



15d-PGJ₂-loaded in nanocapsules enhance the antinociceptive properties into rat temporomandibular hypernociception

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ABSTRACT

Aims: To verify whether the nanoencapsulation of 15d-PGJ₂ in poly(D,L-lactide-co-glycolide) (PLGA) nanocapsules (15d-PGJ₂-NC) might potentialize its antinociceptive activity into rats' temporomandibular joint (TMJ).

Main methods: Transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to evaluate the morphology and suspension of the PLGA nanocapsules. Rats were pretreated (15 min) with an intra-TMJ injection of unloaded 15d-PGJ₂ or 15d-PGJ₂-NC at concentrations of 10, 100 or 1000 pg followed by an ipsilateral intra-TMJ injection of 1.5% formalin. The nociceptive behavioral response was observed during 45 min; animals were then sacrificed and the periarticular tissue was removed for IL-1 β measurements.

Key finding: TEM and AFM analyses showed that 15d-PGJ₂-NC is spherical without any aggregates or adhesion confirming that this formulation is a good drug carrier system for 15d-PGJ₂. Pretreatment with 15d-PGJ₂-NC (100 and 1000 pg/TMJ), but not unloaded 15d-PGJ₂, was found to significantly decrease the release of IL-1 β cytokine and the animals' nociceptive behavioral response induced by intra-TMJ injection of formalin.

Significance: The compound 15d-PGJ₂-NC might be used as a potential antinociceptive and anti-inflammatory agent to treat temporomandibular disorders in clinical practice.

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Introduction

Temporomandibular disorders (TMD) involve multifactorial etiology and might result in temporomandibular joint (TMJ) and/or masticatory muscle pain leading, in many cases, to chronic orofacial pain (Cairns, 2010). Since traditional strategies to control TMD-related pain are unsatisfactory, an alternative and efficient approach to treat such condition is of great interest to both patients and clinicians (Cairns, 2010). Therefore, the development of new drugs and/or new formulation to treat chronic inflammatory diseases continues to be of considerable importance to researchers (Bernardi et al., 2009).

Peroxisome proliferators-activated receptor- γ (PPAR γ) is a ligand-activated transcription factor of the nuclear hormone receptor superfamily (Escher and Wahli, 2000). Synthetic PPAR- γ agonists of the thiazolidinedione class act as insulin sensitizers and have become important in the treatment of type 2 diabetes (Lehrke and Lazar, 2005). PPAR ligands represent a promising therapeutic strategy for other diseases such as arthritis, sepsis, peritonitis, and colitis (Chima et al., 2008; Cuzzocrea et al., 2003; Kaplan et al., 2005; Napimoga et al., 2008a, 2008b; Shan et al., 2004), especially when it involves inflammatory pain (Pena-dos-Santos et al., 2009). Otherwise, PPAR- γ agonists are neuroprotective in animal models of acute central nervous system injury including focal ischemia, spinal cord injury and surgical trauma (Hyong et al., 2008; McTigue et al., 2007; Park et al., 2007; Pereira et al., 2006; Sundararajan et al., 2005; Tureyen et al., 2007; Zhao et al., 2005, 2006). Considering the neuroprotective effect of PPAR- γ agonists, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), which is a natural ligand for PPAR- γ (Ricote et al., 1998; Schoonjans et al., 1997), was found to have a peripheral antinociceptive effect on the TMJ via

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PPAR- γ with the co-participation of κ/δ opioid receptors (Pena-dos-Santos et al., 2009).

Nanomedicine has emerged as a new field of study and is considered one of the most promising pathways for the development of effective targeted therapies (Huynh et al., 2009). Polymeric nanoparticles are colloidal structures below 1 μm and have been used to encapsulate lipophilic drugs to target organs and/or tissues, to avoid drug degradation, to improve its efficacy, and to circumvent its toxicity (Adair et al., 2010; Couvreur et al., 2002). Nanoencapsulation of drugs was observed to greatly prolong their pharmacological activity and decrease their toxicity (Alves et al., 2011; Bernardi et al., 2009; Elron-Gross et al., 2009; Grillo et al., 2010). In particular, the systemic administration of 15d-PGJ₂-NC, when compared to unloaded 15d-PGJ₂, was found to improve the latency and the anti-inflammatory effect of the 15d-PGJ₂ at a much smaller dose (Alves et al., 2011). Therefore, the present study aimed to analyze if the nanoencapsulation of 15d-PGJ₂ might keep or enhance the antinociceptive effects of peripheral injection on acute inflammatory TMJ nociception in a rat model.

Material and methods

Preparation of the PLGA nanocapsules with 15d-PGJ₂

The poly (D,L-lactic-co-glycolic acid, 50:50) (PLGA) nanocapsules were prepared by the nanoprecipitation method (Fessi et al., 1989), which involves mixing an organic phase into an aqueous phase. The organic phase consisted of PLGA polymer (100 mg), acetone (30 mL), 15d-PGJ₂ (100 μg), sorbitan monostereate (40 mg) and caprylic/capric acid triglyceride (200 mg). The aqueous phase was composed of polysorbate 80 (60 mg) and deionized water (30 mL). After dissolution of the components of both phases, the organic phase was gradually added to the aqueous phase, and the suspension maintained under agitation for 10 min. The solvent (acetone) was removed by evaporation and the suspension was concentrated to a volume of 10 mL under low pressure, using a rotary evaporator, in order to obtain a suspension of 15d-PGJ₂ with a final concentration of 10 $\mu\text{g}/\text{mL}$. After evaporation no traces of acetone were observed in the formulation (data not shown). A control formulation (without 15d-PGJ₂) was also prepared, following the methodology described above.

All parameters such as size and polydispersion measurements, Zeta potential measurements and efficiency of association of 15d-PGJ₂ in the PLGA nanocapsules were employed as described previously (Alves et al., 2011).

Transmission electron microscopy (TEM)

The morphology and structure of the PLGA nanocapsules with 15d-PGJ₂ were examined in a JEOL 1200EX II microscope (Jeol ltda, Akishima, Japan) operating at 80 kV. In order to perform the TEM observations, the 15d-PGJ₂-NC was first diluted in water and after that the sample was diluted with a 2% uranyl acetate solution (w/v). One drop of the mixture was deposited on a standard copper grid covered by a carbon film and dried at ambient temperature before TEM analysis.

Atomic force microscopy (AFM)

The microscopy studies of suspension of PLGA nanocapsules with 15d-PGJ₂ were performed with a Nanosurf Easy Scan 2 Basic atomic force microscope (BT02217, Nanosurf, Switzerland). The suspension was deposited onto a silicon surface and the immobilized sample was air-jet dried and analyzed using in contact mode. The analysis was made using a commercial Contr 10 cantilever. The diameters of PLGA nanoparticles were measured and a size distribution was performed using Nanosurf software.

Animals

This study was carried out with male Wistar rats (150–250 g) maintained in a temperature-controlled room (23 \pm 1 $^{\circ}\text{C}$) with a 12-hour light–dark cycle. All experiments were conducted in accordance to the IASP guidelines on using laboratory animals for investigations of experimental pain in conscious animals. All animal experimental procedures and protocols were approved by the Committee on Animal Research of the University of Uberaba (# 047/2009). The animals suffering and number per group were kept at a minimum and each animal was used once.

Testing procedure for TMJ pain

Testing sessions took place during the light phase (between 9:00 AM and 5:00 PM) in a quiet room maintained at 23 $^{\circ}\text{C} \pm 1$ $^{\circ}\text{C}$. Each animal was manipulated for 7 days to be habituated to the experimental manipulation. After this period, the animal was placed in a test chamber (30 \times 30 \times 30 cm mirrored wood chamber with a glass at the front side) for 15 min habituation period to minimize stress. Animals were briefly anesthetized by inhalation of halothane to allow the TMJ injection, which was performed with a 30-gauge needle connected to a 50- μL Hamilton syringe (Roveroni et al., 2001). Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber for counting nociceptive responses. The nociceptive response score was defined as the cumulative total number of seconds that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore or hind paw plus the number of head flinches counted during the observation period as described previously. Since head flinches followed a uniform pattern of 1 s of duration, each flinch was expressed as 1 s. Results are expressed as the duration time of nociceptive behavior (Roveroni et al., 2001). All animals received a final volume of 30 μL into TMJ. All experiments were conducted in a double blind fashion in which the person who injected the solutions was different from the one who made the behavioral assessment.

Experimental protocols

Effect of 15d-PGJ₂-NC on formalin-induced TMJ nociception

Rats were pretreated (15 min) with an intra-TMJ injection of unloaded 15d-PGJ₂ or 15d-PGJ₂-loaded nanocapsules (15d-PGJ₂-NC) in the concentrations of 10, 100 or 1000 pg ($n=6$; 15 $\mu\text{L}/\text{TMJ}$) followed by ipsilateral intra-TMJ injection of 1.5% formalin in a final volume of 30 μL . In order to test whether empty nanocapsules could affect formalin-induced TMJ nociception, a control group of rats were pretreated with empty nanocapsules (the amount of nanocapsules corresponding to the highest dose used, 1000 pg/TMJ , diluted in saline in a final volume of 15 μL) followed by injection of saline or 1.5% formalin into the TMJ. Behavioral nociception response was evaluated for a 45-minute observation period. In order to confirm the peripheral 15d-PGJ₂-mediated antinociception, the highest dose of 15d-PGJ₂ was also injected in the contralateral TMJ that received injection of 1.5% formalin.

Effect of 15d-PGJ₂-NC on formalin-induced IL-1 β cytokine release

After the evaluation of the formalin-induced TMJ nociception, the animals were sacrificed and the periarticular tissues were removed and homogenized in cold RIPA buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 $\mu\text{g}/\text{ml}$ leupeptin, pH 7.5). The samples were centrifuged at 10,000 g for 10 min at 4 $^{\circ}\text{C}$. The supernatant was removed and centrifuged again. IL-1 β levels were detected by ELISA (Enzyme Linked Immunosorbent Assay) using

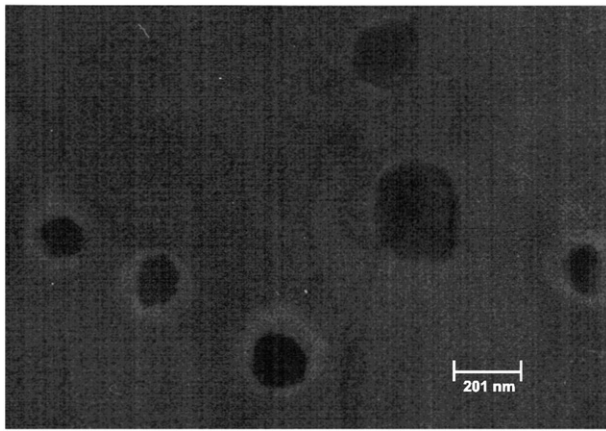


Fig. 1. Transmission electron microscopy images of a spherical PLGA NC observed after negative fixation.

protocols supplied by the manufacturer (R&D Systems, Minneapolis, USA). After all standard procedures, the optical density (O.D.) was measured at 490 nm. Results are expressed as pg/mg of the cytokine, based on the standard curves.

Statistical analysis

To determine if there were significant differences ($p < 0.05$) among treatment groups, the data was analyzed using the *t*-test or one-way ANOVA as appropriate. If there was a significant between-subjects main effect of treatment group following one-way ANOVA, post-hoc contrasts, using the Bonferroni test, were performed to determine the basis of the significant difference. Data are presented in figures as means \pm S.E.M.

Results

Characterization of the 15d-PGJ₂-NC

The images of transmission electron microscopy (Fig. 1) showed that 15d-PGJ₂-NC were spherical, contained no aggregates, and had a size distribution between 182 and 220 nm. This range is lower than that observed by photon correlation spectroscopy and, in this technique, the samples were dried. Another analysis of morphology of the PLGA 15d-PGJ₂-NC was determined by AFM confirming that 15d-PGJ₂-NC were spherical and without aggregates (Fig. 2). An interesting fact observed in this image was that the size distribution of 15d-PGJ₂-NC had a size range between 250 and 600 nm and this increase in the diameter of the nanoparticles can be explained by the flattening or deformation of nanocapsules when the formulation was dripped on the surface of silicon. The 3D view of the AFM image showed that the height of the nanocapsules was only 26 nm, clearly showing that the technique of dripping or a possible interaction with the substrate structure resulted in the formation of the flattened structure of the nanocapsules.

Effect of 15d-PGJ₂-NC on formalin-induced TMJ nociception

Compared with saline administration, the injection of formalin into the TMJ (1.5%) significantly increased the nociceptive behavior (Fig. 3A and B). On the other hand, pretreatment with 15d-PGJ₂-NC (100 and 1000 pg/TMJ) strongly decreased the nociceptive behavior induced by intraarticular injection of formalin (Fig. 3A; $P < 0.05$). Interestingly, pretreatment with unloaded 15d-PGJ₂ (10, 100 and 1000 pg/TMJ) did not reduce the nociceptive behavioral (Fig. 3B). The injection of 15d-PGJ₂-NC at the highest concentration in the contralateral TMJ, did not decrease the formalin-induced

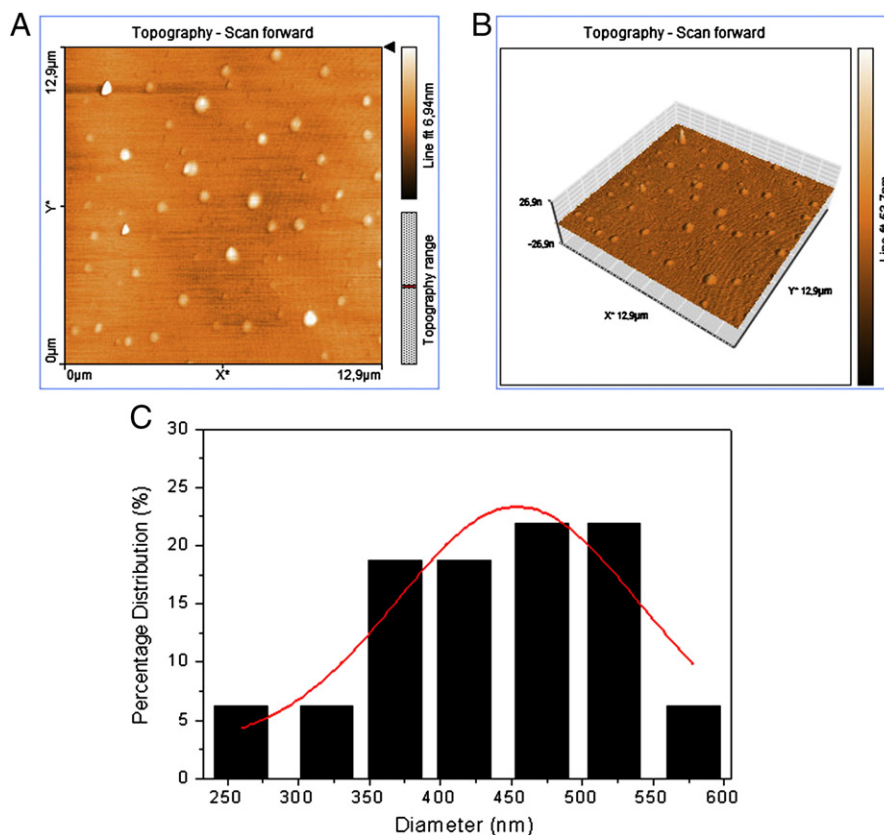


Fig. 2. Atomic force microscopy images of 15d-PGJ₂-NC. A) bi-dimensional image; B) three-dimensional image and C) size distribution of 15d-PGJ₂-NC.

TMJ nociception (data not shown) demonstrating that the anti-nociceptive effect is local.

In order to test whether the empty nanocapsules induced any alteration in the nociceptive behavior, it was injected empty nanocapsules followed by saline or formalin administration. In neither case was there any significant change ($P > 0.05$) in the nociceptive behavior (Fig. 3C).

Effect of low doses of 15d-PGJ₂-NC on IL-1 β releases

The possible interference of 15d-PGJ₂-NC in the release of IL-1 β into the periarticular tissue was investigated since this is an important nociceptive mediator. There was a dose-dependent decrease in the levels of IL-1 β in the periarticular tissue of rats pretreated with

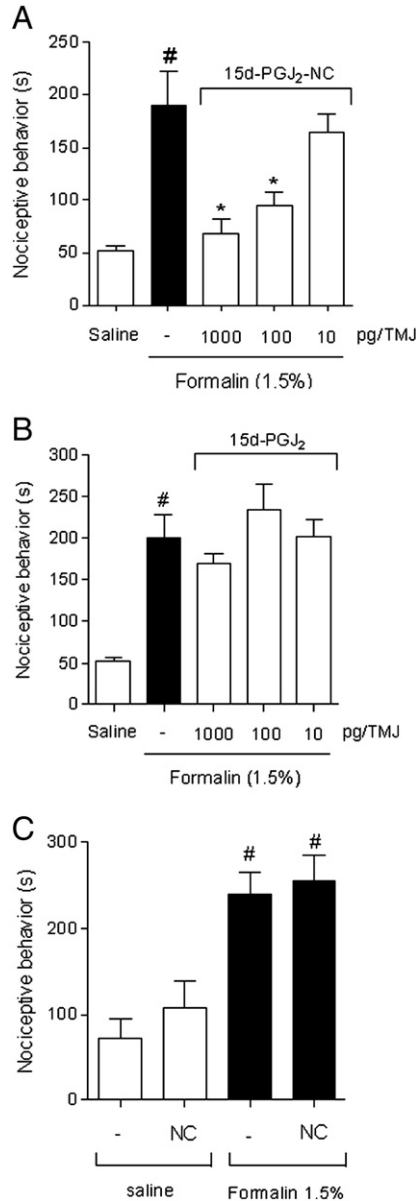


Fig. 3. Effect of 15d-PGJ₂-NC on formalin-induced TMJ nociception. (A) 15d-PGJ₂-loaded nanocapsules (15d-PGJ₂-NC) (1000, 100 but not 10 ng/TMJ) significantly reduced the magnitude of 1.5% formalin-induced nociceptive responses ($p < 0.05$). (B) Pre-treatment with unloaded 15d-PGJ₂ (1000, 100 and 10 ng/TMJ) did not reduce the magnitude of 1.5% formalin-induced nociceptive responses ($p > 0.05$). (C) Pre-treatment with empty nanocapsules (NC) did not change the behavioral response of animal that received intra-TMJ injection of saline or 1.5% formalin ($p > 0.05$). The symbol (#) indicates statistical significance compared to saline; the symbol (*) indicates statistical significance ($p < 0.05$, ANOVA, Bonferroni test) compared to 1.5% formalin.

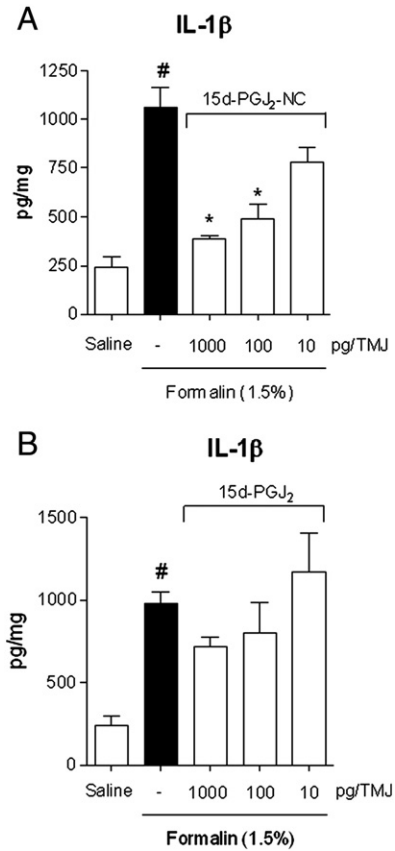


Fig. 4. Effect of low doses of 15d-PGJ₂-NC on IL-1 β release. (A) Pre-treatment with 15d-PGJ₂-NC (1000, 100 but not 10 ng/TMJ) significantly reduced the release of formalin-induced IL-1 β cytokine ($p < 0.05$). (B) Pre-treatment with unloaded 15d-PGJ₂ (1000, 100 and 10 ng/TMJ) did not reduce the release of IL-1 β ($p > 0.05$). The symbol (#) indicates statistical significance ($p < 0.05$, Bonferroni test) compared to saline; the symbol (*) indicates statistical significance ($p < 0.05$, ANOVA, Bonferroni test) compared to 1.5% formalin.

15d-PGJ₂-NC, in contrast to rats pretreated with saline and injected with formalin (Fig. 4A). On the other hand, there was no statistical reduction of cytokine levels in the periarticular tissue of animals treated with the same concentration (10, 100 and 1000 pg/TMJ) of non-encapsulated 15d-PGJ₂ (Fig. 4B).

Discussion

Developing new chemical and biological compounds intended for therapeutics is a great challenge for researchers' worldwide (Huynh et al., 2009). The carrier system is considered a reliable approach to target the drug delivery site (Couvreur et al., 2002; Couvreur and Vauthier, 2006). Among the different nanocarrier systems, biodegradable nanoparticles have been reported as potential drug delivery vehicles over the last few years. A new versatile nanodelivery system for the targeted delivery of therapeutic compounds has shown potential activity against several diseases (Adair et al., 2010).

The biocompatibility of nanoparticles is one of the major concerns in biomedical applications. Lipid nanocapsules are potential vectors for the delivery of drugs into the inner ear after round window membrane application without morphological or cochlear neural functional changes (Zhang et al., 2011). PLGA nanocapsules with 15d-PGJ₂ were used in the present study which is an FDA-approved polymer used for the preparation of nanoparticles. In particular, intra-articular injections of PLGA nanocapsules causes no alteration in articular tissue functions in the knee or TMJ of healthy rats or of those undergoing degenerative or inflammatory conditions such as arthritis and/or osteoarthritis, suggesting that PLGA-nanocapsules

could be used as a safe drug delivery system to treat articular diseases, allowing a wide range of encapsulating molecules (Zille et al., 2010; Mountziaris et al., 2010). In addition, polymers containing PLGA, in previous animal experiments, were found to be biocompatible and to have low immunogenicity and little toxicity (Alves et al., 2011; Ishihara et al., 2010; Shive and Anderson, 1997).

In a previous study, 15d-PGJ₂-containing PLGA-nanoparticles (hydrodynamic diameter: 100 to 400 nm; polydispersity: <0.2; and zeta potential: −30 mV) were reported as an efficient carrier system (Alves et al., 2011). In the present study, morphological analysis of this carrier system using transmission electron microscopy (TEM) and atomic force microscopy (AFM) showed that 15d-PGJ₂-NC is spherical without any aggregates or adhesions, which are important characteristics of colloidal stability in solution.

Relieving TMJ pain is a challenge since temporomandibular disorders involve deep tissues, making it difficult to target the trigeminal neural system (Cairns, 2010). The nanoparticles can accumulate in inflamed tissues due to greater microvascular permeability in those sites (Bernardi et al., 2009). In the present study, an intra-articular injection of 15d-PGJ₂-NC (100 pg/TMJ), at a dose 1000 times lower than that (100 ng/TMJ) used for the unloaded 15d-PGJ₂ (Pena-dos-Santos et al., 2009), enhanced its temporomandibular peripheral antinociceptive effect. This might be due to its spherical morphology, containing no aggregates, as well as the ability of the nanoparticles to reach or release 15d-PGJ₂ in the cells. The literature shows different mechanisms for the endocytosis of nanoparticles, such as pinocytosis, formation of caveolae and clathrin, and caveolae/clathrin-independent uptake (Dobrovolskaia and McNeil, 2007). Thus, the morphological properties of 15d-PGJ₂-NC might allow active compounds to enter the cells via different mechanisms, initiating different interactions with organelles and macromolecules, resulting in different pharmacological activities (Nel et al., 2009; Gratton et al., 2008).

It is well known that 15d-PGJ₂ is a natural ligand for PPAR-γ (Ricote et al., 1998; Schoonjans et al., 1997). PPAR-γ agonists represent a promising therapeutic alternative for inflammatory diseases (Chima et al., 2008; Cuzzocrea et al., 2003; Kaplan et al., 2005; Napimoga et al., 2008a, 2008b; Pena-dos-Santos et al., 2009; Shan et al., 2004) and, in particular, they are extremely neuroprotective (Collino et al., 2006; Hyong et al., 2008; McTigue et al., 2007; Park et al., 2007; Pereira et al., 2006; Sundararajan et al., 2005; Tureyen et al., 2007; Zhao et al., 2005, 2006). PPAR-γ was observed to be more expressive in ischemic neurons in rats with transient cerebral ischemia, suggesting that neuronal injury might alter PPAR-γ signaling (Victor et al., 2006). Pharmacological activation of PPAR-γ in the brain and spinal cord rapidly inhibits the spinal transmission of noxious inflammatory signals and local edema. These results suggest that PPAR-γ plays an important role in pain modulation in the central nervous system (Morgenweck et al., 2010) as well as in peripheral tissues and in peripheral endings of somatic afferents (Napimoga et al., 2008a; Pena-dos-Santos et al., 2009).

With the co-participation of κ/δ opioid receptors mediated by the activation of the intracellular L-Arginine-NO/cGMP/K(+)ATP, 15d-PGJ₂ was found to activate PPAR-γ in the TMJ, inducing a peripheral antinociceptive effect (Pena-dos-Santos et al., 2009). TMJ inflammatory conditions result in the release of several pro-inflammatory cytokines, especially tumor necrosis factor-α (TNF-α) and interleukins (Kopp, 2001), both of which contribute to joint remodeling and cartilage degradation (Vernal et al., 2008). These cytokines induce the release of a number of pro-nociceptive compounds, such as potassium chloride, leukotriene B₄, prostaglandin E₂ (PGE₂), bradykinin, serotonin, histamine, glutamate and adenosine triphosphate (ATP), all of which have been shown to excite and induce spontaneous discharge in the TMJ (Flake and Gold, 2005; Kopp, 2001; Oliveira et al., 2005; Rodrigues et al., 2006). Interestingly, in the present study, low doses of 15d-PGJ₂-NC, but not

unloaded 15d-PGJ₂, were found to inhibit the release of the pro-inflammatory cytokine IL-1β. Since IL-1β is one of the major hypernociceptive mediator (Verri et al., 2006), we may speculate that this reduced levels of IL-1β might enhance the antinociceptive activity of 15d-PGJ₂-NC.

Conclusion

Nanoencapsulation improves drug efficacy and drug bioavailability by providing a more sustained drug release to the inflamed site resulting in facilitating a 15d-PGJ₂ target trigeminal pathway that in turn enhances the peripheral antinociceptive effect of loading 15d-PGJ₂. Taken together with the biodegradable, biocompatible, and low toxic properties of the nanoparticle, these results suggest a strong potential use of this compound as novel pharmacological agents for antinociceptive and anti-inflammatory therapy in clinical practice.

Conflict of interest statement

The manuscript in its submitted form has been read and approved by all authors before submission, and none of them has any potential financial conflict of interests related to this manuscript.

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References

- Adair JH, Parette MP, Altinoğlu EI, Kester M. Nanoparticulate alternatives for drug delivery. *ACS Nano* 2010;4:4967–70.
- Alves C, de Melo N, Fraceto L, de Araújo D, Napimoga M. Effects of 15d-PGJ₂-loaded poly(D,L-lactide-co-glycolide) nanocapsules on inflammation. *Br J Pharmacol* 2011;162:623–32.
- Bernardi A, Zilberstein AC, Jäger E, Campos MM, Morrone FB, Calixto JB, et al. Effects of indomethacin-loaded nanocapsules in experimental models of inflammation in rats. *Br J Pharmacol* 2009;158:1104–11.
- Cairns BE. Pathophysiology of TMD pain—basic mechanisms and their implications for pharmacotherapy. *J Oral Rehabil* 2010;37:391–410.
- Chima RS, Hake PW, Piraino G, Mangeshkar P, Denenberg A, Zingarelli B. Ciglitazone ameliorates lung inflammation by modulating the inhibitor kappaB protein kinase/nuclear factor-kappaB pathway after hemorrhagic shock. *Crit Care Med* 2008;36:2849–57.
- Collino M, Aragno M, Mastrocola R, Gallicchio M, Rosa AC, Dianzani C, et al. Modulation of the oxidative stress and inflammatory response by PPAR-gamma agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. *Eur J Pharmacol* 2006;530:70–80.
- Couvreur P, Vauthier C. Nanotechnology: intelligent design to treat complex disease. *Pharm Res* 2006;23:1417–50.
- Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review. *Crit Rev Ther Drug Carrier Syst* 2002;19:99–134.
- Cuzzocrea S, Ianaro A, Wayman NS, Mazzon E, Pisano B, Dugo L, et al. The cyclopentenone prostaglandin 15-deoxy-delta (12,14)-PGJ₂ attenuates the development of colon injury caused by dinitrobenzene sulphonic acid in the rat. *Br J Pharmacol* 2003;138:678–88.
- Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. *Nat Nanotechnol* 2007;2:469–78.
- Elron-Gross I, Glucksam Y, Biton IE, Margalit R. A novel Diclofenac-carrier for local treatment of osteoarthritis applying live-animal MRI. *J Control Release* 2009;135:65–70.
- Escher P, Wahli W. Peroxisome proliferator-activated receptors: insight into multiple cellular functions. *Mutat Res* 2000;448:121–38.
- Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int J Pharm* 1989;55:R1–4.
- Flake NM, Gold MS. Inflammation alters sodium currents and excitability of temporomandibular joint afferents. *Neurosci Lett* 2005;384:294–9.

- Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, et al. The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A* 2008;105:11613–8.
- Grillo R, de Melo NFS, de Araújo DR, de Paula E, Rosa AH, Fraceto LF. Polymeric alginate nanoparticles containing the local anesthetic bupivacaine. *J Drug Target* 2010;18:688–99.
- Huynh NT, Passirani C, Saulnier P, Benoit JP. Lipid nanocapsules: a new platform for nanomedicine. *Int J Phytoremediation* 2009;379:201–9.
- Hyong A, Jadhav V, Lee S, Tong W, Rowe J, Zhang JH, et al. Rosiglitazone, a PPAR gamma agonist, attenuates inflammation after surgical brain injury in rodents. *Brain Res* 2008;1215:218–24.
- Ishihara T, Takahashi M, Higaki M, Mizushima Y, Mizushima T. Preparation and characterization of a nanoparticulate formulation composed of PEG-PLA and PLA as anti-inflammatory agents. *Int J Pharm* 2010;385:170–5.
- Kaplan JM, Cook JA, Hake PW, O'Connor M, Burroughs TJ, Zingarelli B. 15-Deoxy-delta(12,14)-prostaglandin J(2) (15D-PGJ(2)), a peroxisome proliferator activated receptor gamma ligand, reduces tissue leukosequestration and mortality in endotoxic shock. *Shock* 2005;24:59–65.
- Kopp S. Neuroendocrine, immune, and local responses related to temporomandibular disorders. *J Orofac Pain* 2001;15:9–28.
- Lehrke M, Lazar MA. The many faces of PPARgamma. *Cell* 2005;123:993–9.
- McTigue DM, Tripathi R, Wei P, Lash AT. The PPAR gamma agonist pioglitazone improves anatomical and locomotor recovery after rodent spinal cord injury. *Exp Neurol* 2007;205:396–406.
- Morgenweck J, Abdel-Aleem OS, McNamara KC, Donahue RR, Badr MZ, Taylor BK. Activation of peroxisome proliferator-activated receptor gamma in brain inhibits inflammatory pain, dorsal horn expression of Fos, and local edema. *Neuropharmacology* 2010;58:337–45.
- Mountziaris PM, Sing DC, Mikos AG, Kramer PR. Intra-articular microparticles for drug delivery to the TMJ. *J Dent Res* 2010;89:1039–44.
- Napimoga MH, Souza GR, Cunha TM, Ferrari LF, Clemente-Napimoga JT, Parada CA, et al. 15d-prostaglandin J2 inhibits inflammatory hypernociception: involvement of peripheral opioid receptor. *J Pharmacol Exp Ther* 2008a;324:313–21.
- Napimoga MH, Vieira SM, Dal-Secco D, Freitas A, Souto FO, Mestriner FL, et al. Peroxisome proliferator-activated receptor-gamma ligand, 15-deoxy-Delta12,14-prostaglandin J2, reduces neutrophil migration via a nitric oxide pathway. *J Immunol* 2008b;180:609–17.
- Nel AE, Madler L, Velegol D, Xia T, Hoek EMV, Somasundaran P, et al. Understanding biophysical interactions at the nano-bio interface. *Nat Mater* 2009;8:543–57.
- Oliveira MC, Parada CA, Veiga MC, Rodrigues LR, Barros SP, Tambeli CH. Evidence for the involvement of endogenous ATP and P2X receptors in TMJ pain. *Eur J Pain* 2005;9:87–93.
- Park SW, Yi JH, Miranpuri G, Satriotomo I, Bowen K, Resnick DK, et al. Thiazolidinedione class of peroxisome proliferator-activated receptor gamma agonists prevents neuronal damage, motor dysfunction, myelin loss, neuropathic pain, and inflammation after spinal cord injury in adult rats. *J Pharmacol Exp Ther* 2007;320:1002–12.
- Pena-dos-Santos DR, Severino FP, Pereira SA, Rodrigues DB, Cunha FQ, Vieira SM, et al. Activation of peripheral kappa/delta opioid receptors mediates 15-deoxy-(Delta12,14)-prostaglandin J2 induced-antinociception in rat temporomandibular joint. *Neuroscience* 2009;163:1211–9.
- Pereira MP, Hurtado O, Cardenas A, Bosca L, Castillo J, Davalos A, et al. Rosiglitazone and 15-deoxy-delta12, 14-prostaglandin J2 cause potent neuroprotection after experimental stroke through noncompletely overlapping mechanisms. *J Cereb Blood Flow Metab* 2006;26:218–29.
- Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998;391:79–82.
- Rodrigues LR, Oliveira MC, Pelegrini-da-Silva A, de Arruda Veiga MC, Parada CA, Tambeli CH. Peripheral sympathetic component of the temporomandibular joint inflammatory pain in rats. *J Pain* 2006;7:929–36.
- Roveroni RC, Parada CA, Cecilia M, Veiga FA, Tambeli CH. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. *Pain* 2001;94:185–91.
- Schoonjans K, Martin G, Staels B, Auwerx J. Peroxisome proliferator activated receptors, orphans with ligands and functions. *Curr Opin Lipidol* 1997;8:159–66.
- Shan ZZ, Masuko-Hongo K, Dai SM, Nakamura H, Kato T, Nishioka K. A potential role of 15-deoxy-delta(12,14)- prostaglandin J2 for induction of human articular chondrocyte apoptosis in arthritis. *J Biol Chem* 2004;279:37939–50.
- Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev* 1997;28:5–24.
- Sundarajan S, Gamboa JL, Victor NA, Wanderi EW, Lust WD, Landreth GE. Peroxisome proliferator-activated receptor-gamma ligands reduce inflammation and infarction size in transient focal ischemia. *Neuroscience* 2005;130:685–96.
- Tureyen K, Kapadia R, Bowen K, Satriotomo I, Liang J, Feinstein D, et al. Peroxisome proliferator-activated receptor-gamma agonists induce neuroprotection following transient focal ischemia in normotensive, normoglycemic as well as hypertensive and type-2 diabetic rodents. *J Neurochem* 2007;101:41–6.
- Vernal R, Velasquez E, Gamonal J, Garcia-Sanz JA, Silva A, Sanz M. Expression of proinflammatory cytokines in osteoarthritis of the temporomandibular joint. *Arch Oral Biol* 2008;53:910–5.
- Verri Jr WA, Cunha TM, Parada CA, Poole S, Cunha FQ, Ferreira SH. Hypernociceptive role of cytokines and chemokines: targets for analgesic drug development? *Pharmacol Ther* 2006;112:116–38.
- Victor NA, Wanderi EW, Gamboa J, Zhao X, Aronowski J, Deininger K, et al. Altered PPARgamma expression and activation after transient focal ischemia in rats. *Eur J Neurosci* 2006;24:1653–63.
- Zhang Y, Zhang W, Löbler M, Schmitz KP, Saulnier P, Perrier T, et al. Inner ear biocompatibility of lipid nanocapsules after round window membrane application. *Int J Pharm* 2011;404:211–9.
- Zhao Y, Patzer A, Gohlke P, Herdegen T, Culman J. The intracerebral application of the PPARgamma-ligand pioglitazone confers neuroprotection against focal ischaemia in the rat brain. *Eur J Neurosci* 2005;22:278–82.
- Zhao Y, Patzer A, Herdegen T, Gohlke P, Culman J. Activation of cerebral peroxisome proliferator-activated receptors gamma promotes neuroprotection by attenuation of neuronal cyclooxygenase-2 overexpression after focal cerebral ischemia in rats. *FASEB J* 2006;20:1162–75.
- Zille H, Paquet J, Henrionnet C, Scala-Bertola J, Leonard M, Six JL, et al. Evaluation of intra-articular delivery of hyaluronic acid functionalized biopolymeric nanoparticles in healthy rat knees. *Biomed Mater Eng* 2010;20:235–42.