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ORIGINAL ARTICLE

Low-level laser therapy (LLLT) reduces the COX-2 mRNA expression in both subplantar and total brain tissues in the model of peripheral inflammation induced by administration of carrageenan

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Abstract In the classical model of edema formation and hyperalgesia induced by carrageenan administration in rat paw, the increase in prostaglandin E2 (PGE_2) production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses. Prostaglandins are generated by the cyclooxygenase (COX). There are two distinct COX isoforms, COX-1 and COX-2. In inflammatory tissues, COX-2 is greatly expressed producing proinflammatory prostaglandins (PGs). Low-level laser therapy (LLLT) has been used in the treatment of inflammatory pathologies, reducing both pain and acute inflammatory process. Herein we studied the effect of LLLT on both COX-2 and COX-1 messenger RNA (mRNA) expression in either subplantar or brain tissues taken from rats treated with carrageenan. The experiment was designed as follows: A1 (saline), A2 (carrageenan—0.5 mg/paw), A3 (carrageenan—0.5 mg/paw + LLLT), A4 (carrageenan—1.0 mg/paw), and A5 (carrageenan—1.0 mg/paw + LLLT). Animals from the A3 and A5 groups were irradiated at 1 h after carrageenan administration, using a diode laser with an output power of 30 mW and a wavelength of 660 nm. The laser beam covered an area of 0.785 cm^2, resulting in an energy dosage of 7.5 J/cm^2. Both COX-2 and COX-1 mRNAs were measured by RT-PCR. Six hours after carrageenan administration, COX-2 mRNA expression was significantly increased both in the subplantar (2.2–4.1-fold) and total brain (8.65–13.79-fold) tissues. COX-1 mRNA expression was not changed. LLLT (7.5 J/cm^2) reduced significantly the COX-2 mRNA expression both in the subplantar (~2.5-fold) and brain (4.84–9.67-fold) tissues. The results show that LLLT is able to reduce COX-2 mRNA expression. It is possible that the mechanism of LLLT decreasing hyperalgesia is also related to its effect in reducing the COX-2 expression in the CNS.

Keywords COX-2 · COX-1 · LLLT · Peripheral inflammation · Carrageenan

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Introduction

The classical model of edema formation and hyperalgesia induced by carrageenan administration in the rat paw has been used in the development of nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase (COX)-2 inhibitors. It has been demonstrated that the increase in prostaglandin E_2 production in the central nervous system (CNS) contributes to the severity of the inflammatory and pain responses in this model [1]. Prostaglandins (PGs) are end products of fatty acid metabolism produced at elevated levels during pain and inflammation that are products of the COX pathway of arachidonic acid metabolism [2, 3]. There are two distinct COX isoforms, COX-1 and COX-2 [4]. COX-1 is expressed constitutively in most cell types, regulating normal physiological functions, while COX-2 is expressed in different types of activated cells, although it is also constitutively expressed in the brain and spinal cord [5, 6]. It has been

demonstrated that in inflammatory tissues, the inducible isoform, COX-2, is greatly expressed, producing proinflammatory prostaglandins [7, 8]. Ichitani et al. [9] demonstrated a strong expression of COX-2 messenger RNA (mRNA) in the spinal cord and other regions of the CNS, suggesting an augmented PG production inside and around the spinal cord under peripheral inflammatory process induced by carrageenan administration in the rat paw. The increase in the COX-2 mRNA expression in the spinal cord has been demonstrated by different authors, using the model of inflammation induced by endotoxin administration [10–12]. Also, an increase in the COX-2 expression has been demonstrated in spinal cord neurons and in other regions of the CNS in adjuvant-induced peripheral inflammation [6]. Carrageenan-induced inflammation is potentiated by intrathecal administration of prostaglandin E2 (PGE_2) [13], and the direct microinjection of PGE_2 in the brain promotes hyperalgesia [14]. Narita et al. [15] demonstrated that COX-2, but not COX-1, within the spinal cord plays an important role in the development of chronic pain following peripheral inflammation. Altogether, these studies indicate the upregulation of COX-2 in the CNS following peripheral inflammation induction. Using rats receiving injection of complete Freund's adjuvant (CFA) into the hind paw, a condition of hyperalgesia to mechanical stimuli was described, which was associated with an increase in the levels of PGE_2 and TNF-α in the cerebrospinal fluid (CSF) [16]. These data indicate that spinal PGE_2 is involved in pain initiated by acute peripheral inflammation.

Presenting minimal side effects, low-level laser therapy (LLLT) has been used in the treatment of inflammatory pathologies since it reduces both pain [17–21] and the acute inflammatory process [22–26]. It has been suggested that the ability of LLLT in reducing the inflammatory responses can be related to its effect decreasing the production of inflammatory factors, including COX-2 expression and its product, PGE_2 [22, 27–31]. Thus, in order to study the ability of LLLT in reducing both acute and peripheral inflammation induced by carrageenan, in the present work, we studied the effect of LLLT on the expression of both COX-2 and COX-1 mRNA, in either subplantar or total brain tissues obtained from rats submitted to the model of edema formation and hyperalgesia induced by carrageenan administration.

Materials and methods

Animals

All the experiments were carried out according to the guidelines of the University of Vale do Paraíba for animal care. The experiments were performed using male Wistar rats (150–200 g), supplied with food and water ad libitum provided by the Central Animal House of the Research and Development Department of the Vale do Paraíba University (UNIVAP). The rats were placed in appropriate cages and randomly divided into experimental groups with six animals per group.

Experimental groups

Rats received subplantar injections (0.05 or 0.1 ml) of carrageenan (Sigma Chemical Co., St Louis, MO, USA), using a stock concentration of 1 % (saline 0.85 %), in the left hind paw under brief anesthesia with halothane. The experiment was designed with 30 rats divided into five groups, hereafter designated as: A1 (control—saline), A2 (carrageenan—0.5 mg/paw), A3 (carrageenan—0.5 mg/paw + LLLT), A4 (carrageenan—1.0 mg/paw), and A5 (carrageenan—1.0 mg/paw + LLLT). The animals from the A3 and A5 groups were irradiated at 1 h after carrageenan administration.

Laser irradiation

A diode laser with an output power of 30 mW and a wavelength of 660 nm (model: laser unit, Kondortech) was used. The laser beam covered an area of 0.785 cm^2, resulting in an energy dosage of 7.5 J/cm^2. The time of irradiation used was 232 s, maintaining an application distance of 1.2 cm. Spectroscopic measurements were carried out with the laser, showing no thermal drift. The optical power was calibrated by using a Newport Multifunction Optical Meter (model 1835C).

RT-PCR

The animals were sacrificed 6 h after carrageenan subplantar administration, and both subplantar and total brain tissues were immediately removed from the rats and stored in liquid nitrogen until use. Total RNA was isolated from both subplantar and brain tissues by TRIzol reagent (Gibco BRL, Gaithersburg, MD), according to the manufacturer's protocol. The RNA was subjected to DNAse digestion, followed by reverse transcription to cDNA, as previously described [32]. PCR was performed in a 7000 Sequence Detection System (ABI Prism, Applied Biosystems, Foster City, CA) using the SYBRGreen core reaction kit (Applied Biosystems). The primers used were as follows: COX-1 forward primer 5′-TCATGTTCTGCATGTGGCTG-3′ and reverse primer 5′-GCAGCCATCTCCTTCTCTCC-3′ (GenBank accession number S67721.1); COX-2 forward primer 5′-TGTATGCT ACCATCTGGCTTCGG-3′ and reverse primer 5′-GTTTGG AACAGTCGCTCGTCATC-3′ (GenBank TM accession number J00691); and β-actin forward primer 5′-AAGTCC CTCACCCTCCCAAAAG-3′ and reverse primer 5′-AAGC AATGCTGTCACCTTCCC-3′ (GenBank accession number V01217.1). The PCR primer efficiencies were calculated using standard curves, and the relative expression levels of COX-2 in real time were analyzed using the $2^{\Delta\Delta Ct}$ method,

presented as the ratio to the expression of the housekeeping gene β-actin. Each sample was replicated twice from three independent sets of RNA preparations.

Statistical analysis

Statistical differences were evaluated by analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test to determine differences between groups. The results were considered significant when $p<0.005$.

Results

Effect of LLLT on both COX-2 and COX-1 mRNA expression in subplantar tissue

Initially, the expression of both COX-2 and COX-1 mRNA was determined in subplantar tissue obtained from animals submitted to a classical model of edema formation and hyperalgesia induced by carrageenan. Six hours after carrageenan-induced inflammation in rat paw, COX-2 mRNA expression was significantly increased in the subplantar tissue, when compared to the control group (Fig. 1). Compared to the control group, an increase in the "inducible isoform" COX-2 mRNA expression of ~2.2-fold and ~4.1-fold was observed in animals receiving subplantar administration of carrageenan 0.5 and 1.0 mg/paw, respectively. The levels of COX-2 mRNA expression were 0.309±0.081, 0.665±0.123, and 1.23±0.320, respectively, in the control group and in groups receiving either carrageenan 0.5 or 1.0 mg/paw. Figure 1 also shows that LLLT (7.5 J/cm^2) was able to reduce significantly

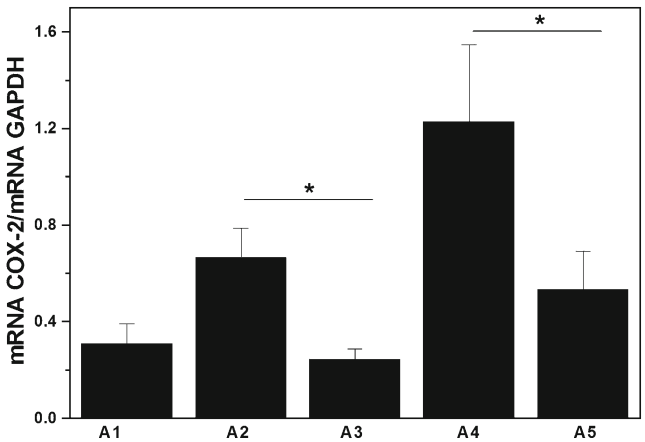

Fig. 1 Effect of LLLT on COX-2 mRNA expression in subplantar tissue obtained from animals receiving carrageenan in paw. The experimental conditions are described under "Materials and methods." The experimental groups were divided into five groups: A1 (control—saline), A2 (carrageenan—0.5 mg/paw), A3 (carrageenan—0.5 mg/paw + LLLT), A4 (carrageenan—1.0 mg/paw), and A5 (carrageenan—1.0 mg/paw + LLLT). The data are mean ± SE ($n=6$). Statistical analysis indicated $p<0.005$ (*)

the COX-2 mRNA expression in animals submitted to the classical model of edema formation and hyperalgesia induced by carrageenan. In the animal group receiving carrageenan 0.5 mg/paw, the LLLT promoted a decrease in COX-2 mRNA expression of ~2.7-fold. The LLLT was able to reduce the COX-2 mRNA expression values from 0.665±0.123 to 0.243 ±0.044, similar values observed in the control group (Table 1). Also, in the animal group receiving carrageenan 1.0 mg/paw, LLLT decreased the COX-2 mRNA expression from 1.230± 0.320 to 0.532±0.160 (~2.3-fold) (Table 1). The COX-2 mRNA expression levels were not significantly different, comparing the control group and the experimental groups receiving both carrageenan 1.0 mg/paw and LLLT. These results showed the efficiency of LLLT in reducing the inflammatory process, probably due to the decrease in the expression of COX-2 mRNA, in animals receiving carrageenan either 0.5 or 1.0 mg/paw. The mRNA expression of the "constitutive isoform" COX-1 was also measured in the subplantar tissue obtained from animals receiving subplantar administration of carrageenan. Figure 2 shows that the values of COX-1 mRNA expression were similar to those observed in COX-2 mRNA expression in the control group. However, in contrast to that observed in COX-2 mRNA expression, the administration of carrageenan either 0.5 or 1.0 mg/paw was not able to promote significant changes in the COX-1 mRNA expression (Fig. 2 and Table 1). Also, LLLT did not modify significantly the expression of COX-1 mRNA. These results show that in the classical model of edema formation and hyperalgesia induced by carrageenan, the mRNA expression of the "constitutive isoform" COX-1 is not modified; however, the expression of the "inducible isoform" COX-2 presents a pronounced increase. In addition, LLLT was able to reduce the levels of COX-2 mRNA expression, although it did not modify the COX-1 mRNA expression.

Effect of LLLT on both COX-2 and COX-1 mRNA expression in brain tissue

An increase in the level of COX-2, both in the subplantar tissue and in the central nervous system (CNS) after inflammation induced by carrageenan, has been demonstrated. The increase in the expression of COX-2 mRNA in brain tissue obtained from rats submitted to the carrageenan-induced acute inflammation has been described, indicating their involvement in the CNS during peripheral inflammation.

Figure 3 shows a pronounced increase in the COX-2 mRNA expression in total brain tissue 6 h after carrageenan-induced inflammation in rat paw. An increase in the COX-2 mRNA expression of ~8.65-fold and ~13.79-fold was observed in animals receiving subplantar administration of carrageenan 0.5 and 1.0 mg/paw, respectively, compared to the control group. The levels of COX-2 mRNA expression were 0.703±0.155, 6.086±1.020, and 9.698±1.747, respectively,

Table 1 Summary of LLLT effect on both COX-1 and COX-2 mRNA expression in either subplantar or brain tissue

	A1	A2	A3	A4	A5
mRNA COX-1					
Subplantar tissue	0.304±0.07	0.260±0.08	0.294±0.06	0.477±0.12	0.346±0.12
Brain tissue	1.898±0.58	1.974±0.42	1.609±0.51	1.498±0.65	1.107±0.38
mRNA COX-2					
Subplantar tissue	0.309±0.08	0.665±0.12*	0.243±0.04**	1.230±0.32*	0.532±0.16**
Brain tissue	0.703±0.15	6.086±1.02*	1.256±0.43**	9.698±1.75*	1.003±0.24**

Statistical analysis indicated $p<0.005$

* represents statistical analysis comparing experimental groups (A2 or A4) to the control group (A1)

** represents statistical analysis comparing experimental groups: A3 to A2 and A5 to A4

in the control group and in the groups receiving either carrageenan 0.5 or 1.0 mg/paw. The increase in the COX-2 mRNA expression levels produced by carrageenan administration in the paw was greatly higher in brain tissue than in subplantar tissue (compare Figs. 1 and 3). The effect of LLLT on COX-2 mRNA expression in brain tissue was also determined. A significant decrease was observed in the COX-2 mRNA expression in animal groups submitted to carrageenan administration and treated with LLLT (Fig. 3). LLLT was able to reduce the COX-2 mRNA expression in brain tissue from 6.086±1.020 to 1.256±0.432 (~4.84-fold) in animals receiving carrageenan 0.5 mg/paw (Table 1). Curiously, the effect of LLLT to reduce the COX-2 mRNA expression in brain tissue was higher in animals receiving carrageenan 1.0 mg/paw, decreasing the COX-2 mRNA expression from 9.698±1.747 to 1.003±0.238 (~9.67-fold) (Table 1). Statistical analyses demonstrated that the COX-2 mRNA expression levels did not present significant differences comparing the control group to groups receiving carrageenan either 0.5 or 1.0 mg/paw and treated with LLLT. These results showed the efficiency of LLLT in reducing carrageenan-induced inflammation in rat paw, decreasing the local effects of acute inflammation at the same time as peripheral inflammation produced in this model.

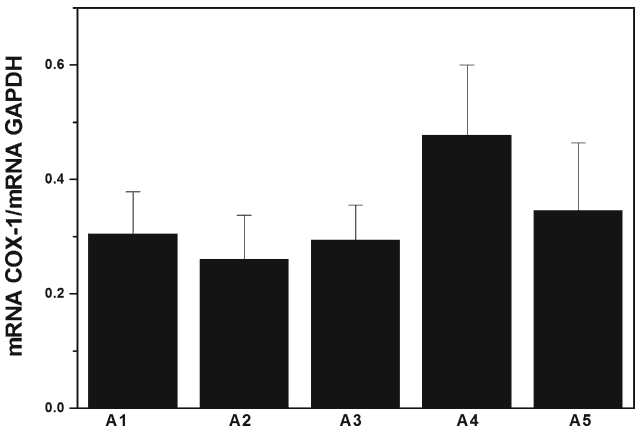

Fig. 2 Effect of LLLT on COX-1 mRNA expression in subplantar tissue obtained from animals receiving carrageenan in paw. The experimental conditions are described under "Materials and methods." The data are mean ± SE ($n=6$)

The expression of COX-1 isoform was also measured in brain tissue. Figure 4 shows that animals receiving carrageenan either 0.5 or 1.0 mg/paw did not present significant changes in the COX-1 mRNA expression compared to the control group. It was observed that LLLT did not modify the expression of COX-1 (Table 1). These results show for the first time the ability of LLLT in decreasing the expression of "inducible isoform" COX-2 mRNA in brain tissue from animals submitted to the classical model of edema formation and hyperalgesia induced by carrageenan.

Discussion

During inflammation, the central production of PGE_2, mediated by COX-2, represents an important contribution to hyperalgesia at the site of peripheral inflammation. Following carrageenan administration in the paw, the induction of COX-2 in the spinal cord and other regions of the CNS has been demonstrated [9, 33]. In addition, a pronounced increase in PGE_2 concentration was determined in the CSF, in the spinal cord, and in brain extracts after carrageenan-induced paw edema [33]. The administration of selective COX-2 inhibitors decreases both the levels of PGE_2 in the CSF and hyperalgesia [34–36]. These data demonstrated that following carrageenan administration in the paw, the increase in the COX-2 expression observed in the subplantar tissue is also observed in the CNS, promoting the production and release of high concentrations of PGE_2, contributing to hyperalgesia observed in peripheral inflammation. Our results corroborate these papers, showing an increase in the COX-2 mRNA expression, but not COX-1, in both subplantar and total brain tissues. As previously demonstrated [22], carrageenan administration was able to produce a significant increase in the expression of COX-2 mRNA in subplantar tissue. No effect was observed in COX-1 mRNA expression after carrageenan administration. These results are according to literature, indicating the evolvement

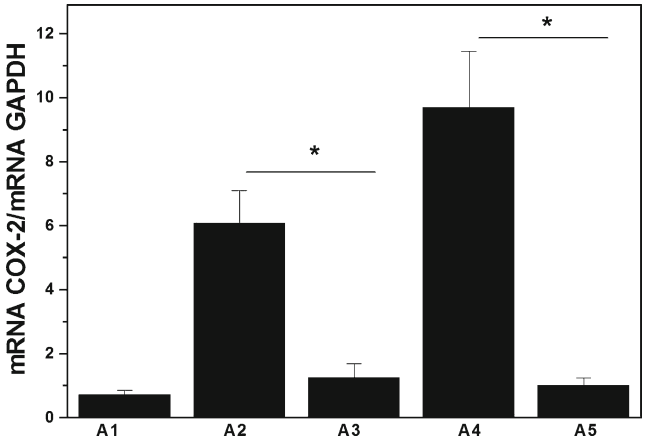

Fig. 3 Effect of LLLT on COX-2 mRNA expression in total brain tissue obtained from animals receiving carrageenan in paw. The experimental conditions are described under "Materials and methods." The data are mean ± SE (n=6). Statistical analysis indicated p<0.005 (*)

of the "inducible isoform" COX-2, but not the "constitutive isoform" COX-1 in the site of peripheral inflammation. The augment in the COX-2 mRNA expression was dose-dependent, being to a great extent pronounced in animals receiving carrageenan 1 mg/paw. In the CNS, the same profile was observed, showing the role of "inducible isoform" COX-2, but not COX-1, in the brain during peripheral inflammation. The evolvement of COX-2, but not of COX-1, in both PGE_2 increase and hyperalgesia has been previously demonstrated [9, 14–16, 34, 37–39].

The ability of LLLT in reducing inflammation responses can be related to its effect in decreasing the production of different inflammatory mediators, such as PGE_2, produced by COX-2 metabolism [22, 27–31]. In this work, we studied the effect of LLLT on the expression of both COX-2 and COX-1 mRNA, in both subplantar and brain tissues obtained from rats submitted to the model of edema formation and hyperalgesia induced by carrageenan administration. A marked decrease in the expression of COX-2 mRNA, but not COX-1 mRNA, was observed either in the subplantar or brain tissues. LLLT significantly reduced the levels of COX-2 mRNA to levels equivalent to the control group. The potential of LLLT in decreasing the inflammatory signals, such as edema formation, pain, and release of inflammatory mediators, has been extensively demonstrated [17–26]. Presenting minimal side effects, LLLT has been presented as a very efficient tool to reduce acute inflammation. Our results indicate that LLLT administrated directly in the site of inflammation is able to reduce COX-2 mRNA expression in the CNS and, consequently, the peripheral inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are nonselective COX inhibitors, presenting an inhibitory effect on both COX-1 and COX-2. Their side effects have been presented as a clinical limitation to the use of these drugs. Our results demonstrate evidence that LLLT presents specific effects on COX-2 expression, promoting no effect on COX-1 expression. This is a great positive property in LLLT administration, reducing specifically COX-2 expression and, consequently, the inflammation process, at the same time, not producing the negative effects observed when COX-1 is inhibited by NSAIDs use. We suggest that the PGE_2 release in both spinal cord and brain tissue can be reduced by decreasing the expression of COX-2 as in the site of inflammation as in the CNS following LLLT. It is possible that the mechanism of LLLT decreasing hyperalgesia is also related to its effect in reducing the COX-2 expression in the CNS leading to a decrease in the PGE_2 spinal cord release.

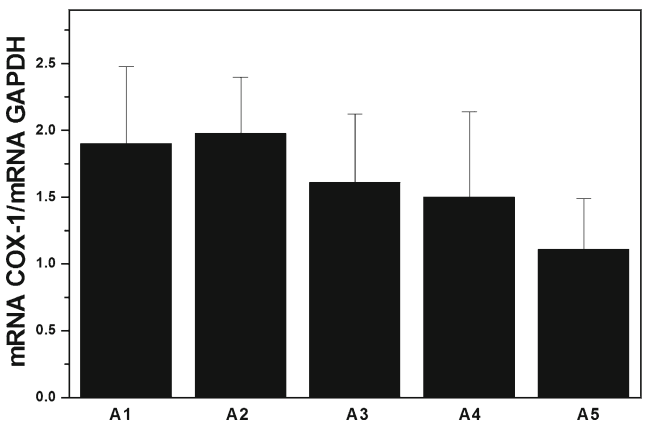

Fig. 4 Effect of LLLT on COX-1 mRNA expression in total brain tissue obtained from animals receiving carrageenan in paw. The experimental conditions are described under "Materials and methods." The data are mean ± SE (n=6)

Conclusion

We conclude that LLLT is able to reduce both acute and peripheral inflammation in animals submitted to the inflammation model induced by carrageenan administration. We suggest that LLLT is able to reduce both inflammation and hyperalgesia observed in this model, by reducing COX-2, but not COX-1 expression, both locally and centrally by decreasing PGE_2 release.

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References

1. Ibuki T, Matsumura K, Yamazaki Y, Nozaki T, Tanaka Y, Kobayashi S (2003) Cyclooxygenase-2 is induced in the endothelial cells throughout the central nervous system during carrageenan-induced hind paw inflammation; its possible role in hyperalgesia. J Neurochem 86(2):318–328
2. Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. Physiol Rev 79(4):1193–1226

3. Rao P, Knaus EE (2008) Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J Pharm Pharm Sci 11(2):81s–110s
4. Feng L, Sun W, Xia Y, Tang WW, Chanmugam P, Soyoola E, Wilson CB, Hwang D (1993) Cloning two isoforms of rat cyclooxygenase: differential regulation of their expression. Arch Biochem Biophys 307(2):361–368
5. Vane JR, Bakhle YS, Botting RM (1998) Cyclooxygenases 1 and 2. Ann Rev Pharmacol Toxicol 38:97–120
6. Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A, Poole S, Bonventre JV, Woolf CJ (2001) Interleukin-1 beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature 410:471–475
7. Masferrer JL, Seibert K, Zweifel B, Needleman P (1992) Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. Proc Natl Acad Sci U S A 89(9):3917–3921
8. Hla T, Neilson K (1992) Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci U S A 89(16):7384–7388
9. Ichitani Y, Shi T, Haeggstrom JZ, Samuelsson B, Hökfelt T (1997) Increased levels of cyclooxygenase-2 mRNA in the rat spinal cord after peripheral inflammation: an *in situ* hybridization study. Neuroreport 8:2949–2952
10. Schuligoi R, Ulcar R, Peskar BA, Amann R (2003) Effect of endotoxin treatment on the expression of cyclooxygenase-2 and prostaglandin synthases in spinal cord, dorsal root ganglia, and skin of rats. Neuroscience 116(4):1043–1052
11. Grill M, Heinemann A, Hoefler G, Peskar BA, Schuligoi R (2008) Effect of endotoxin treatment on the expression and localization of spinal cyclooxygenase, prostaglandin synthases, and PGD2 receptors. J Neurochem 104(5):1345–1357
12. Grill M, Peskar BA, Schuligoi R, Amann R (2006) Systemic inflammation induces COX-2 mediated prostaglandin D2 biosynthesis in mice spinal cord. Neuropharmacology 50(2):165–173
13. Daher JB, Tonussi CR (2003) A spinal mechanism for the peripheral anti-inflammatory action of indomethacin. Brain Res 962:207–212
14. Hosoi M, Oka T, Hori T (1997) Prostaglandin E receptor EP3 subtype is involved in thermal hyperalgesia through its actions in the preoptic hypothalamus and the diagonal band of Broca in rats. Pain 71(3): 303–311
15. Narita M, Shimamura M, Imai S, Kubota C, Yajima Y, Takagi T, Shiokawa M, Inoue T, Suzuki M, Suzuki T (2008) Role of interleukin-1beta and tumor necrosis factor-alpha-dependent expression of cyclooxygenase-2 mRNA in thermal hyperalgesia induced by chronic inflammation in mice. Neuroscience 152(2):477–486
16. Bianchi M, Martucci C, Ferrario P, Franchi S, Sacerdote P (2007) Increased tumor necrosis factor-alpha and prostaglandin E2 concentrations in the cerebrospinal fluid of rats with inflammatory hyperalgesia: the effects of analgesic drugs. Anesth Analg 104(4): 949–954
17. Walker JB (1983) Relief from chronic pain by low power laser irradiation. Neurosci Lett 43:339–344
18. Ribas ES, Paiva WS, Pinto NC, Yeng LT, Okada M, Fonoff ET, Chavantes MC, Teixeira MJ (2012) Use of low intensity laser treatment in neuropathic pain refractory to clinical treatment in amputation stumps. Int J Gen Med 5:739–742
19. Chow R, Armati P, Laakso EL, Bjordal JM, Baxter GD (2011) Inhibitory effects of laser irradiation on peripheral mammalian nerves and relevance to analgesic effects: a systematic review. Photomed Laser Surg 29(6):365–381
20. Hagiwara S, Iwasaka H, Hasegawa A, Noguchi T (2008) Preirradiation of blood by gallium aluminum arsenide (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. Anesth Analg 107(3):1058–1063
21. Hagiwara S, Iwasaka H, Okuda K, Noguchi T (2007) GaAlAs (830 nm) low-level laser enhances peripheral endogenous opioid analgesia in rats. Lasers Surg Med 39(10):797–802
22. Albertini R, Aimbire F, Villaverde AB, Silva JA Jr, Costa MS (2007) COX-2 mRNA expression decreases in the subplantar muscle of rat paw subjected to carrageenan-induced inflammation after low level laser therapy. Inflamm Res 56(6):228–229
23. Xavier M, de Souza RA, Pires VA, Santos AP, Aimbire F, Silva JA Jr, Albertini R, Villaverde AB (2014) Low-level light-emitting diode therapy increases mRNA expressions of IL-10 and type I and III collagens on Achilles tendinitis in rats. Lasers Med Sci 29(1):85–90
24. de Lima FM, Vitoretti L, Coelho F, Albertini R, Breithaupt-Faloppa AC, de Lima WT, Aimbire F (2013) Suppressive effect of low-level laser therapy on tracheal hyperresponsiveness and lung inflammation in rat subjected to intestinal ischemia and reperfusion. Lasers Med Sci 28(2):551–564
25. Bjordal JM, Lopes-Martins RA, Iversen VV (2006) A randomised, placebo controlled trial of low level laser therapy for activated Achilles tendinitis with microdialysis measurement of peritendinous prostaglandin E2 concentrations. Br J Sports Med 40:76–80
26. Fukuda TY, Tanji MM, Silva SR, Sato MN, Plapler H (2013) Infrared low-level diode laser on inflammatory process modulation in mice: pro- and anti-inflammatory cytokines. Lasers Med Sci 28(5):1305–1313
27. Lim W, Lee S, Kim I, Chung M, Kim M, Lim H, Park J, Kim O, Choi H (2007) The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors. Lasers Surg Med 39(7):614–621
28. Assis L, Moretti AI, Abrahão TB, Cury V, Souza HP, Hamblin MR, Parizotto NA (2012) Low-level laser therapy (808 nm) reduces inflammatory response and oxidative stress in rat tibialis anterior muscle after cryolesion. Lasers Surg Med 44(9):726–735
29. de Almeida P, Lopes-Martins RÁ, Tomazoni SS, Albuquerque-Pontes GM, Santos LA, Vanin AA, Frigo L, Vieira RP, Albertini R, de Tarso Camillo de Carvalho P, Leal-Junior EC (2013) Low-level laser therapy and sodium diclofenac in acute inflammatory response induced by skeletal muscle trauma: effects in muscle morphology and mRNA gene expression of inflammatory markers. Photochem Photobiol 89(2):501–507
30. Marcos RL, Leal-Junior EC, Arnold G, Magnenet V, Rahouadj R, Wang X, Demeurie F, Magdalou J, de Carvalho MH, Lopes-Martins RÁ (2012) Low-level laser therapy in collagenase-induced Achilles tendinitis in rats: analyses of biochemical and biomechanical aspects. J Orthop Res 30(12):1945–1951
31. Pires D, Xavier M, Araújo T, Silva JA Jr, Aimbire F, Albertini R (2011) Low-level laser therapy (LLLT; 780 nm) acts differently on mRNA expression of anti- and pro-inflammatory mediators in an experimental model of collagenase-induced tendinitis in rat. Lasers Med Sci 26(1):85–94
32. Arganaraz GA, Silva JA Jr, Perosa SR, Pessoa LG, Carvalho FF, Bascands JL, Bader M, da Silva TE, Amado D, Cavalheiro EA, Pesquero JB, da Graça N-MM (2004) The synthesis and distribution of the kinin B1 and B2 receptors are modified in the hippocampus of rats submitted to pilocarpine model of epilepsy. Brain Res 23:114–125
33. Guay J, Bateman K, Gordon R, Mancini J, Riendeau D (2004) Carrageenan-induced paw edema in rat elicits a predominant prostaglandin E2 (PGE2) response in the central nervous system associated with the induction of microsomal PGE2 synthase-1. J Biol Chem 279(23):24866–24872
34. Riendeau D, Percival MD, Boyce S, Brideau C, Charleson S, Cromlish W, Ethier D, Evans J, Falgueyret JP, Ford-Hutchinson AW, Gordon R, Greig G, Gresser M, Guay J, Kargman S, Léger S, Mancini JA, O'Neill G, Ouellet M, Rodger IW, Thérien M, Wang Z, Webb JK, Wong E, Chan CC et al (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. Br J Pharmacol 121(1):105–117
35. Francischi JN, Chaves CT, Moura AC, Lima AS, Rocha OA, Ferreira-Alves DL, Bakhle YS (2002) Selective inhibitors of cyclooxygenase-2 (COX-2) induce hypoalgesia in a rat paw model of inflammation. Br J Pharmacol 137(6):837–844

36. Doyle T, Chen Z, Muscoli C, Obeid LM, Salvemini D (2011) Intraplantar-injected ceramide in rats induces hyperalgesia through an NF-κB- and p38 kinase-dependent cyclooxygenase 2/prostaglandin E2 pathway. FASEB J 25(8):2782–2791
37. Yaksh TL, Dirig DM, Conway CM, Svensson C, Luo ZD, Isakson PC (2001) The acute antihyperalgesic action of nonsteroidal, anti-inflammatory drugs and release of spinal prostaglandin E2 is mediated by the inhibition of constitutive spinal cyclooxygenase-2 (COX-2) but not COX-1. J Neurosci 21(16):5847–5853
38. Dolan S, Hastie P, Crossan C, Nolan AM (2011) Co-induction of cyclooxygenase-2 [correction of cyclooxyenase-2] and early growth response gene (Egr-1) in spinal cord in a clinical model of persistent inflammation and hyperalgesia. Mol Pain 7:91–100
39. Lenz H, Raeder J, Draegni T, Heyerdahl F, Schmelz M, Stubhaug A (2011) Effects of COX inhibition on experimental pain and hyperalgesia during and after remifentanil infusion in humans. Pain 152(6):1289–1297