Lasers Med Sci
DOI 10.1007/s10103-016-2089-2

ORIGINAL ARTICLE

Photobiomodulation therapy in the modulation of inflammatory mediators and bradykinin receptors in an experimental model of acute osteoarthritis

Vanessa Lima Cavalcante de Oliveira[1] · José Antonio Silva Jr.[2] · Andrey Jorge Serra[3] · Rodney Capp Pallotta[3] · Evela Aparecida Pereira da Silva[3] · Anna Cristina de Farias Marques[1] · Regiane dos Santos Feliciano[2] · Rodrigo Labat Marcos[3] · Ernesto Cesar Pinto Leal-Junior[1,3] · Paulo de Tarso Camillo de Carvalho[1,3]

Received: 4 May 2016 / Accepted: 27 September 2016
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Abstract The objective of this study was to evaluate the effects of photobiomodulation therapy (PBMT) on inflammatory indicators, i.e., inflammatory mediators (TNF-α and CINC-1), and pain characterized by hyperalgesia and B1 and B2 receptor activation at 6, 24, and 48 h after papain-induced osteoarthritis (OA) in rats. Fifty-four rats were subjected to hyperalgesia evaluations and then divided randomly into three groups—a control group and two groups OA and OA PBMT group by using laser parameters at wavelength (808 nm), output power (50 mW), energy per point (4 Joules), power density (1.78 W/cm^2), laser beam (0.028 cm^2), and energy density (144 J/cm^2)—the induction of osteoarthritis was then performed with 20-μl injections of a 4 % papain solution dissolved in 10 μl of saline solution, to which 10 μl of cysteine solution (0.03 M). The statistical analysis was performed using two-way ANOVA with Bonferroni's post hoc test for comparisons between the 6, 24, and 48 h and team points within each group, and between the control, injury, and PBMT groups, and $p < 0.05$ was considered to indicate a significant difference. The hyperalgesia was evaluated at 6, 24, and 48 h after the injury. PBMT at a wavelength of 808 nm and doses of 4 J, administered afterward, promotes increase at the threshold of pressure stimulus at 6, 24, and 48 h after application and promote cytokine attenuation levels (TNF and CINC-1) and bradykinin receptor (B1 and B2) along the experimental period. We conclude that photobiomodulation therapy was able to promote the reduction of proinflammatory cytokines such as TNF-α and CINC-1, to reduce the gene and protein expression of the bradykinin receptor (B1 and B2), as well as increasing the stimulus response threshold of pressure in an experimental model of acute osteoarthritis

Keywords Osteoarthritis · Hyperalgesia · Cytokines · Photobiomodulation therapy

✉ Paulo de Tarso Camillo de Carvalho
paulo.tarso@uninove.br

[1] Postgraduate Program in Rehabilitation Sciences, Universidade Nove de Julho (UNINOVE), Rua Vergueiro 235, São Paulo, SP, Brazil

[2] Postgraduate Program in Medicine Universidade Nove de Julho (UNINOVE), São Paulo, SP, Brazil

[3] Postgraduate Program in Biophotonics, Universidade Nove de Julho (UNINOVE), São Paulo, SP, Brazil

Introduction

Osteoarthritis (OA) is a degenerative joint disease that affects most of the elderly population, causing chronic pain and joint disability. In particular, it is characterized by cartilage breakdown that is attributed to an imbalance between the synthetic (anabolic) and resorptive (catabolic) activities of resident chondrocytes and by synovial inflammation that is directly linked to clinical symptoms such as joint swelling, synovitis, and inflammatory pain [1, 2].

The inflammation of the synovial membrane that occurs in both the early and late phases of OA is associated with alterations in the adjacent cartilage that are similar to those seen in rheumatoid arthritis. Catabolic and pro-inflammatory mediators such as cytokines, nitric oxide, prostaglandin E2, and neuropeptides are produced by the inflamed synovium and alter the balance of cartilage

Published online: 10 October 2016

matrix degradation and repair, leading to excess production of the proteolytic enzymes responsible for cartilage breakdown [3, 4]. The spectrum of these factors includes different cytokines such as interleukins (IL-1P), (IL-6), and (TNF-α), chemokines such as (IL-8), numerous metalloproteinases (MMPs) (MMP-3, MMP-9, and MMP-13), prostaglandin E2 (PGE2), and nitric oxide (NO) [5]. Recent evidence suggests the involvement of the bradykinin (BK) B1 and B2 receptors in the pathophysiology of OA [2].

BK belongs to a family of oligopeptides derived from the enzymatic action of kallikreins on kininogens [5]. BK is a vasodilator and inflammatory nonapeptide, which is generated in the synovium in OA.

The kallikrein system is activated in several arthropathies such as osteoarthritis, rheumatoid arthritis, gout, and psoriatic arthritis. BK can contribute to inflammation and structural alterations in arthritic joints, as its intra-articular administration in animals causes excitation and sensitization of sensory nerves, evoking pain and hyperalgesia, leukocyte recruitment, and an increase in vascular permeability and vasodilation, producing local heating and edema [6].

It contributes to the initiation and maintenance inflammation, to excitation and sensitization of sensory nerve fibers, thus producing pain, and to activation of synoviocytes and chondrocytes, which are the main cells involved in maintaining the homeostasis of the synovial fluid and cartilage, respectively. Moreover, BK synergistically potentiates the effects produced by pro-inflammatory cytokines [7–9].

Several studies [10–13] have indicated that joint inflammation can be reduced by blocking bradykinin receptors, and according to Valenti et al. [6], kinin B2 receptor antagonists could represent a new class of anti-inflammatory drugs that may act synergistically with steroids in acute arthritis, suggesting the possibility of reducing their effective doses and side effects.

Photobiomodulation therapy (PBMT) is a therapy involving the application of low-power monochromatic coherent light to injuries and lesions. PBMT is used to treat pain, although the biological mechanisms of the beneficial results observed in clinical trials remain unclear [14].

Albertini et al. [14, 15] demonstrated the reduction of TNF-α cytokines, IL-1β, IL-6, and COX-2 in a rat paw edema model after a treatment with PBMT and concluded that PBMT has an anti-inflammatory effect, which may modulate transcription factors linked to pro-inflammatory cytokines and messenger RNA (mRNA) gene expression. In previous studies, we found that PBMT can reduce protein expression of cytokines IL-1β, IL-6, and TNF-α [16, 17] as well as that of MMPs 2 and 9 [18] in a model of inflammation-induced joint pain. Other researchers have also reported about the effect of PBMT in proinflammatory cytokines [19–21] and in its anti-inflammatory effects [22, 23] in different experimental and clinical models. In concordance with results indicating that PBMT can modulate the activity of cytokines, studies by Bortone et al. [24], Manchini et al. [25], and Silva et al. [26] have also demonstrated the effectiveness of PBMT in reducing B2 receptor expression, demonstrating that PBMT may attenuate expression of the B2 receptor.

Considering the important role of Kinin inflammation and its activity on different kinds of joint cells, attenuation of B2 receptor expression by PBMT may represent a new therapeutic strategy in OA. This study aimed to test the hypothesis that PBMT can reduce levels of pro-inflammatory cytokines and mononuclear cells, and modulate bradykinin levels and the B1 and B2 receptors, besides changing the mechanical hyperalgesia reaction. As the null hypothesis, photobiomodulation is not able to alter the inflammatory markers and bradykinin receptors B1 and B2, thus not changing the mechanical hyperalgesia reaction in rats subjected to experimental osteoarthritis. In this light, the objective of this study was to investigate the action of PBMT on hyperalgesia (nociception) pressure threshold, gene and protein expression of the B1 and B2 receptors, and monitor the expression of TNF-α, CINC-1 at the protein levels.

Materials and methods

Animals and ethics statement

The sample population consisted of 54 male Wistar rats (*Norvegicus albinus*), 90 days old, weighing 250–300 g. The animals were obtained from the animal housing facility from the Universidade Nove de Julho (Brazil), and they were kept under controlled light and temperature conditions, with free access to water and chow. Animals were handled in compliance with national guidelines for the human treatment of laboratory animals, and the Research Ethics Committee of the UNINOVE (AN 0016/2011) approved all experimental procedures.

Experimental groups

Animals ($n = 54$) were randomly distributed into three groups of 18 animals each. The first group (control) did not receive any kind of intervention, the second group (OA) received induction but did not receive any treatment, and the third group (OA PBMT) was treated with PBMT. All the three groups were evaluated at 6, 24, and 48 h post-injury (six animals per group, at each experimental time point).

Papain-induced inflammation

The animals were anesthetized with an intramuscular injection of a 7 % ketamine solution (Cetamin; Syntec, Cotia, SP,

Brazil) and 0.3 % xylene solution (Xilazin; Syntec Syntec, Cotia, SP, Brazil) at a proportion of 2:1 (0.2 ml/100 g). The OA induction was then performed following previously published methods [15, 16]. Specifically, 20-μl injections of a 4 % papain solution (Sigma–Aldrich, St. Louis, MO, USA) dissolved in 10 μl of saline solution (Aster Produtos Médicos Ltda, Sorocaba, SP, Brazil) to which 10 μl of cysteine solution (0.03 M) (Sigma–Aldrich, St. Louis, MO, USA); then prepared injections were administered in the right knee of the hind leg of each animal. This solution was used as the activator to produce cartilage injury.

Photobiomodulation therapy

The PBMT was applied using a gallium aluminum arsenide (GaAlAs) diode laser device with a wavelength of 808 nm from Photon Laser III DMC (Sao Carlos, SP, Brazil) was used. The dose and parameters are summarized in Table 1.

Irradiation

Laser irradiation was given transcutaneously at two points: medial and lateral. Laser irradiation was performed immediately after the papain-cysteine injection, on the right knee, at a power output of 50 mW. The control and injury groups received no treatment and served as the negative and positive control groups, respectively, for the comparative analysis. Animals were manually immobilized and were irradiated at an angle of 90° to the surface of the tissue [15].

Hyperalgesia evaluation

The evaluation was carried out before randomization of the groups and at 6, 24, and 48 h after the second papain induction. Mechanical hyperalgesia was evaluated with a digital analgesimeter (Insight, Ribeirão Preto, São Paulo, Brazil). The procedures were performed in a quiet room, with no sounds that might stimulate the animals. A trained, experienced examiner applied the tip to the plantar surface of the rat, at a 90° angle, through one of the hollow floor platforms. Pressure was increased linearly until the mouse showed a paw withdrawal response [27, 28].

Table 1 Summary of the laser parameters

Wave length (nm)	Output power (mW)	Power density (W/cm^2)	Laser beam (cm^2)	Energy density (J/cm^2)	Energy per point (J)	Irradiation time per point (s)
808	50	1.78	0.028	144	4	80

Euthanasia and sample collection

At the end of each experimental period (6, 24 and 48 h) the animals were identified, weighed and then euthanized by intraperitoneal administration of thiopental (Thiopentax, Cristália, Itapira, São Paulo, Brazil) at a dose of 100 mg/kg (DL), with lidocaine at 10 mg/ml (Xylestesin, Cristália Cristália, Itapira, São Paulo, Brazil). After euthanasia, a procedure for obtaining the washed articular synovial fluid was performed [16].

Evaluation of the B1 and B2 receptors and the cytokines TNF-α and CINC-1

The amount of B1 and B2 receptors (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and TNF-α and CINC-1 in washed articular synovium was quantified using an enzyme-linked immunosorbent assay, as per the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The manufacturer reports this kit, when run in accordance with standard Quantikine protocols, to be extremely sensitive (minimum detectable dose ranged from 2.2 to 18.3 pg/ml), specific (no significant cross-reactivity or interference was observed), precise (intra- and inter-assay CVs were 3.7 % and 6.7 %), and linear (all diluted samples fell with the dynamic range of the assay). Since sample concentrations were expected to fall outside the range of provided standards, serum was diluted 100-fold by adding 5 μl of sample to 495 μl of calibrator diluent [19].

B1 and B2 gene expression quantification

Total mRNA was isolated from cartilage by TRIzol reagent (*Trizol reagent*, *Gibco BRL*, *Gaithersburg*, MD, USA), according to the manufacturer's protocol. The mRNA was subjected to DNase I digestion, followed by reverse transcription to cDNA, as previously described [15]. PCR was performed in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the SYBRGreen core reaction kit (Applied Biosystems, Foster City, CA, USA) (Table 2).

Statistical analysis

The data were found to be normally distributed (Shapiro-Wilk test), and a two-way ANOVA with Bonferroni's post hoc test was used for comparisons between the 6-, 24-, and 48-h time points within each group, and between the control, injury, and PBMT groups. All data are expressed as the mean ± standard deviation (SD). The GraphPad Prism 5 software program (GraphPad Software, San Diego, CA, USA) was used, and $P < 0.05$ was considered to indicate a significant difference.

Table 2 Rat primers used for real-time PCR for mRNA quantification

Gene	Forward primer	Reverse primer	GenBank accession number
Kinin B1 receptor	5′-CCTTCCAGGCTT AAACGATTCTC-3′	5′-GGTTGGAGGATT GGAGCTCTAGA-3′	NM-030851.1
Kinin B2 receptor	5′-CCACCACGGCCT CTTTCAG-3′	5′-CGAACAGCACC CAGAGGAA-3′	NM-001270713.1
GAPDH	5′-TGCACCACCAAC TGCTTAGC-3′	5′-GCCCCACGGCC ATCA-3′	NM-017008

The calculation of sample power using the G * Power software found 80 % power for all outcomes analyzed.

Results

Hyperalgesia evaluation

At 6 h, hyperalgesia from OA group (17.20 ± 1.1) differed significantly ($P < 0.001$) from that observed in controls (22.74 ± 1.2). The control group differed significantly ($P < 0.001$) from the OA group; however, no significant difference was observed compared to the PBMT OA group ($P > 0.05$). At 24 h, significant differences were observed between the control group and the OA group ($P < 0.001$), and between the OA group and the PBMT OA group ($P < 0.001$). At 48 h, significant differences were observed ($P < 0.001$) between the control group (22.74 ± 1.2) and the OA group (18.02 ± 1.30), and between the OA group and the PBMT OA group (22.64 ± 0.9) ($P < 0.001$) (Fig. 1).

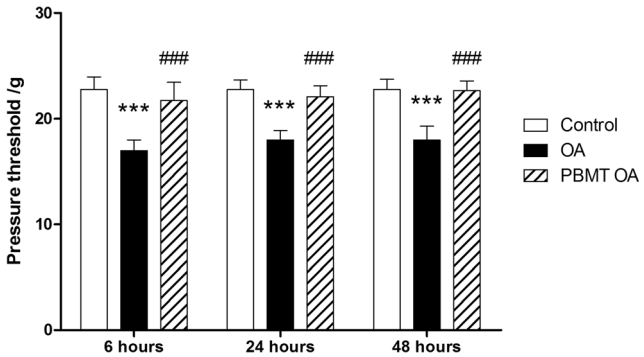

Fig. 1 Comparison of hyperalgesia in the knee joint before and after AO induction with papain, at 6, 24, and 48 h; data are shown as mean ± SD. The figure shows the basal pressure threshold, and the threshold at 6 h after treatment in OA; ***$P < 0.001$, using the Bonferroni test for comparison with the basal pressure threshold; ###$P < 0.001$ for comparisons with the OA group. We can still see the comparison in the 24-h experimental time, using the Bonferroni test for comparison with the basal pressure threshold ***$P < 0.001$ and ###$P < 0.001$ for comparisons with the OA group. For the experimental time of 48 h after treatment in OA, we observed using the Bonferroni test for comparison with the basal pressure threshold ***$P < 0.001$ and ###$P < 0.001$ for comparisons with the OA group

Quantification of TNF-α protein expression

Protein expression of TNF-α at 6 h showed significant differences ($P < 0.001$) from that observed at baseline (1.00 ± 0.07 pg/ml) from OA group (1.40 ± 0.10 pg/ml), but not in the PBMT OA group (0.96 ± 0.05 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group. At 24 h, significant differences were observed ($P < 0.001$) at baseline from OA group (1.68 ± 0.09 pg/ml), but not in the OA PBMT group (1.09 ± 0.10 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group. At 48 h, significant differences were observed ($P < 0.001$) at baseline from OA group (1.90 ± 0.10 pg/ml) and the OA PBMT group (1.17 ± 0.12 pg/ml) ($P < 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group (Fig. 2).

CINC-1 protein expression quantification

CINC-1 protein expression at 6 h showed significant differences ($P < 0.05$) from that observed at baseline from OA group (1.20 ± 0.10 pg/ml), but not from OA PBMT group (0.99 ± 0.05 pg/ml) ($P > 0.05$). Significant differences ($P < 0.01$) were also observed between the OA group and the PBMT OA group. At 24 h, there was a significant difference ($P < 0.001$) between the baseline and the OA group and between the baseline and the PBMT OA group ($P < 0.05$). The OA group showed no significant difference ($P > 0.05$) from the PBMT OA group. At 48 h, significant differences from baseline were observed from OA group (1.29 ± 0.20 pg/ml) ($P < 0.001$), but the OA PBMT group (1.02 ± 0.10 pg/ml) showed no significant difference ($P > 0.05$). The OA group showed a significant difference ($P < 0.001$) from the PBMT OA group (Fig. 3).

B1 protein expression

Protein expression of B1 at 6 h showed significant differences ($P < 0.001$) from that observed at baseline (1.00 ± 0.08 pg/ml) from OA group (1.27 ± 0.08 pg/ml), but not in the OA PBMT group (1.05 ± 0.09 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the

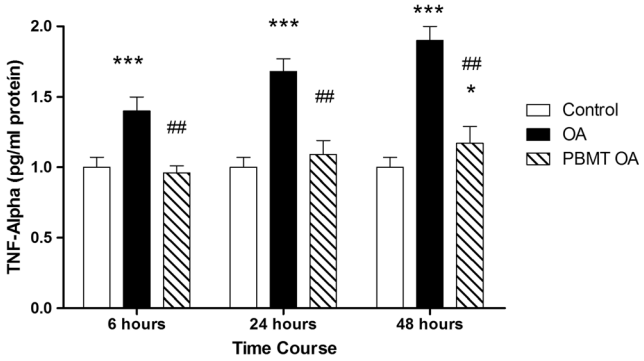

Fig. 2 Comparison of TNF-α level as measured using an enzyme-linked immunosorbent assay (ELISA). Protein concentrations in washed articular fluid after AO induction with papain are shown, at 6, 24, and 48 h; data are shown as mean ± SD. *$P < 0.05$ and ***$P < 0.001$ using the Bonferroni post hoc test for comparison with the control group

OA PBMT group. At 24 h, significant differences were observed ($P < 0.001$) from baseline from OA group (1.36 ± 0.09 pg/ml) but not from the OA PBMT group (1.09 ± 0.10 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group. At 48 h, significant differences were observed ($P < 0.001$) at baseline from the OA group (1.40 ± 0.05 pg/ml) but not from the OA PBMT group (1.10 ± 0.08 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group (Fig. 4a).

B2 protein expression

B2 protein expression at 6 h showed significant differences ($P < 0.001$) from that observed at baseline (1.00 ± 0.05 pg/ml) from OA group (1.28 ± 0.02 pg/ml), but not from OA PBMT group (1.10 ± 0.09 pg/ml) ($P > 0.05$). Significant differences ($P < 0.01$) were also observed between the OA group and the PBMT OA group. At 24 h, significant differences were

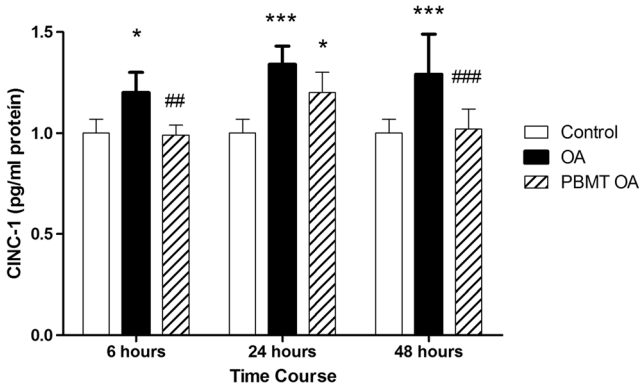

Fig. 3 Comparison of CINC-1 concentrations as measured using an enzyme-linked immunosorbent assay (ELISA). Protein concentrations in washed articular fluid after AO induction with papain are shown, at 6, 24, and 48 h; data are shown as mean ± SD. *$P < 0.05$ and ***$P < 0.001$using the Bonferroni post hoc test for comparison with the control group

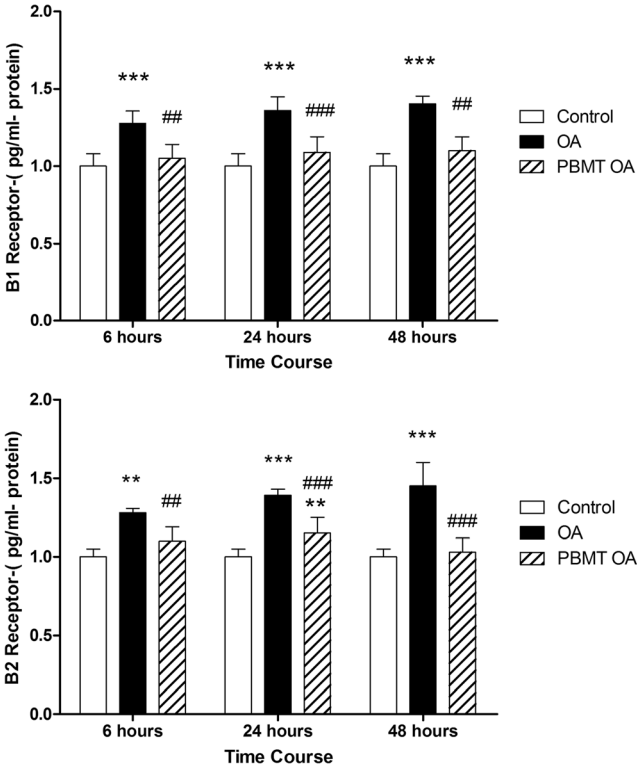

Fig. 4 Comparison of B1 and B2 concentrations as measured using an enzyme-linked immunosorbent assay (ELISA). Protein concentrations in washed articular fluid after AO induction with papain are shown, at 6, 24, and 48 h; data are shown as mean ± SD. *$P < 0.05$ and ***$P < 0.001$ using the Bonferroni post hoc test for comparison with the control group

observed ($P < 0.001$) at baseline from OA group (1.39 ± 0.03 pg/ml) and from OA PBMT group (1.15 ± 0.10 pg/ml, $P < 0.05$). Significant differences were also observed between the OA group and the PBMT OA group ($P < 0.001$). At 48 h, significant differences were observed ($P < 0.001$) at baseline from OA group (1.45 ± 0.15 pg/ml), but not from the OA PBMT group (1.03 ± 0.08 pg/ml) ($P > 0.05$). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group (Fig. 4b).

B1 gene expression quantification

B1 protein expression at 6 h was significantly different ($P < 0.001$) from that observed at control (1.0 ± 0.03) from OA (2.05 ± 0.15) and PBMT OA groups (0.63 ± 0.17). The control group showed a significant difference ($P < 0.001$) from the OA group, but not from the PBMT OA group ($P > 0.05$). At 24 h, the OA group differed significantly from the PBMT OA group ($P < 0.001$). At 48 h, the OA group (9.86 ± 0.50) and the PBMT OA group (2.10 ± 0.90) differed significantly ($P < 0.001$) from the control group (1.0 ± 0.03). Significant differences were also observed between the OA and the PBMT OA groups ($P < 0.001$) (Fig. 5).

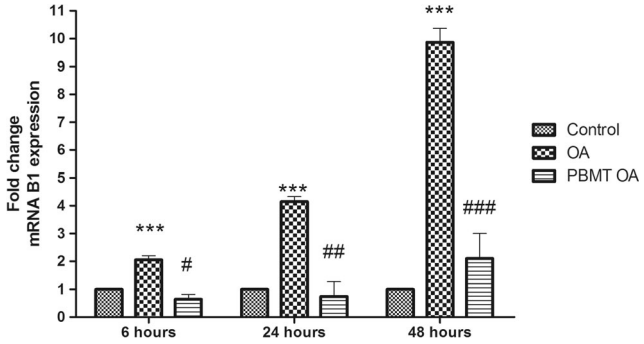

Fig. 5 Comparison of B1 levels as measured using real-time PCR for mRNA quantification. Gene concentrations in washed articular fluid after AO induction with papain are shown, at 6, 24, and 48 h; data are shown as mean ± SD. *$P < 0.05$ and ***$P < 0.001$ using the Bonferroni post hoc test for comparison with the control group

B2 gene expression quantification

B2 protein expression at 6 h showed significant differences ($P < 0.001$) from that observed at baseline (1.00 ± 0.10 pg/ml) from OA group (1.37 ± 0.08 pg/ml), but not from OA PBMT group (1.14 ± 0.13 pg/ml) ($P > 0.05$). Significant differences ($P < 0.01$) were also observed between the OA group and the PBMT OA group. At 24 h, significant differences at baseline were observed ($P < 0.001$) in the OA (1.60 ± 0.04 pg/ml) and the OA PBMT groups (1.24 ± 0.07 pg/ml). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group. At 48 h, significant differences at baseline were observed ($P < 0.001$) from OA group (1.77 ± 0.11 pg/ml) and the OA PBMT group (1.19 ± 0.08 pg/ml). Significant differences ($P < 0.001$) were also observed between the OA group and the PBMT OA group (Fig. 6).

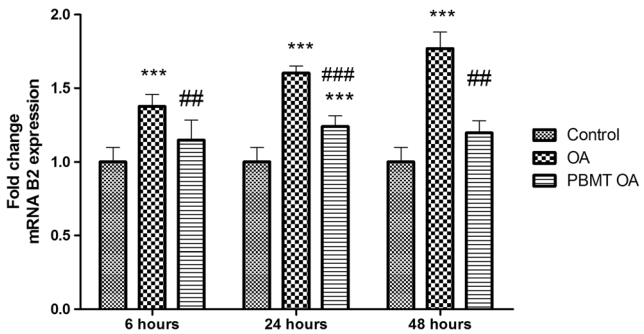

Fig. 6 Comparison of B2 levels as measured using real-time PCR for mRNA quantification. Gene concentrations in washed articular fluid after AO induction with papain are shown, at 6, 24, and 48 h; data are shown as mean ± SD. *$P < 0.05$ and ***$P < 0.001$ using the Bonferroni post hoc test for comparison with the control group

Discussion

Synovial membrane inflammation plays an important role in the OA pathophysiology. Mononuclear cells infiltration and proinflammatory cytokines production and other mediators of inflammation and joint pain characterize the initial OA stages. OA pain is often comprised of hyperalgesia, referred pain, and ongoing pain. PBMT therapy has great potential utility in this regard, since several studies have shown that it can regulate expression of the inflammatory mediator interleukin and can reduce the inflammatory signs and symptoms that are present in osteoarthritis.

Preclinical studies conducted by our and other research groups [17, 18, 20, 29] have shown that PBMT can decrease cytokines levels such as IL-1β and TNF, resulting in decreased expression of MMPs and increased collagen [18, 29]. Several clinical studies have also been conducted on the ability of PBMT to reduce pain in chronic conditions such as OA [30]. This study aimed to test the hypothesis that PBMT can reduce levels of pro-inflammatory cytokines and mononuclear cells, and modulate bradykinin levels and the B1 and B2 receptors, besides changing the mechanical hyperalgesia reaction.

Nociceptors sensitization occurs as a secondary process, following the initiation of a cytokine cascade. During inflammation, TNF-α release induces the liberation of IL-1β and IL-6, as well as the production of cyclooxygenase products. TNF-α may also trigger the release of IL-8, which induces the production of sympathomimetic mediators.

In rats, induced mechanical hypernociception is mediated by the release of a cytokine cascade initiated by bradykinin, which is rapidly generated when plasma components reach the extravascular tissue, although neutrophils are able to produce and release cytokines (TNF-α, IL-1α, and CINC-1/CXCL1) involved in the mediation of hypernociception that might be released by resident cells such as macrophages and mast cells. Indeed, these cells are able to release cytokines when activated by inflammatory stimuli [31]. In this study, we conducted a protein expression analysis of TNF-α, cytokines, and cytokine-induced neutrophil chemoattractant 1 (CINC-1) in washed coordinate at 6, 24, and 48 h. We observed a significant reduction of the expression of both cytokines (TNF-α and CINC-1) in the AO PBMT group compared with the AO group, which did not receive PBMT.

Alves et al. [18] and dos Santos et al. [17] have shown a reduction in TNF-α levels, using the same dose of PBMT that was effective in reducing intra-articular TNF-α levels. In this study, we also measured hypernociception of the inflamed joint, using an electronic version of the von Frey hair test, in which the force required to evoke a behavioral withdrawal is automatically recorded by an electronic pressure meter. We observed that the AO PBMT group showed a gradual reduction of hypernociception over time (6, 24, and 48 h), but the AO group did not.

TNF-α is considered to be a pivotal cytokine in the hypernociception of inflamed joints [24]. For instance, during inflammation, TNF-α release induces the liberation of IL-1β and IL-6 as well as the production of cyclooxygenase products. TNF-α may also trigger the release of IL-8, which induces the production of sympathomimetic mediators. Rats do not express IL-8, but the chemokine CINC-1 is considered homolog of IL-8 in rats, and CINC-1 and IL-8 are ligands for the same receptor [31].

In rats, the release of TNF-α is preceded by the generation of BK. Thus, BK stimulates the release of TNF-α, which in turn stimulates two distinct hypernociceptive pathways. TNF-α stimulates IL-1β production, which induces the expression of COX-2, which is responsible for prostanoid biosynthesis. TNF-α also stimulates release of CXC chemokines (CINC-1/IL-8), which induce the release of sympathomimetic amines [32].

In this study, we quantified the mRNA expression of the BK receptors, B1 and B2, since the literature demonstrates evidence of their role in both the hyperalgesia with joint inflammation.

Although the relative contribution of the B1 and B2 kinin receptors to the genesis of inflammatory pain is not completely understood, it has been demonstrated that the activation of B2 kinin receptors is associated with direct activation of small-diameter afferent nociceptive fibers that also induces the release of prostanoids and sympathetic amines, which trigger nociceptor sensitization [33].

The involvement of the B1 and B2 receptors in joint hyperalgesia was demonstrated in this study, since increased levels of the B1 and B2 receptors decreased the pressure required to trigger mechanical hyperalgesia. mRNA quantification in the joint wash fluid from control rats revealed that both B1 and B2 receptors are continuously synthesized, even in the absence of AO injury, and that there is a constant turnover of these receptors in washed articulate. An increase in the synthesis of both B1 and B2 receptors was identified 6 h after injection of papain in the knee joint. This upregulation was observed at all of the time points investigated (6, 24, and 48 h). The next step was to investigate whether the overexpression of the B1 and B2 receptors was also observed at the protein level, using the ELISA technique. We observed similar B1 and B2 overexpression at the protein level as at the gene level. However, the group treated with PBMT showed lower values for mRNA and protein expression of B1 and B2.

Our results for the bradykinin receptors B1 and B2 are similar to those reported by Bortone et al. [24], who evaluated mRNA expression of the B1 and B2 receptors in a rat experimental model of paw edema induced by carrageenan, with PBMT at two different wavelengths (660 and 684 nm). The authors concluded that PBMT at both wavelengths reduced the mRNA expression of the B1 and B2 receptors, producing an anti-inflammatory effect.

However, Manchini et al. [25] conducted a study of the effect of PBMT using an experimental model of myocardial infarction; PBMT was administered using the following parameters: wavelength = 660 nm, power = 15 mW, laser beam spot size = 0.785 cm^2, energy density = 22.5 J/cm^2, irradiation time = 60 s, and energy delivered = 1.1 J. The authors observed the effect of PBMT in reduction of the infarcted area and attenuation of systolic dysfunction, together with mRNA upregulation of the protective kinin B2 receptor, a reduction of the kinin receptor B1, and mRNA expression of pro-inflammatory interleukins. The authors concluded that PBMT treatment increased protein and mRNA levels of the B1 and B2 receptors, but the mRNA expression of kinin B2 receptors and the circulating levels of plasma kallikrein compared to non-treated post-myocardial infarction rats. mRNA expression of the kinin B1 receptor decreased after PBMT. It is noteworthy that the results obtained by Manchini [25] regarding B1 receptor are similar to our findings, even with different models and experimental times.

Our results show that PBMT, in addition to its activity in the modulation of cytokines such as TNF-α and CINC-1, can contribute to a reduction in the protein and mRNA expression of the B1 and B2 receptors, decreasing mechanical hyperalgesia.

This study also has limitations that prevent us from stating that PBMT directly influences the B1 and B2 receptors and is responsible for altering the intensity of hyperalgesia, as determined by the von Frey test; ideally, future study would use mouse models in which these receptors would be blocked, as controls.

Author contributions VO, PTCC, and AJS wrote the paper. Statistical analyses: ELJ, RLM, and PTCC. The design and performance of the experiments were overseen by Oversaw: VO, EAPS, RCP, and ACM.

Compliance with ethical standards

Role of the funding source This work was supported by grants from the São Paulo Research Foundation (FAPESP, grant number (2015/13677-4) and the National Council for Scientific and Technological (grant number 309065/2015-1).

Conflict of interest The authors have no conflict of interest.

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