% ethylenediami-netetraacetic acid (EDTA) for approximately 10 weeks. Samples were placed in formalin, dehydrated in a graded series of ethanol and xylol, embedded in paraffin blocks and histological sagittal serial sections (5 μm) were done, starting from the medial margin of the joint using a micrometer (Leica Microsystems SP 1600, Nussloch, Germany). Laminas were stained with hematoxylin e eosin (HE- Merck, Germany) and Safranin-O (Merck, Germany). Moreover, other sections were obtained for the immunohistochemical analysis.

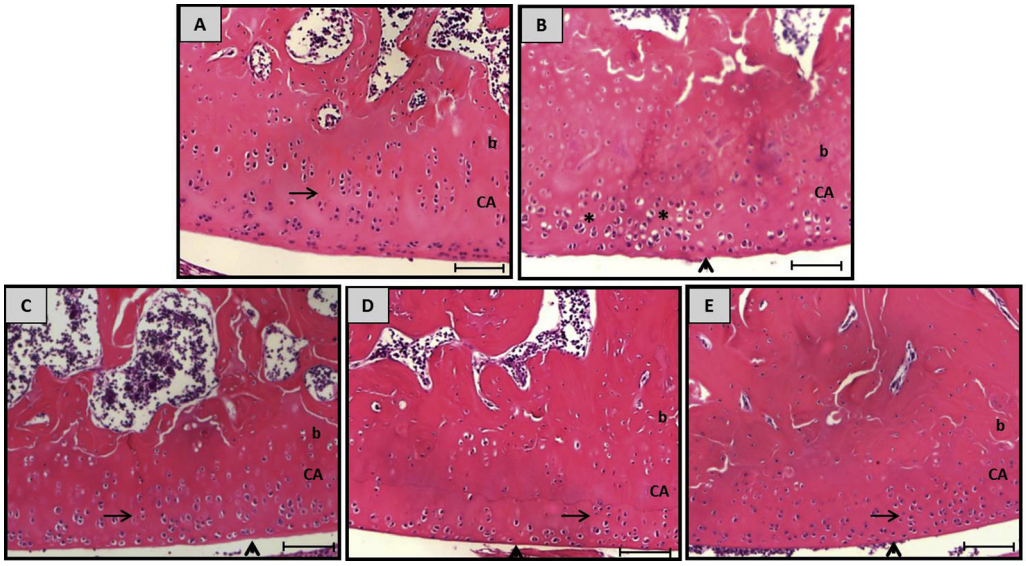

Fig. 1. Representative photomicrographs of the experimental groups. Organization of chondrocytes (arrow), fibrillation and irregularities (arrowhead), joint cartilage (JC), subchondral bone (b). (A) Control – CG; (B) Osteoarthritis – OAC; (C) Osteoarthritis plus exercise training – OAT; (D) Osteoarthritis plus LLLT – OAL; (E) Osteoarthritis plus exercise training associated with LLLT – OATL. (Stain: H.E.; Scale Bar: 100 μm).

OARSI score system

The progression of OA in all samples was assessed and compared according to the Osteoarthritis Research Society International (OARSI)[5]. The system uses a 24-point scale based on a combination of OA grade (0–6 points) and OA stage (0–4 points). Samples were evaluated histopathologically for the presence of osteoarthritis grade (OARSI grades 0–6), osteoarthritis stage (OA stages 0–4) and osteoarthritis scoring (OA scoring: [OARSI grade] × [OA stage]) according to the recommendation of the OARSI and cartilage histopathology grading and staging system. Two experienced observers (LA and LP) performed the scoring in a blinded manner.

Morphometric analysis

The morphometric study was carried out using one slide stained with HE per animal. The number of chondrocytes and cartilage thickness in each area were quantitatively scored using the computer-based image analysis Axiovision 3.1 Image Analysis (Carl Zeiss, Oberkochen, Germany). To count the density of chondrocytes, three areas of 80.000 μm^2, at the anterior, central and posterior region of each slide were chosen[18]. Within each area, cells were marked and the chondrocytes average was calculated. Thickness was also measured in three regions, one central and two lateral (300 mm left and right from the first region) from the subchondral bone to articular surface[26]. Two experienced observers (LA and LP) performed the scoring in a blinded manner.

Immunohistochemistry analysis

For IL-1β, MMP-13 and caspase-3 expression analysis the paraffin was removed with xylene from serial sections of 5 μm. After this procedure, the sections were rehydrated in graded ethanol and pretreated in a microwave with 0.01 M citric acid buffer (pH 6) for three cycles of 5 min each at 850 W for antigen retrieval. Subsequently, the material was pre-incubated with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) solution for 5 min to inactivate the endogenous peroxidase and then blocked with 5% normal goat serum in PBS solution for 10 min. The specimens were incubated with anti-IL-1β polyclonal primary antibody (Santa Cruz Biotechnology, USA) at a concentration of 1:50, anti-MMP-13 polyclonal primary antibody (Santa Cruz Biotechnology, USA) at a concentration of 1:100 and anti-caspase-3 polyclonal primary antibody (Santa Cruz Biotechnology, USA) at a concentration of 1:100. Incubation was performed overnight at 4°C in the refrigerator and followed by two washes in PBS for 10 min. Afterwards, the sections were incubated with biotin conjugated secondary antibody anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) at a concentration of 1:200 in PBS for 1 h. The sections were washed two times with PBS followed by the application of avidin biotin complex conjugated to peroxidase (Vector Laboratories) for 45 min. The visualization of the bound complexes was realized by the application of a 0.05% solution of 3-3′-diaminobenzidine solution and counterstained with Harris Hematoxylin. Finally, for control analyses of the antibodies, the serial sections were treated with rabbit IgG (Vector Laboratories, USA) at a concentration of 1:200 instead of the primary antibody.

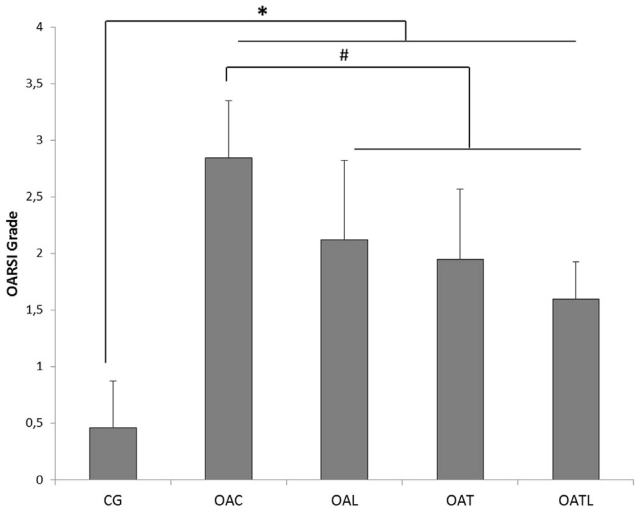

Fig. 2. Histopathological evaluation using OARSI Score: CG – Control; OAC – Osteoarthritis; OAT – Osteoarthritis plus exercise training; OAL – Osteoarthritis plus LLLT; OATL – Osteoarthritis plus exercise training associated with LLLT. (Scale Bar: 50 μm) $^{*}P < 0.0001$ vs CG; $^{\#}P < 0.05$ vs OAC.

Futhermore, internal positive controls were performed with each staining bath. Digital images of the 200× magnification were captured by optical microscope (Leica Microsystems AG, Wetzlar, Germany). Brown chondrocytes nucleuses marked were considered positive for IL-1β, MMP-13 and caspase-3 expression. The results were evaluated both qualitatively (presence of the immunomarkers) and semi quantitatively according to the percentage of positive cells in the randomly selected fields in each sample using a light microscopy (Leica Microsystems AG, Wetzlar, Germany), according to a previously described scoring scale from 1 to 4 (1 = absent, 2 = weak, 3 = moderate, and 4 = intense for immunohistochemical analysis[27]. Two experienced observers (LA and LP) performed the scoring in a blinded manner.

Statistics

Data are expressed as the mean ± standard deviation and 95% confidence interval (CI) where N indicated the number of animals in each group. Shapiro–Wilk's and Levene's test were applied to evaluate the normality and homogeneity of the results, respectively. For the variables that exhibited normal distribution (OARSI Scoring System; thickness; IL-1, Caspase-3 and MMP-13 expression) comparisons between experimental groups were performed by analysis of variance (one-way ANOVA), and the Tukey *post hoc* used to compare individual groups. For the variables that exhibited non-normal distribution, Kruskal-Wallis test with subsequent post hoc Dunns was used. A P value < 0.05 was regarded as statistically significant difference. All analyzes were performed using a GraphPad Prism 6.0 (GraphPad Software, San Diego CA, USA).

Results

General observation of the experimental animals

From the 50 animals available for this study, two animals were lost due to an anesthesia-induced respiratory depression. The remaining animals recovered uneventfully from the OA induction, exercise training and laser procedure. No signs of macroscopic adverse tissue responses were observed during the experimental period. At final of the experiment, the rats were about 5 months old with a mean weigh ±400 g. There was no significant difference between the rat groups at surgery and 12 weeks post-surgery.

Histological descriptive analysis

For CG, histopathologic analysis revealed a normal cartilage tissue structure, with chondrocytes displayed in a parallel arrangement in the superficial region and in columns in the intermediate region [Fig. 1(A)]. For OAC animals, morphological changes were observed, including tissue degradation, intense signs of fibrillation along the articular surface and increased number of chondrocytes [Fig. 1(B)]. All treated groups presented similar histological findings, with slight signs of fibrillation and irregularities on the articular surface [Fig. 1(C), (D), (E)]. Furthermore, moderate number of chondrocytes was observed, displayed in organized columns resembling the organization of a normal tissue [Fig. 1(C)].

OARSI scoring system

Fig. 2 shows the OARSI score for cartilage degeneration. For CG (0.45 ± 0.41, n = 10), OARSI analysis demonstrated a statistical lower value compared to the OA experimental groups (treated and untreated animals) (2.83 ± 0.47, 95% CI −3.17–1.84, n = 10, P < 0.0001 [OAC]; 2.11 ± 0.69, 95% CI −2.25–0.89, n = 9, P < 0.0001 [OAL]; 1.95 ± 0.69, 95% CI −2.24–0.88, n = 9, P < 0.0001 [OAT]; 1.61 ± 0.24, 95% CI −1.88–0.55, n = 10, P < 0.0001 [OATL]; Kruskal-Wallis, Dunns post-hoc). Furthermore, OAC score was higher compared to OAL (95% CI = 0.25–1.61, P = 0.003), OAT (95% CI 0.26–1.62, P = 0.002) and OATL (95% CI 0.62–1.95, P < 0.0001). No other difference among the other experimental periods was observed.

Morphometric analysis – density of chondrocytes and thickness

Fig. 3(A) and (B) shows the morphometric evaluation of the cellularity and thickness.

For CG (156.3 ± 7.5, n = 10), the density of chondrocytes was statistically lower compared to OAC (190 ± 16, 95% CI −45.52–21.88, n = 10, P = 0.037; one-way ANOVA, Tukey post-hoc Fig. 3(A)). Interestingly, no other difference was observed between OAC and the treated groups.

For the thickness analysis, OAC (138 ± 18) presented significant lower values compared to the other groups (175.7 ± 28, 95% CI

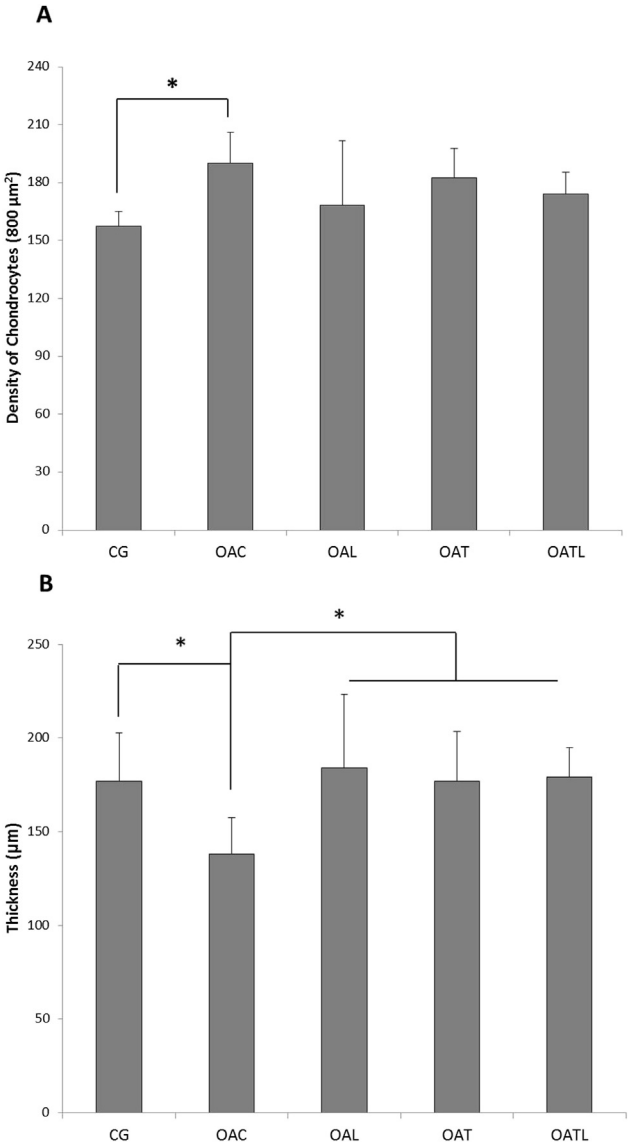

Fig. 3. (A) Results of the morphometric analysis of cellularity *P = 0.037 vs CG. (B) Results of morphometric analysis of thickness. CG – Control; OAC – Osteoarthritis; OAT – Osteoarthritis plus exercise training; OAL – Osteoarthritis plus LLLT; OATL – Osteoarthritis plus exercise training associated with LLLT. *P < 0.05 vs OAC.

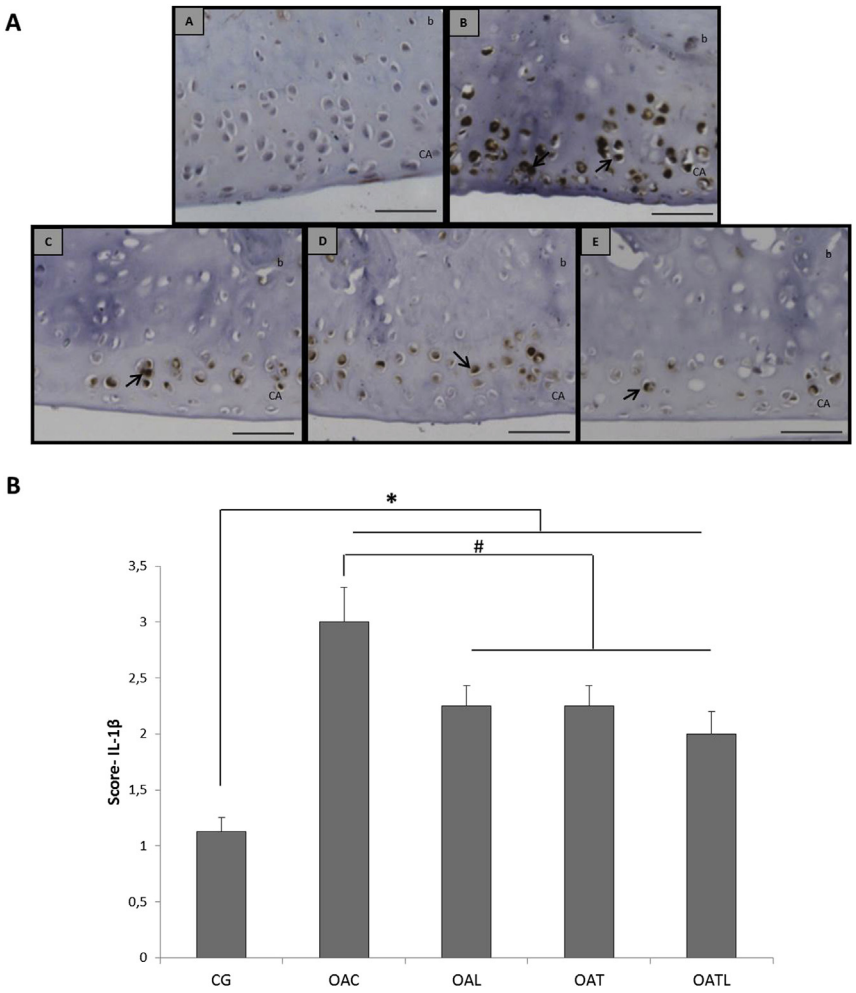

Fig. 4. (A) Representative sections of IL-1β immunohistochemistry. Immunolabeled chondrocytes (arrow). (A) Control – CG; (B) Osteoarthritis – OAC; (C) Osteoarthritis plus exercise training – OAT; (D) Osteoarthritis plus LLLT – OAL; (E) Osteoarthritis plus exercise training associated with LLLT – OATL. (Scale Bar: 50 μm); **(B)** Results of semi-quantitative analysis of the IL-1β expression $^{*}P < 0.05$ vs CG; $^{\#}P < 0.05$ vs OAC.

6.4–68.8, $n = 10$, $P = 0.01$ [CG]; 184.2 ± 28, 95% CI −78.2–14.1, $n = 9$, $P = 0.001$ [OAL]; 176.2 ± 30, 95% CI −38,22–70.27, $n = 9$, $P = 0.012$ [OAT]; 179.1 ± 14, 95% CI −72.27–9.87, $n = 10$, $P = 0.004$ [OATL]; one-way ANOVA, Tukey post-hoc; Fig. 3(B)).

Immunohistochemistry analysis

IL-1β expression

Immunohistochemistry evaluation demonstrated that IL-1β expression was not detected in CG. For all OA animals, IL-1β expression was mainly marked in the nucleus of the chondrocytes [Fig. 4(A)]. Moreover, the semi-quantitative analysis showed a lower IL-1β expression in CG (1.25 ± 0.19) compared to all of the other experimental groups (3.2 ± 0.53, 95% CI − 2.75–1.17, $n = 7$, $P < 0.0001$ [OAC]; 2.23 ± 0.48, 95% CI −1.74–0.20, $n = 7$, $P = 0.008$ [OAL]; 2.22 ± 0.41, 95% CI −1.73–0.19, $n = 7$, $P < 0.0087$ [OAT]; 2.1 ± 0.71, 95% CI −1.62–0.078, $n = 7$, $P < 0.025$ [OATL]; one-way ANOVA, Tukey post-hoc). Additionally, for OAC, IL-1β expression was significantly higher compared to OAL (95% CI 0.19–1.74, $P = 0.084$), OAT (95% CI 0.20–1.75, $P = 0.0078$) and OATL (95% CI 0.31–1.86; $P = 0.0025$; Fig. 4(B)). Similar findings for the OA treated groups were demonstrated.

Caspase-3 expression

Caspase-3 immunoexpression was observed mainly in the nucleous of the chondrocytes for all experimental groups (however, in a limited intensity for CG) [Fig. 5(A)]. Additionally, immunohistochemistry semi-quantitative analysis showed that a significantly lower caspase-3 expression was observed in CG (1.34 ± 0.32) compared to the other experimental groups (3.76 ± 0.27, 95% CI −3.01–1.80, $n = 7$, $P < 0.0001$ [OAC]; 2.54 ± 0.45, 95% CI −1.8–0.59, $n = 7$, $P < 0.0001$ [OAL]; 1.70 ± 0.43, 95 % CI −1.679–0.4608, $n = 7$, $P < 0.0001$ [OAT]; one-way ANOVA, Tukey post-hoc). For OAC a significantly higher expression of caspase-3 was observed compared to OAL (95% CI 0.61–1.82, $P < 0.0001$), OAT (95% CI 0.70 to 1.91, $P < 0.0001$) and OATL (95% CI 1.44–2.65, $P < 0.0001$). Furthermore, a lower caspase-3 expression was observed in OATL compared to OAL (95% CI 0.23–1.44, $P = 0.0032$) and OAT (95% CI 0.13–1.34, $P = 0.01$; Fig. 5(B)).

MMP-13 expression

MMP-13 expression was detected in the nucleus of chondrocytes in all groups, mainly for the OA animals [Fig. 6(A)]. Semi-quantitative analysis showed that CG (1.18 ± 0.21) presented a

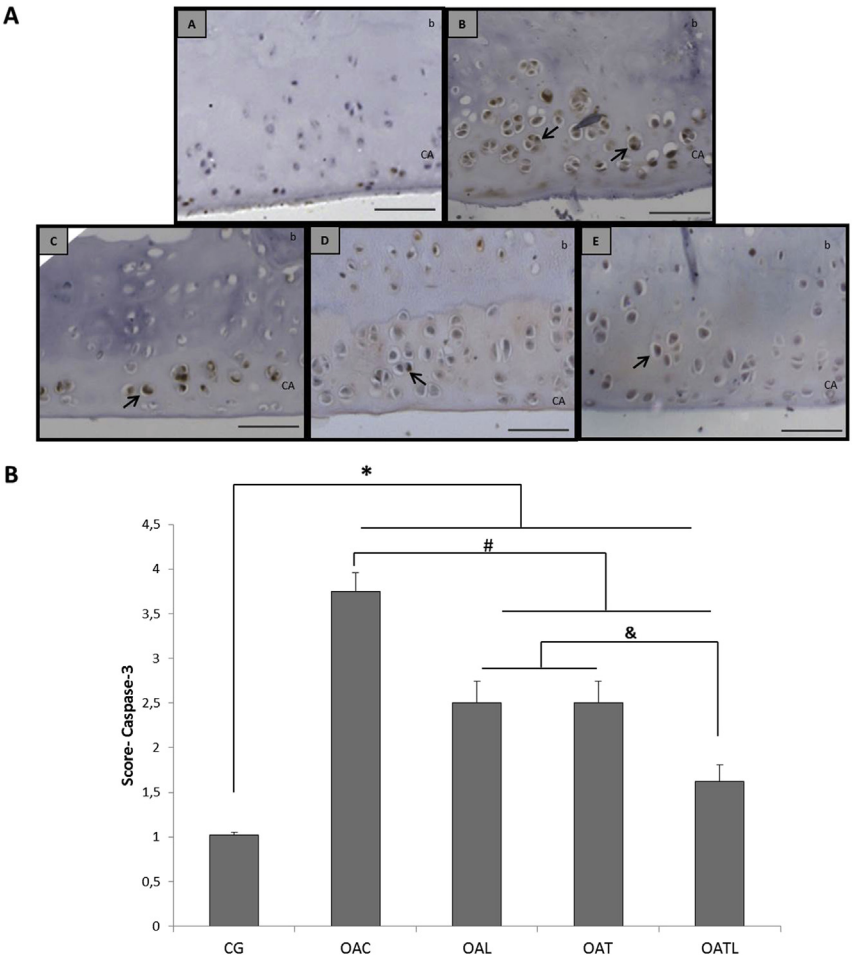

Fig. 5. (A) Representative sections of caspase-3 immunohistochemistry. Immunolabeled chondrocytes (arrow). (A) Control – CG; (B) Osteoarthritis – OAC; (C) Osteoarthritis plus exercise training – OAT; (D) Osteoarthritis plus LLLT – OAL; (E) Osteoarthritis plus exercise training associated with LLLT – OATL. (Scale Bar: 50 μm); **(B)** Results of semi-quantitative analysis of the caspase-3 expression $^{*}P < 0.0001$ vs CG; $^{\#}P < 0.05$ vs OAC; $^{\&}P < 0.05$ vs OATL.

lower expression of the immunmarker compared to the other experimental groups (3.20 ± 0.47, 95% CI −2.67–1.36, $n = 7$, $P < 0.0001$ [OAC]; 2.32 ± 0.36, 95% CI −1.79–0.48, $n = 7$, $P = 0.0002$ [OAL]; 2.35 ± 0.54, 95% CI −1.82–0.51, $n = 7$, $P = 0.008$ [OAT]; 2.22 ± 0.43, 95 % CI −1.69–0.38, $n = 7$, $P = 0.0251$ [OATL], one-way ANOVA, Tukey post-hoc). Also, it is possible to observe in Fig. 6(B), a lower MMP-13 immunostainning in OAL (95% CI 0.22–1.52, $P = 0.004$), OAT (95% CI 0.19–1.53, $P = 0.0059$) and OATL (95% CI 0.32–1.63, $P = 0.0013$) compared to OAC. No other difference was observed between the treated groups.

Discussion

This study aimed to evaluate the articular cartilage response to an aerobic exercise training and LLLT (associated or not), in an experimental model of OA in the knee of rats. The results of the present study demonstrated that signs of tissue degeneration, increased number of chondrocytes and a decrease of thickness were observed in OAC 12-weeks post-surgery. The histological findings demonstrated that all treated groups showed a better pattern of tissue organization, with less fibrillation and irregularities along the articular surface and with a similar chondrocyte organization compared to CG. Furthermore, a lower OARSI score and higher thickness values were shown in the treated groups compared to OAC. Immunohistochemistry analysis demonstrated that IL1-B, caspase-3 and MMP-13 expression were also lower in the treated groups compared to the osteoarthritic animals without treatments. Moreover, LLLT was able of decreasing the expression of caspase-3 in OA animals compared to the other treated groups.

The high prevalence of OA highlighted the importance of the development of improved therapeutic strategies able of treating this disease[28]. In this context, experimental and clinical studies have investigated the effects of physical exercise training and electrophysical treatments, such as LLLT, on osteoarthritic cartilage tissue[20–23]. Several authors have suggested that exercises such as joint-specific strength and general aerobic conditioning are effective for OA treatment[10,14,15]. Moreover, some authors demonstrated that LLLT was able of decreasing the level of cartilage damage and producing a better tissue structure, biomodulating the inflammatory process and reducing swelling[2,27,29,30].

Histological findings (qualitative analysis and OARSI) showed that both treatments, used isolated or in association, were efficient to avoid the progression of the degenerative process related to OA. The initial signs of tissue modification related to OA can be characterized by degeneration of the cartilage, fibrillation and abnormal cell proliferation[5,31]. It is well known that exercise training affects the articular cartilage metabolism and modifies the cartilaginous structure by a mechanotransduction response[32]. Biomechanical stimulus generated by dynamic compression during a moderate exercise, can reduce the synthesis of proteolytic enzymes,

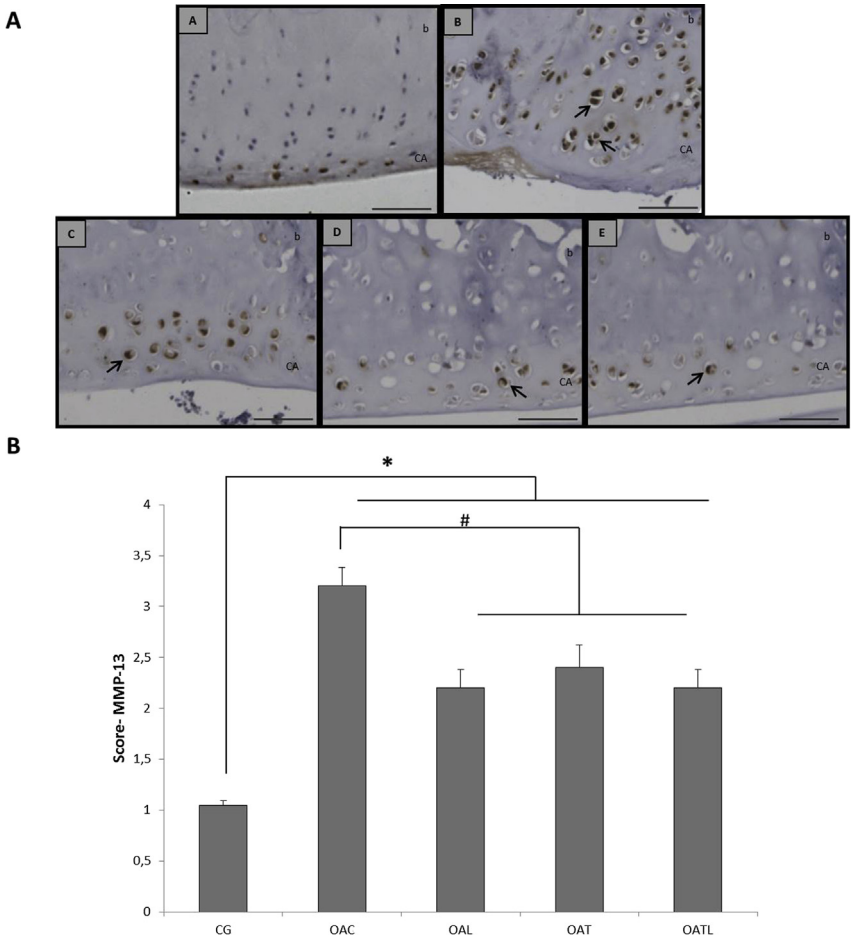

Fig. 6. (A) Representative sections of MMP-13 immunohistochemistry. Immunolabeled chondrocytes (arrow). (A) Control – CG; (B) Osteoarthritis – OAC; (C) Osteoarthritis plus exercise training – OAT; (D) Osteoarthritis plus LLLT – OAL; (E) Osteoarthritis plus exercise training associated with LLLT – OATL. (Scale Bar: 50 μm). **(B)** Results of semi-quantitative analysis of the caspase-3 expression $^{*}P < 0.05$ vs CG; $^{\#}P < 0.05$ vs OAC.

regulating the metabolic balance and preventing the progression of the disease[33,34]. In this context, it may be suggested that aerobic exercise training used in the present study had protective and anabolic effects, minimizing the articular degenerative process and increasing the deposition of proteoglycans and collagen[32,33]. Similarly, the laser treated animals also presented a biomodulation of the degenerative process related to the OA. Possibly, laser energy acted on the stimulation of tissue metabolism, preventing the evolution of the degenerative process in the articular cartilage. These results are in agreement with Oliveira *et al.*[11] and Gottlieb *et al.*[35] who also observed a decreased of the joint damage and an increased amount of proteoglycans in osteoarthritic cartilage of rabbit and rats, respectively. Interestingly, the association of both treatments did not present any additional effect the morphological aspects of the OA knees.

Furthermore, the initial phase of cartilage injury is marked by the action of inflammatory cytokines and increased chondrocyte metabolic rate to try to prevent the degradation of the tissue[36,37]. This phase is followed by an abnormal increase in the number of cells, with cellular disorganization and cell death by apoptosis[5,31,38]. The morphometric analysis demonstrated that the chondrocytes number was similar between all the OA treated groups and CG. Moreover, treated groups showed a lower cartilage thickness compared to the OA control. At an early stage of the OA process, there is an apparent increase in cartilage volume due to swelling, which supports a pathophysiological role of inflammation, followed by a significant decrease[39]. This phenomenon may be related to physical exercise training and LLLT capacity of modulating cell metabolism in an attempt to delay the evolution of the degenerative process in the articular cartilage.

In addition, during the course of OA, many inflammatory mediators are expressed[37]. Biochemical pathways are activated within joint tissues in OA especially IL-1β and Caspase 3. These cytokines induce chondrocytes to produce proteases to drive catabolic pathways, inhibit matrix synthesis and promote cellular apoptosis[36]. Also, the degradation of the articular cartilage matrix is related to the increased of collagenases and matrix metalloproteinase (MMP), mainly MMP-1, 8 and 13[31,40]. In the present study, both the exercise training and LLLT were able of decreasing the expression of IL-1β, Caspase 3 and MMP-13. These findings corroborate those authors who affirm that mechanical and photochemical stimulus induced by exercise and LLLT present anti-inflammatory effects in OA experimental models[15,18,27]. Furthermore, some studies have evidenced that physical exercise and LLLT exert chondroprotective properties through its anti-apoptotic capacities[14,41,42]. Interestingly, the exercise training associated with LLLT was able of reducing the expression of caspase-3 when compared to other treatments. Thus, the highest down-regulation of caspase-3 observed in OATL leads us to infer that the association of both therapies may have provided a direct positive effect on the cell death in OA pathogenesis, reducing the severity of cartilage lesions in experimental OA.

In this context, the aerobic exercise training and LLLT might be promising to improve the cartilage tissue regeneration in degenerative conditions. However, before studying the present exercise training in OA patients, some modifications such as the intensity and duration of each session, needs to be done to adapt better to the physical conditions of volunteers. Furthermore, the association of both treatments did not produce any significant extra effect on cartilage tissue. As this study was limited to relatively short-term evaluation of the performance of physical exercise and LLLT under optimal conditions to provide full control over the degenerative process, information on the long-term performance of this therapeutic modality remains to be provided.

Conclusion

In conclusion, our findings suggest that the aerobic exercise training and the 808 nm laser prevented articular degenerative morphological modifications and modulated inflammatory process in OA rats. Consequently, these data highlight the potential of both therapies to be used as promising effective therapeutic strategies to treat OA in the clinical setting. Further additional studies are needed to understand the molecular interaction of both therapies in the presence of degenerative articular diseases and to evaluate different protocols of physical exercise and laser parameters to define the ideal protocol of treatment to be used in patients with this degenerative disease. Such future studies will undoubtedly contribute to a better understanding of the safety and efficacy of LLLT and physical exercise in clinical OA.

Authors' contributions

LA and LPM contributed equally to the conception and design, acquisition of data, analysis and interpretation of data and drafting the manuscript. TA, HWK, CT and KR were involved in data interpretation, statistical analysis and manuscript preparation. ACMR conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors contributed to revising the manuscript critically for important intellectual content, and read and approved the manuscript for publication.

Conflict of interest

No competing financial interests exist.

Acknowledgements

We thank to Brazilian funding agencies Fapesp and CNPq for the financial support of this research (grant number: FAPESP-2012-20145-0).

References

1. Fibel KH, Hillstrom HJ, Halpern BC. State-of-the-Art management of knee osteoarthritis. World J Clin Cases 2015;16(3):89–101.
2. Hamblin MR. Can osteoarthritis be treated with light? Arthritis Res Ther 2013;29(15):120.
3. Bijlsma JW, Berenbaum F, Lafeber FP. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011;18(377):2115–26.
4. Aguiar GC, Do Nascimento MR, De Miranda AS, Rocha NP, Teixeira AL, Scalzo PL. Effects of an exercise therapy protocol on inflammatory markers, perception of pain, and physical performance in individuals with knee osteoarthritis. Rheumatol Int 2015;35:525–31.
5. Pritzker KP, Gay S, Jimenez SA, Ostergaard K, Pelletier JP, Revell PA, *et al*. Osteoarthritis cartilage histopathology: grading and staging. Osteoarthritis Cartilage 2006;14:13–29.
6. Maly MR, Robbins SM. Osteoarthritis year in review 2014: rehabilitation and outcomes. Osteoarthritis Cartilage 2014;22:1958–88.
7. Assis L, Almeida T, Milares LP, Dos Passos N, Araújo B, Bublitz C, *et al*. Musculoskeletal atrophy in an experimental model of knee osteoarthritis: the effects of exercise training and low-level laser therapy. Am J Phys Med Rehabil 2014;(8) [Epub ahead of print].
8. Sutton AJ, Muir KR, Mockett S, Fentem P. A case-control study to investigate the relation between low and moderate levels of physical activity and osteoarthritis of the knee using data collected as part of the Allied Dunbar National Fitness Survey. Ann Rheum Dis 2001;60:756–64.
9. Cifuentes DJ, Rocha LG, Silva LA, Brito AC, Rueff-Barroso CR, Porto LC, *et al*. Decrease in oxidative stress and histological changes induced by physical exercise calibrated in rats with osteoarthritis induced by monosodium iodoacetate. Osteoarthritis Cartilage 2010;18:1088–95.
10. Clausen B, Holsgaard-Larsen A, Søndergaard J, Christensen R, Andriacchi TP, Roos EM. The effect on knee-joint load of instruction in analgesic use compared with neuromuscular exercise in patients with knee osteoarthritis: study protocol for a randomized, single-blind, controlled trial (the EXERPHARMA trial). Trials 2014;15(15):444.
11. Fransen M, McConnell S, Harmer AR, Van der Esch M, Simic M, Bennell KL. Exercise for osteoarthritis of the knee. Cochrane Database Syst Rev 2015;9:1. CD004376.
12. Fernandes L, Hagen KB, Bijlsma JW, Andreassen O, Christensen P, Conaghan PG, *et al*. European League Against Rheumatism (EULAR). EULAR recommendations for the non-pharmacological core management of hip and knee osteoarthritis. Ann Rheum Dis 2013;72:1125–35.
13. Zhang W, Nuki G, Moskowitz RW, Abramson S, Altman RD, Arden NK, *et al*. OARSI recommendations for the management of hip and knee osteoarthritis: part III: changes in evidence following systematic cumulative update of research published through January 2009. Osteoarthritis Cartilage 2010;18:476–99.
14. Galois L, Etienne S, Grossin L, Watrin-Pinzano A, Cournil-Henrionnet C, Loeuille D, *et al*. Dose-response relationship for exercise on severity of experimental osteoarthritis in rats: a pilot study. Osteoarthritis Cartilage 2004;12:779–86.
15. Zhang SL, Liu HQ, Xu XZ, Zhi J, Geng JJ, Chen J. Effects of exercise therapy on knee joint function and synovial fluid cytokine levels in patients with knee osteoarthritis. Mol Med Rep 2013;7:183–6.
16. Gomes WF, Lacerda AC, Mendonça VA, Arrieiro AN, Fonseca SF, Amorim MR, *et al*. Effect of aerobic training on plasma cytokines and soluble receptors in elderly women with knee osteoarthritis, in response to acute exercise. Clin Rheumatol 2012;31:759–66.
17. Samut G, Dinçer F, Özdemir O. The effect of isokinetic and aerobic exercises on serum interleukin-6 and tumor necrosis factor alpha levels, pain, and functional activity in patients with knee osteoarthritis. Mod Rheumatol 2015;27:1–6 [Epub ahead of print] PubMed PMID: 25849853.
18. Oliveira P, Santos AA, Rodrigues T, Tim CR, Pinto KZ, Magri AM, *et al*. Effects of phototherapy on cartilage structure and inflammatory markers in an experimental model of osteoarthritis. J Biomed Opt 2013;18:128004.
19. dos Santos SA, Alves AC, Leal-Junior EC, Albertini R, Vieira R de P, Ligeiro AP, *et al*. Comparative analysis of two low-level laser doses on the expression of inflammatory mediators and

on neutrophils and macrophages in acute joint inflammation. Lasers Med Sci 2014;29:1051–8.
20. Alves AC, Albertini R, dos Santos SA, Leal-Junior EC, Santana E, Serra AJ, *et al*. Effect of low-level laser therapy on metalloproteinase MMP-2 and MMP-9 production and percentage of collagen types I and III in a papain cartilage injury model. Lasers Med Sci 2014;29:911–9.
21. Paolillo AR, Paolillo FR, João JP, João HA, Bagnato VS. Synergic effects of ultrasound and laser on the pain relief in women with hand osteoarthritis. Lasers Med Sci 2015;30:279–86.
22. Moon CH, Kwon O, Woo CH, Ahn HD, Kwon YS, Park SJ, *et al*. Therapeutic effect of irradiation of magnetic infrared laser on osteoarthritis rat model. Photochem Photobiol 2014;90:1150–9.
23. Wang P, Liu C, Yang X, Zhou Y, Wei X, Ji Q, *et al*. Effects of low-level laser therapy on joint pain, synovitis, anabolic, and catabolic factors in a progressive osteoarthritis rabbit model. Lasers Med Sci 2014;29:1875–85.
24. Coelho C de F, Leal-Junior EC, Biasotto-Gonzalez DA, Bley AS, de Carvalho Pde T, Politti F, *et al*. Effectiveness of phototherapy incorporated into an exercise program for osteoarthritis of the knee: study protocol for a randomized controlled trial. Trials 2014;11(15):221.
25. Kheshie AR, Alayat MS, Ali MM. High-intensity versus low-level laser therapy in the treatment of patients with knee osteoarthritis: a randomized controlled trial. Lasers Med Sci 2014;29:1371–6.
26. Renner AF, Carvalho E, Soares E, Mattiello-Rosa S. The effect of a passive muscle stretching protocol on the articular cartilage. Osteoarthritis Cartilage 2006;14:196–202.
27. Bublitz C, Medalha C, Oliveira P, Assis L, Milares LP, Fernandes KR, *et al*. Low-level laser therapy prevents degenerative morphological changes in an experimental model of anterior cruciate ligament transection in rats. Lasers Med Sci 2014;29:1669–78.
28. Vincent KR, Conrad BP, Fregly BJ, Vincent HK. The pathophysiology of osteoarthritis: a mechanical perspective on the knee joint. PM R 2012;4(5 Suppl):S3–9.
29. Castano AP, Dai T, Yaroslavsky I, Cohen R, Apruzzese WA, Smotrich MH, *et al*. Low-level laser therapy for zymosan-induced arthritis in rats: importance of illumination time. Lasers Surg Med 2007;39:543–50.
30. Cho HJ, Lim SC, Kim SG, Kim YS, Kang SS, Choi SH, *et al*. Effect of low-level laser therapy on osteoarthropathy in rabbit. In Vivo 2004;18:585–91.
31. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007;213:626–34.
32. Knobloch TJ, Madhavan S, Nam J, Agarwal Jr S, Agarwal S. Regulation of chondrocytic gene expression by biomechanical signals. Crit Rev Eukaryot Gene Expr 2008;18:139–50.
33. Madhavan S, Anghelina M, Rath-Deschner B, Wypasek E, John A, Deschner J, *et al*. Biomechanical signals exert sustained attenuation of proinflammatory gene induction in articular chondrocytes. Osteoarthritis Cartilage 2006;14:1023–32.
34. Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. Curr Med Chem 2001;8(7):829–38.
35. Gottlieb T, Jorgensen B, Rohde E. The influence of irradiation low-level diode laser on the proteoglycan content in arthrotically cartilage in rabbits. Med laser Appl 2006;21:53–9.
36. Cuellar VG, Cuellar JM, Golish SR, Yeomans DC, Scuderi GJ. Cytokine profiling in acute anterior cruciate ligament injury. Arthroscopy 2010;26(10):1296–301.
37. Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediat Inflamm 2014;2014:561459.
38. Thomas CM, Fuller CJ, Whittles CE, Sharif M. Chondrocyte death by apoptosis is associated with cartilage matrix degradation. Osteoarthritis Cartilage 2007;15(1):27–34.
39. Ding L, Heying E, Nicholson N, Stroud NJ, Homandberg GA, Buckwalter JA, *et al*. Mechanical impact induces cartilage degradation via mitogen activated protein kinases. Osteoarthritis Cartilage 2010;18:1509–17.
40. Troeberg L, Nagase H. Proteases involved in cartilage matrix degradation in osteoarthritis. Biochim Biophys Acta 2012;1824:133–45.
41. Guo H, Luo Q, Zhang J, Lin H, Xia L, He C. Comparing different physical factors on serum TNF-α levels, chondrocyte apoptosis, caspase-3 and caspase-8 expression in osteoarthritis of the knee in rabbits. Joint Bone Spine 2011;78:604–10.
42. Lin HD, He CQ, Luo QL, Zhang JL, Zeng DX. The effect of low-level laser to apoptosis of chondrocyte and caspases expression, including caspase-8 and caspase-3 in rabbit surgery-induced model of knee osteoarthritis. Rheumatol Int 2012;32:759–66.