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

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A R T I C L E   I N F O

Article history:
Received 24 March 2015
Accepted 27 July 2015

Keywords:
Low-level laser therapy
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.

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Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the loss of articular cartilage, bone alterations and joint inflammation. The knee is the most frequently joint affected in OA and it is a major cause of pain and functional disability among older adults. This disease is characterized by progressive degeneration of the extracellular matrix of articular cartilage, subchondral bone remodeling and inflammation of periaricular tissues, culminating in chronic pain and reduction in functional capacity of the patient. 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.

OA treatment is based mainly in the use of analgesics, non-steroidal anti-inflammatory drugs and COX-2 inhibitors. 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. Among the innumerable range of therapeutic interventions, the positive effects of physical exercise programs on cartilage metabolism can be highlighted.

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. It is well established that regular physical
exercise can improve function and reduce pain and muscle fatigue. 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. According to Gomes et al., 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. Moreover, Samut et al. 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. A wide range of works have showed LLLT is effective in treating OA in both experimental models and clinical studies. Anti-inflammatory and chondroprotective effects and inhibition of cartilage degradation after laser-irradiation treatment were observed in several experimental model of OA in rats. 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.

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 determine 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 animals submitted to aerobic exercise training and LLLT treatment (OATL).

The knee OA induction was performed as previously described by Galois et al. 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.

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 cm² spot area, dose 50 J/cm², Irradiance 1.7 W/cm², 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.

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 fixed in 10% buffered formalin for 24 h, followed by decalcified in 10% ethylenediaminetetraacetic 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.
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 to 6 points) and OA stage (0 to 4 points). Samples were evaluated histopathologically for the presence of osteoarthritis grade (OARSI grades 0 to 6), osteoarthritis stage (OA stages 0 to 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 \(\mu\)m\(^2\), 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-1\(\beta\), MMP-13 and caspase-3 expression analysis the paraffin was removed with xylene from serial sections of 5 \(\mu\)m. 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-1\(\beta\) 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. 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 \(\mu\)m).

**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 \(\mu\)m) *\(P < 0.0001\) 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). 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 test was 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
6.4–68.8, n = 10, P = 0.01 [CG]; 184.2 ± 28, 95% CI –78.2–141.1, 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 –72.27–90.27, 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 immuneeexpression was observed mainly in the nucleus 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 –1.67–0.4608, 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 = 0.01; 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
lower expression of the immunomarker 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 immunostaining 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\(^20\)\(^\text{e}\)\(^23\). Several authors have suggested that exercises such as joint-specific strength and general aerobic conditioning are effective for OA treatment\(^10\)\(^\text{e}\)\(^14\)\(^\text{e}\)\(^15\). 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\(^2\)\(^3\)\(^27\)\(^29\)\(^30\).

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\(^3\)\(^3\)\(^1\). 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,
regulating the metabolic balance and preventing the progression of the disease\textsuperscript{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\textsuperscript{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.\textsuperscript{11} and Gottlieb et al.\textsuperscript{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\textsuperscript{36,37}. This phase is followed by an abnormal increase in the number of cells, with cellular disorganization and cell death by apoptosis\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{15,18,27}. Furthermore, some studies have evidenced that physical exercise and LLLT exert chondroprotective properties through its anti-apoptotic capacities\textsuperscript{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


