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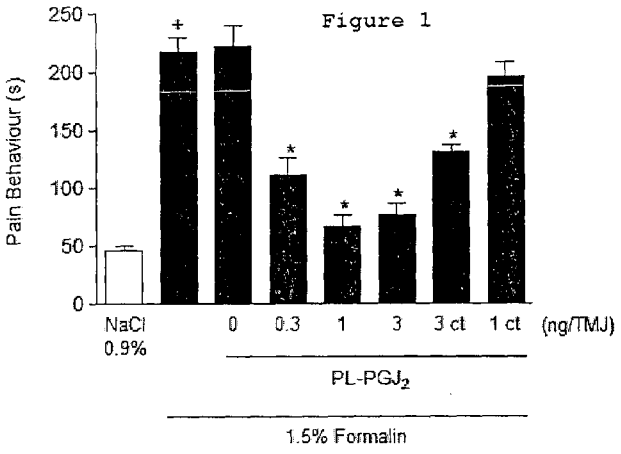
Figure 1



(57) Abstract: The present invention is related to a pharmaceutical composition comprising a poloxamer-based micellar system, 15-deoxy-Δ-12,14-prostaglandin J2 as the active ingredient and an aqueous carrier, with prolonged residence time at the site of injection, inducing potent and sustained analgesic and anti-inflammatory effect, being particularly useful for treatment of inflammatory conditions of joints and arthritis.

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PHARMACEUTICAL COMPOSITION OF 15-DEOXY-DELTA-12,14-PROSTAGLANDIN J2 IN A POLOXAMER-BASED MICELLAR SYSTEM AND ITS USE FOR TREATMENT OF INFLAMMATORY CONDITIONS

The field of the invention

The present invention is directed to a poloxamer-based micellar system as a new pharmaceutical composition with prolonged residence of 15-deoxy-Δ-12,14-prostaglandin J2 at the site of administration and its use in therapy, particularly as an analgesic and anti-inflammatory composition that has a potential effect in joints.

Invention Background

In response to inflammatory signals, cells migrate and accumulate at the sites of inflammation. A multifaceted signaling cascade initiated by the inflammatory insult leads to the activation of neutrophils and production of a vast array of pro-inflammatory cytokines and chemokines. Although they have a protective role in inflammation, tissue damage is a consequence of intense leukocyte migration, as observed in immune inflammatory diseases. The challenge in the development of new anti-inflammatory drugs has been to find appropriate targets that are essential in the inflammatory process but are mostly dispensable in terms of the host's defense against pathogens.

Pain is a consequence of the inflammatory process, being one of its classical signals. It is now accepted that the sensitization of the primary sensory neurons is essential to inflammatory pain. Experimentally, the peripheral pharmacologic control of inflammatory pain is based on two main strategies. The first is the use of drugs that prevent the nociceptor sensitization, such as non-steroidal anti-inflammatory drugs (NSAIDs) (aspirin and aspirin-like drugs), which inhibit prostaglandin synthesis, and therefore, prevent

the development of hypernociception. The second strategy is the direct blockade of the ongoing nociceptor sensitization, which can be achieved by the use of peripheral morphine (opioids), dipyrone and diclofenac. In fact, these drugs reverse the already established hypernociception induced by prostaglandin E2 (PGE2).

Arthritis refers to a group of rheumatic diseases and other conditions that are characterized as a chronic and progressive inflammatory disorder with synovitis and severe joint destruction which can cause pain, stiffness and swelling in the joint.

Being a synovial joint, the temporomandibular joint (TMJ) is subject to the same disorders affecting other synovial joints, including rheumatoid arthritis (RA). Inflammation of the TMJ induced by RA have often resulted in persistent pain and caused distress to many patients. There was a high prevalence of temporomandibular disorders in RA patients from 67 to 92.9% in which the most common clinical findings are pain in the TMJ area, tenderness of the masticatory muscles, joint sounds and limited joint function (Lin et al., J. Clin. Med. Assoc. 70: 527-34, 2007; Aliko et al., Int. J. Oral Maxilofac. Surg. 40: 704-9, 2011).

The management of articular pain includes a large number of conservative treatments with limited efficacy.—Mild articular pain may be controlled initially by the use of simple analgesics such as acetaminophen, propoxyphene and tramadol, which are the first line of articular treatment. If these analgesics do not sufficiently control the pain, a NSAID can be used, with similar effectiveness, but with significant side effects on gastrointestinal tract, including stomach upset, cramping diarrhea, ulcers and even bleeding. The cyclooxygenase-(COX)2-specific NSAIDs have lower rates of gastrointestinal side effects but are associated with higher rates of cardiovascular disease including myocardial

infarction. Because of these side effects of NSAIDs, physicians are increasingly considering intra-articular (IA) treatment when oral therapies are not effective or show considerable systemic side effects.

Intra-articular drug delivery can be very useful when a small number of joints are affected or when the disease does not respond to systemic medications. IA treatment has several advantages, for example, acting directly on selected joints, lower total drug dose, initial high local drug concentrations, reducing the exposure of unaffected sites to the drug, diminishing systemic side effects, fewer drug interactions, and enabling the administration of drugs with efficacy but low oral bioavailability, such as proteins or drugs with low solubility. Therefore, IA therapy not only reduces the costs of treatment but also improves the efficacy of therapy for patients suffering from articular pain.

The more recent method for treating osteoarthritis (the most common form of arthritis and the most frequent form of joint disease) by the means of anti-inflammatory drugs directed at cytokines to prevent the progression of structural changes of the joint has been disappointing for a number of reasons and needs further investigation in regards to delivery form and reduction of toxicity.

Another group of drugs is disease-modifying osteoarthritis drugs, which has the ability to slow down the disease process, including compounds that inhibit matrix-metalloproteinases, bisphosphonates, cytokine blockers, calcitonin, inhibitors of inducible nitric oxide synthase (iNOS), doxycycline, glucosamine and diacerein. Surgical treatments including joint replacement are indicated in advanced cases with significant disability and for those patients in whom more conservative management has failed.

Infiltrations with glucocorticoids and viscosupplementation with sodium hyaluronate/hyaluronic acid

(HA) are widely used. However, their use is often contraindicated, especially in small joints.

Numerous researches drug delivery system have been directed to the treatment of inflammatory conditions of the joint. For example, liposomes have been developed to encapsulate drugs for sustained local delivery and are believed to have promise for intra-articular drug therapy, although they generally exhibit a burst release due to instability in physiologic environments. Microspheres have also been used to encapsulate drugs for arthritis (e.g., methotrexate, paclitaxel), using degradable polymers such as poly(lactic) or poly(lactic-coglycolic acid), albumin, N-N-dicarboxymethyl chitosan and gelatin/chondroitin sulfate. These approaches have been found to improve the residence time of synthetic drugs or active compounds within the joint space by as much as 10-fold. One strategy to achieve long-term drug retention within the synovial joint is the concept of thermogelling polymers, such as polyethylene glycol (PEG) copolymers or chitosans that have been evaluated as "drug depot"-forming materials for sustained release in systemic drug delivery.

Besides new strategies to obtain a better performance of intra-articular medicines, also new therapeutic entities are of great interest to improve the arthritis treatment. In this regard, prostaglandin derivatives have emerged as a promising class of anti-inflammatory substances.

Prostaglandins (PGs) are autacoids synthesized in response to appropriate stimulus from 20 carbon-containing polyunsaturated fatty acids, principally arachidonic acid (AA), from cell membranes, a reaction catalyzed by phospholipase A2 and derived from dietary sources. The eicosanoids (C20-hydroxy-fatty acids) have been detected in almost every tissue and body fluid. With the exception of seminal fluid, PGs are not stored in tissues or cells.

Instead, their production may increase in response to diverse stimuli, and they produce a broad spectrum of biological effects. Once synthesized, PGs are released and/or exported to the extracellular space. Owing to their instability, PGs exert their functions mainly in proximity to their sites of synthesis, contributing to inflammation, smooth muscle tone, hemostasis, thrombosis, parturition and gastrointestinal secretion, among others. Prostanoids play a critical role in inflammation and for many years, COX enzymes and their products have been considered mainly pro-inflammatory agents.

Released AA is converted to an unstable oxygenated intermediate prostaglandin H2 (PGH2) by COX. Once formed, the PGH2 intermediate may be converted into various prostaglandins such as PGE2, prostacyclin, prostacyclin F2α or prostaglandin D2 (PGD2), by a range of specific enzymes called prostaglandin synthases (e.g. PGE synthase or PGE-S, PGD synthase or PGD-S etc.). PGD2 is very short lived, and undergoes dehydration *in vivo* and *in vitro* to yield biologically active prostaglandins of the J2 series, including prostaglandin 15-deoxy-Δ-12,14-prostaglandin J2 (herein after PGJ2). In contrast to most PGs, accumulating data suggests that PGJ2 may exert anti-inflammatory effects.

Indeed, this prostaglandin is a natural, chemically stable anti-inflammatory derivative of PGD2 with high-affinity ligand for the peroxisome proliferator-activated receptor (PPAR) subtype PPAR-γ, which regulates gene expression of enzymes associated with lipid homeostasis, inflammation, cell proliferation, and malignancy. PPAR-γ is found in many cell types, including macrophages/monocytes, lymphocytes, neutrophils, mast cells, myocytes, fibroblasts, breast cells, human bone marrow precursors, and hepatocytes, and adipocytes.

PPAR-γ represents a major anti-inflammatory mediator through which PGJ2 is able to suppress immune reactivity.

PPAR-γ agonists have been reported to attenuate a variety of pathologies, including inflammation in arthritis models, neuronal cytotoxicity, inflammatory bowel disease, ischemia-reperfusion injury, Alzheimer's disease, glomerulonephritis, and activation of PPAR-γ on cardiac myocytes has been shown to reduce cardiac hypertrophy. Moreover, PGJ2-mediated PPAR-γ activation has been shown to promote inflammatory resolution through the apoptotic induction of macrophages as well as blocking LPS-induced NO production in macrophages.

Gilroy et al. (Nat. Rev. Drug Discov. 3:401-416, 2004) have already proposed a role of COX2 in inflammatory resolution, since they verified that an exacerbating effect of NSAIDS at late stage of the inflammatory response was associated to the production of PGD2 and its dehydration product, 15-deoxy-Δ-12-14-prostaglandin J2.

In fact, pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are central mediators in RA. Particularly, it is well established that cytokines, such as TNF-α, IL-1β, IL-6 and the chemokines IL-8, chemokine-induced neutrophil chemoattractant-1 and keratinocyte-derived chemokine, trigger the release of prostanoids or sympathetic amines that act directly on the nociceptors to cause hypernociception.

In vitro data shows that the PGJ2 reduced, almost completely, the synthesis of PGE2 stimulated by cytokines and the expression of PGE2 on rats chondrocytes, indicating that PGJ2 acts as an anti-inflammatory messenger turning the production of the pro-inflammatory prostaglandin PGE2 off in these cells (Boyault et al. FEBS Letters 572:33-40, 2004). Intraperitoneal administration of PGJ2 (20 or 40 μg/kg) has also been shown to reduce the development of experimental colitis in rat, a model of inflammatory bowel disease. Agents and conditions that tip the balance of prostaglandin production in favor of PGD2 and PGJ2 would therefore be

expected to have anti-inflammatory effects (Cuzzocrea et al., Br. J. Pharmacol. 138: 678-88, 2003).

Kawahito et al. (J. CLin. Invest. 106: 189-97, 2000) showed intraperitoneal administration of 15-deoxy-Δ-12,14-prostaglandin J2 (1mg/kg/day - ~350µg/day) suppressed the chronic inflammation in adjuvant-induced arthritis (AIA) in rats. The mechanism of the anti-inflammatory effects of PGJ2 in AIA can be explained, at least in part, by the activation of PPAR-γ, which leads to the apoptosis of several cells such as synovial cells and inhibition of macrophage function.

Data from Napimoga et al. (J. Immunol. 180: 609-617, 2008) also indicate that 15-deoxy-Δ-12,14 prostaglandin J2 (PGJ2) is as promising drug for the treatment of inflammatory diseases since it down-regulated most cells involved in the innate and adaptive immune response. Furthermore, the group estimated that more than 50% of the PGJ2 added exogenously to a biological system binds to albumin and more than 80% added to cell culture bind to the culture medium. For this reason, it is necessary to administer large amounts of free PGJ2 (1 mg/kg) to observe an anti-inflammatory effect in systemic inflammatory models in rats.

In a further study (Alves et al., Br. J. Pharmacol. 162: 623-32, 2011) the anti-inflammatory and analgesic effects of exogenously-administered 15-deoxy-Δ-12,14 prostaglandin J2 (PGJ2) loaded into nanocapsules of poly (DL-lactide-co-glycolide) - PLGA - as drug delivery system was investigated. This PLGA delivery system enabled the administration of 30 µg/kg of PGJ2 in mouse, systemically, with the additional advantage of prolonging the release of PGJ2, as observed by the detection of significant amounts of PGJ2 in circulation 24h following the administration.

In this context, micelles have an enormous importance for drug-delivery. The main features to be considered are the small size, the particular nanostructure, the capacity to

solubilize hydrophobic drugs in the core and the presence of a hydrophilic shell for the stabilization and protection of the drug from the external medium, pointing them as depot for hydrophobic drugs and also providing advantages for sustained release.

One of the most used components for micellar delivery systems are the copolymers of the poloxamer class.

Poloxamers, which are also known as Kolliphor® (formerly Pluronics® (US) and Lutrols® (Europe)), have been introduced in 1950 as a non-ionic triblock copolymer consisting of propylene oxide (PO) and ethylene oxide (EO) blocks-specifically, i.e., poly (a-oxyethylene-b-oxypropylene-a-oxyethylene) triblock copolymers, consisting of two hydrophilic chains of ethylene oxide chains (PEO) that sandwiched one hydrophobic propylene oxide chain (PPO) giving a chemical formula $HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$ where a and b values varies and gives rise to different polymers identified as 124, 188, 237, 338, 403, and 407 showing a slight difference in their properties.

Poloxamers, in general, are non-toxic polymeric surfactants and have been incorporated into drug delivery systems likely to be administered through oral, parenteral, topical routes and has been used as solubilizer, emulsifier and stabilizer. Poloxamers exhibit a fascinating range of structures in solution depending on concentration, copolymer composition, cosolutes, and temperature.

Micellar carriers composed of poloxamers have been employed in different studies as drug carriers (anti-inflammatories, local anesthetics)due to their self-assembly properties and micellar nanostructure (Sharma and Bathia, Int. J. Pharm. 278:361-77, 2004; Sharma et al., Colloids Surf. B Biointerfaces. 63:229-35, 2008).

Also, the US Patent No. 6,153,193 (Supratek Pharma) discloses the use of poloxamers with a targeting molecule,

for the delivery of selected drugs, such as benzodiazepines. Combinations of poloxamers are disclosed with the aim of manipulating micelle properties between room temperature and body temperature.

In a different point of view, the effectiveness of delivery systems composed of poloxamers, in special poloxamer 407 and poloxamer 188, have been investigated on local and systemic inflammation. Particularly, poloxamer 407 prolonged the residence time of the Ag85A antigen optimizing the immune response to *Mycobacterium turbeculosis* following pulmonary delivery (Todoroff et al., Eur. J. Pharm. Biopharm. 84:40-8, 2013). In addition, poloxamer 188 has been shown to reduce the inflammation and tissue damage after brain injury (Curry et al., J. Neurosurg. 101(1 Suppl):91-6, 2004).

A synergistic mixed poloxamer systems for solubilizing drugs was disclosed in the US Patent No. 8,460,644 (Maelor Pharmaceuticals Ltd), incorporated herein by reference. Particularly, it discloses combinations of poloxamers manipulating micelle properties between room temperature and body temperature, with significant advantages over solutions of single poloxamers, mainly regarded to its capability of solubilizing more drug, often substantially more, than single poloxamer systems, intended to be injectable, directed mainly to drugs that causes pain on injection, or that are difficult to formulate as injectable or in aqueous formulations in general, for example, sedatives, anaesthetic agents, anti-schizophrenic agents, antiseptic and anti-fungal agents. The poloxamers are selected from P188, P234, P237, P338 and P407, and the ratio of poloxamers used is between 7:3 and 3:7 (w/w), and the total concentration of poloxamer is between 3-15% (w/v).

In the light of those observations and considering that poloxamer thermoreversible systems have presented promising results regarding the improvement in

biopharmaceutical, pharmacodynamic and pharmacokinetic properties of the incorporated drugs and the capability to form stable systems *in vivo*, the present invention describes a new pharmaceutical composition comprising a poloxamer-based micellar system containing PGJ2 as active ingredient incorporated into the micellar core for enhancing its analgesic and anti-inflammatory effects. Then, the object of the present invention is to provide a new pharmaceutical composition for local injection of PGJ2 presenting thermal responsively and biocompatibility, being possible the administration of reduced doses of PGJ2 which is released for prolonged period of time.

Summary of the invention

In one aspect, the present invention provides a pharmaceutical composition comprising a poloxamer-based micellar system, PGJ2 as the active ingredient, and an aqueous carrier, with enhanced analgesic and anti-inflammatory efficacy.

In another aspect, the present invention refers to a pharmaceutical composition comprising a poloxamer-based micellar system comprising an association of P407 and P188, PGJ2 and water for injection.

In a further aspect, the present invention provides the use of the pharmaceutical composition of the instant invention for the manufacture of a medicament for treatment or prophylaxis of acute or chronic inflammatory conditions of temporomandibular joint (TMJ), arthritis and other conditions mediated by inflammatory intermediates (for example, enhanced levels of prostaglandin E2 or other metabolites of COX-2).

In another aspect, the present invention provides a method for the treatment or prophylaxis of acute or chronic inflammatory conditions of temporomandibular joint (TMJ), arthritis or other conditions mediated by inflammatory

intermediates characterized by comprising the administration of an effective amount of the composition of claim 1 to a subject in need thereof.

The pharmaceutical composition comprising a poloxamer-based micellar system and PGJ2 (PL-PGJ2) presents advantages in relation to plain PGJ2 or PGJ2-loaded polymeric nanocapsules in inflammation animals models, namely:

i) local stimulus, since they are thermorreversible and self-assembled in response to physiological temperature;

ii) local retention (enhancing the permanence of the system into the injection site) and, finally,

iii) their size (< 100 nm) is sufficiently large to avoid the elimination via glomerular filtration, but sufficiently small to permeated the blood vessels and penetrate into the inflammated tissue.

Brief Description of the illustrations

Aspects of the invention are explained or depicted by the following figures:

Fig. 1 shows the analgesic effect of different doses of a composition comprising poloxamer-based micellar system and PGJ2 (PL-PGJ2), injected directly into the joint, in the formalin-induced inflammatory pain model. PL-PGJ2 was injected into the rats TMJ followed by the administration of formalin, into the same joint, and their pain behavior monitored. As control, rats were treated with PL-PGJ2 in the contralateral joint (ct).

Fig. 2 shows the comparative efficacy of pretreatment with plain PGJ2 (100 ng/TMJ) and PL-PGJ2 (1 ng/TMJ) injected directly into the joint 15 minutes before the administration of formalin, in the formalin-induced inflammatory pain.

Fig. 3 shows the effects of pretreatment with a single injection of PL-PGJ2 (1 ng/TMJ) at different time intervals

(15 minutes, 24 hours, 48 hours, 72 hours, 7 days, 10 days, and 14 days) in the formalin-induced inflammatory painful behavior at temporomandibular joint (TMJ).

Fig. 4 shows the effect of the pretreatment with the PL-PGJ2 (1 ng/TMJ) at different time intervals (15 min, 24 hours, 7 days, 10 days, and 14 days) in plasma extravasation induced by formalin into the TMJ.

Fig. 5 shows the effect of pretreatment with single injection of PL-PGJ2 (1 ng/TMJ) at different time intervals (15 min, 24 hours, 7 days, 10 days, and 14 days) in leukocyte migration into joints.

Fig. 6 shows the experimental design of rheumatoid arthritis induced TMJ inflammatory hypernociception.

Fig. 7 shows the effect of a single injection of PL-PGJ2 (1 ng/TMJ) on rheumatoid arthritis-induced acute and chronic pain, compared to non-arthritic animals (control), and to arthritic animals which did not received PL-PGJ2 treatment.

Fig. 8 shows the comparative efficacy of pretreatment with plain PGJ2 (100 ng/TMJ) and PL-PGJ2 (1 ng/TMJ) injected directly into the joint, on rheumatoid arthritis-induced acute pain.

Detailed description of the invention

The development of new drugs and/or new formulations to treat chronic inflammatory diseases continues to be of considerable research interest. The pharmacological response to a drug is directly associated with its concentration at the required site of action. A non-specific distribution leads to high drug concentrations in healthy organs, tissues and cells, resulting in toxicity. To address these concerns, several drug delivery systems have increasingly been employed.

The present invention is directed to a new pharmaceutical composition comprising a poloxamer-based micellar system containing PGJ2 as the active ingredient, and an aqueous carrier, with increased analgesic and anti-inflammatory effect.

Several poloxamer and poloxamer associations were evaluated regarding their properties on the pharmaceutical composition described herein, such as viscosity, and capacity to control de release of the active component. Preferably, the poloxamer-based micellar system of the instant invention composition comprises the hydrophilic poloxamers P188, P407 or associations thereof. More preferably the poloxamer-based micellar system of the instant invention composition comprises an association between poloxamers P188 and P407 with different hydrophilic-lipophilic balances.

The ratio between poloxamer 407 and poloxamer 188 can vary since adequate fluidity for injection is maintained. Preferably the ratio between poloxamer 407 and poloxamer 188 is 1:1. The amount of poloxamer in the pharmaceutical composition as described herein ranges from 7.5% w/v to 15% w/v of the total composition. More preferred are pharmaceutical compositions in which the total amount of poloxamer is 15% (w/v) of the total composition.

It is important to point out that the pharmaceutical compositions of the instant invention were prepared in order to obtain a micellar system, avoiding micellar aggregation, and prevent the formation of a hydrogel. For this reason, final poloxamer concentration was maintained up to 15% (w/v).

The pharmaceutical composition of the present invention comprises a therapeutically effective amount of PGJ2. "Therapeutically effective" means any amount of PGJ2 that, when administered to a subject, is sufficient to produce clinical analgesic or anti-inflammatory effect. This amount may vary over a considerable range depending on the

inflammatory condition being treated, the age and relative health of the subject, the route of administration (systemic or local) and other factors apparent to one skilled in the art.

The aqueous carrier used in the present invention composition is selected from water for injection, saline physiological solution or an appropriate buffer, being water for injection the preferred carrier.

For the micellar systems preparation, poloxamers were dispersed in water under 4°C, under magnetic stirring, until the complete poloxamer solubilization (transparent solution). The active ingredient PGJ2 is solubilized in dimethyl sulfoxide (DMSO), or in a poloxamer solution, and an appropriated volume of the resulting PGJ2 solution is dispersed into the micellar solution of poloxamers.

In the preparation of the pharmaceutical composition of the present invention, further pharmaceutical additives may be used, if desired. Such pharmaceutical additives include for example preservatives, antioxidants, stabilizers and agents to adjust pH or osmolarity.

The pharmaceutical composition of the instant invention presents as a liquid or a viscous-liquid, with final pH of 5.5-6.0 (in order to achieve the pH of the inflammated tissue), suitable for both systemic and local administration through injection.

Advantageously, the pharmaceutical composition comprising PL-PGJ2 as described herein presents the ability to incorporate hydrophobic molecules, such as PGJ2, in association with its solvent, without modifying the micelles thermoreversible properties.

The pharmaceutical composition of the instant invention is thermodynamically stable in a temperature ranging from 0 to 150°C, enabling the sterilization by autoclaving. Moreover, it is also advantageous that the

instant composition presents micellar diameter in the range of 50 to 70 nm, depending on the temperature, making it also possible the sterilization by filtration.

Due to the reduced micelles dimensions (< 100 nm diameter) the proposed pharmaceutical composition PL-PGJ2 remains at the site of injection for a long period of time.

The pharmaceutical composition of the present invention comprising a poloxamer-based micellar system and PGJ2 functions as a depot formulation, with prolonged residence of the composition at the site of injection, controlling the release of PGJ2 during prolonged period of time.

In particular, the PL-PGJ2 compositions described herein showed improved characteristics when injected into inflammated joints, providing good efficacy, good tolerability, and prolonged release, allowing single injection each 7-10 days.

The pharmaceutical composition PL-PGJ2 as describe herein, have significant advantages of being able to maintain PGJ2 stability and enhanced therapeutic efficacy for long period of time, in a dose-dependent manner.

The pharmaceutical composition PL-PGJ2, as described herein, has the advantage of permitting the administration of very low frequency dosing, very small doses of the PGJ2, minimizing the side effects due to high doses of the active substance. Advantageously, the compositions described herein have a rapid onset of action, which lasts up to 7 to 10 days.

Particularly, PL-PGJ2 composition has been shown to potentiate PGJ2 analgesic and anti-inflammatory activity, and reducing the inflammatory pain due to rheumatoid arthritis.

The pharmaceutical composition of the present invention PL-PGJ2 is intended to deliver PGJ2 directly to the injured site, enabling the use of very small doses, with increased efficacy, reduced side effects, and extended

residence at the site of injection, consequently allowing greater intervals between dosages.

Therefore the present invention also provides a pharmaceutical composition comprising a poloxamer-based micellar system, PGJ2 as the active ingredient, and an aqueous carrier (PL-PGJ2), for use in treatment or prophylaxis of acute or chronic inflammatory conditions in human or animal, with enhanced PGJ2 analgesic and anti-inflammatory effect.

In accordance with other embodiment, the present invention provides the use of the pharmaceutical composition of the instant invention PL-PGJ2 for the manufacture of a medicament for treatment or prophylaxis of acute or chronic inflammatory conditions of TMJ, arthritis or other conditions mediated by inflammatory intermediates.

The present invention also provides a method for the treatment or prophylaxis of acute or chronic inflammatory conditions of temporomandibular joint (TMJ), arthritis or other conditions mediated by inflammatory intermediates characterized by comprising the administration of an effective amount of the composition PL-PGJ2 to a subject in need thereof.

The analgesic and anti-inflammatory potential of the pharmaceutical composition PL-PGJ2 was investigated employing an animal model.

A pharmaceutical composition PL-PGJ2 prepared as described in the Example 1 was used in antinociceptive assays, as described in Example 4. Nociceptive behavior was evaluated after the injection of PL-PGJ2 in the rats TMJ, followed by the nociceptive stimulus (formalin). Fig. 1 depicts the effect of PL-PGJ2 in formalin-induced inflammatory pain. The pre-treatment with PL-PGJ2 at doses of 0.3, 1 and 3 ng injected directly into the joint significantly decreased the pain response induced by intra-articular injection of formalin. Some effect following the injection of PL-PGJ2 at the

contralateral joint (ct) was also observed. This find suggests some systemic effect with doses of 3 ng/TMJ (Fig. 1) or greater (not shown).

The pharmaceutical composition PL-15-PGJ2 of the present invention has shown analgesic and anti-inflammatory activity almost 100 times greater than plain PGJ2, as shown in Fig. 2, thus confirming the ability of the pharmaceutical composition disclosed herein in potentiate the analgesic and anti-inflammatory activity of PGJ2 following the administration of very smaller doses.

Besides this important analgesic effect using very small quantity of PL-PGJ2, another advantage of the instant invention is the long-term effect promoted by PL-PGJ2 following a single injection (1 ng/TMJ). As shown in Fig. 3, the PL-PGJ2 antinociceptive effect was observed until 10 days after a single injection, showing the prolonged residence of the poloxamer-based micellar system at the site of injection together with the controlled release of the active compound from the micelles.

This analgesic action demonstrated by the PL-PGJ2 was due to its ability to reduce the inflammation in the injured site.

As described in Examples 5 and 6, plasma extravasation and leucocyte migration, respectively, were evaluated to confirm the antinociceptive and anti-inflammatory effect of the PL-PGJ2 composition. Plasma extravasation was significantly reduced until 24h after the administration of the PL-PGJ2 composition (Fig. 4), while the inflammatory cells migration was reduced until 10 days after de injection (Fig. 5) confirming the anti-inflammatory effect of the composition of the instant invention.

Specifically, the pharmaceutical composition of the instant invention was evaluated on experimental arthritis model, as described in the Example 7 and 8. As depicted in

Fig. 7, the PL-PGJ2 composition advantageously presents significantly reduced rheumatoid arthritis-induced acute and chronic pain upon administration of 1 ng of PGJ2 in poloxamer-based micelles, as shown by the decreased nociceptive pain.

Previous work (Quinteiro et al., Eur. J. Pain. 16:1106-15, 2012) has demonstrated the intra-TMJ injection of plain PGJ2 was able to inhibit the arthritis-induced hypernociception into TMJ in doses of 30, 100 or 300 ng/TMJ (in a total volume injected of 15 µL) in rats.

Advantageously, the pharmaceutical composition as described in this invention presented the same effect as the dose of 100 ng/TMJ of plain PGJ2 but with a dose 100 times smaller (1 ng/TMJ) (Fig.8), confirming that the poloxamer-based micellar composition enable the administration of very small doses with the same effect than the free drug.

Thus, the present invention describes a pharmaceutical composition comprising poloxamer-based micellar system, PGJ2 as the active ingredient, and an aqueous carrier, as a new promising composition that can overcome some drawbacks of the arthritis treatment, since it promotes analgesic and anti-inflammatory effect in both acute and chronic phases, allowing reduced dosing schedule together with reduced doses, and consequently, reduced systemic and side effects.

The poloxamer-based micellar system as described herein presents an increased residence time at the site of injection (joints), leading a longer period of pain relief to the patient.

The examples below are merely illustrative, and must be applied solely for a better understanding of the present invention embodiments, and are not to be used with the intention of limiting the described objects.

Example 1

Preparation of Samples:

A) PGJ2 Stock Solutions (10 mL):

0.01% w/v PGJ2 (Calbiochem, San Diego, CA, USA) solution was prepared by adding 10 mL of dimethyl sulfoxide (DMSO) to 1.0 mg of PGJ2. Following, the obtained solution can be aliquoted and frozen or stored at -20 °C for up to 3 months.

B) Poloxamer Stock Solutions (500 mL):

15 % w/v poloxamer solutions were prepared by adding 75 g of total poloxamer to 350 mL of water for injection at 4°C, under magnetic stirring (100 rpm), until the complete poloxamer solubilization (transparent solution). This solution was then made up to 500 mL with cold water for injection. Table 1 presents the different formulations prepared as described.

Table 1. Different poloxamer formulations

Formulation	**Poloxamer 407 (% w/v)**	**Poloxamer 188 (% w/v)**
F1	15.0	-
F2	7.5	-
F3	-	15.0
F4	-	7.5
F5	7.5	7.5

C) Pharmaceutical composition (20 mL):

0.015 to 0.15 µg/mL PGJ2 pharmaceutical compositions on poloxamer-based micellar system were prepared by dispersing 30 to 300 µL, respectively, of PGJ2 stock solution 0.01%, obtained as described in A) to 20 ml of the poloxamer

stock solution, as prepared B) **F5**, above. After PGJ2 incorporation, compositions were stirred (100 rpm) at 4°C, for at least 6 hours. The obtained compositions must be stored at 2-8°C until further use. All compositions presented total DMSO concentration of 0.15 to 1.5 % v/v.

Example 2

The micellar hydrodynamic diameter was determined using Dynamic Light Scattering (DLS) to evaluate micelle-PGJ2 interaction and its influence on micellar assembly. Measurements were performed using a particle analyzer Zetasizer ZS (Malvern®, UK) at a fixed angle of 173° and temperatures of 25°C and 37°C. All measurements were determined at least three times for each sample.

The hydrodynamic diameter determination was used as a parameter for monitoring the formation of mixed micelles composed of poloxamer 407 and poloxamer 188 in the presence or absence of PGJ2. In general, no significant changes were observed on micellar hydrodynamic diameter for PL407-PL188 systems after PGJ2 incorporation (Table 2). Micellar diameters of ~ 70 nm were observed at 25 °C and when the temperature was raised to 37 °C, a reduction on micellar dimensions (~ 50 nm) was obtained (with a 99.5 % average distribution). Polydispersity values were from 0.25 at 25 °C to 0.15 at 37 °C. These parameters showed an important contribution of temperature on micellar diameter. Considering that the micellar dimension will be reduced at physiological temperature, micelles can remain for long periods of time at the site of administration and also should be small enough (<100 nm) to avoid the uptake by the reticuloendothelial system, favoring the therapeutic efficacy of the drug carrier (Wei et al., Int. J. Pharm. 376:176-185, 2009).

Table 2 - Micellar hydrodynamic diameter - at two different temperatures

Formulations	Medium diameter (nm)	
	25°C	37°C
Poloxamer 407-188	69.1 ± 2.3	48.2 ± 3.4
Poloxamer 407-188 (0.1 ng PGJ2)	68.9 ± 1.5	45.7 ± 2.8
Poloxamer 407-188 (1 ng PGJ2)	68.9 ± 2.5	48.4 ± 1.6
Poloxamer 407-188 (3 ng PGJ2)	69.7 ± 2.1	47.1 ± 2.2

Example 3

To determine the temperature and enthalpy relatively to the micellization process, Differential Scanning Calorimetry (DSC) analysis was performed using 50 mg of poloxamer formulations (in the presence or absence of PGJ2) placed in sealed aluminum pans. Samples were analyzed according to three successive thermal cycles of heating and cooling (0 °C to 50 °C) at heat-cooling rate of 5 °C/min with an empty pan as reference. Data were expressed in thermograms represented by heat flux ($k.J.mol^{-1}$) versus temperature (°C).

Results showed that there were no significant variations in the temperature on the micelles formation, which indicates the stability of the thermoreversible micellar systems and also its preservation even after the addition of DMSO and PGJ2. In addition, samples containing PGJ2 had higher enthalpy changes, suggesting the insertion of DMSO and PGJ2 into the micelles. Regarding thermodynamic parameters, considering that the enthalpy variation in enthalpy of the system is greater than zero, the micellization process is endothermic (Table 3). The association of hydrophilic polymers (such as poloxamer 188 with HLB = 29) induces conformational changes in the micellar self-assembly and increases the hydration of the micellar corona (due to the larger number of units of ethylene oxide), justifying the minor enthalpy variations observed during the micellization

process for the poloxamer 407-poloxamer 188 with no PGJ2. However, the main advantage of this system is the capability for incorporating hydrophobic molecules, such as PGJ2, in association with its solvent, without modifying thermoreversible properties at physiological temperature (since the final temperature for micelles formation is below to the body temperature for all formulations).

Table 3 - Temperatures and enthalpy variations ($\Delta H°$) relatively to the micellization process

Formulations	T_{onset} (°C)	$T_{micellization}$ (°C)	T_{endset} (°C)	$\Delta H°$ (cal/g)
Poloxamer 407-188	17.15	20.16	25.02	0.03201
Poloxamer 407-188 (PGJ2 0.015 µg/mL)	17.06	20.83	27.02	0.1780
Poloxamer 407-188 (PGJ2 0.05 µg/mL)	16.98	20.03	26.49	0.1383
Poloxamer 407-188 (PGJ2 0.15 µg/mL)	19.94	19.94	26.54	0.1238

Note: T_{onset} = initial temperature for micelles formation, $T_{micellization}$ = peak temperature for micelles formation, T_{endset} = final temperature for micelles formation

Experimental Procedures

All experimental protocols and procedures were conducted in accordance to the guidelines of National Council for Control of Animal Experimentation (CONCEA) and International Association for the Study of Pain (IASP) in conscious animals (Zimmermann, Pain. 16:109-10, 1983). The animals suffering and number per group were kept at a minimum and each animal was used once. Male Wistar rats (*Rattus norvegicus*), weighing about 150-250 g, obtained from the Multidisciplinary Center for Biological Research (CEMIB) at

the State University of Campinas (Campinas, São Paulo, Brazil). The animals were housed in temperature-controlled rooms (23 ± 1°C) with a 12/12 h light-dark cycle (lights on at 06:00 a.m.), with access to water and food *ad libitum*.

Example 4: Nociceptive Behavior

Testing sessions took place during light phase (between 09:00 a.m. and 05:00 p.m.) in a quiet room maintained at 23°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 (30x30x30 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., Pain. 94:185-91, 2001). Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber for counting nociceptive responses. Rats were pretreated (15 min) with an intra-TMJ injection of PL-PGJ2 (0.3, 1.0 or 3.0 ng/TMJ) followed by ipsilateral intra-TMJ injection of 1.5% formalin in a final volume of 45 µL. Behavioral nociception response was evaluated for a 45 min observation period. In order to confirm the peripheral PGJ2-mediated antinociception, the two highest dose of PL-PGJ2 was also injected in the contralateral TMJ (ct) that received injection of 1.5% formalin. 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 shown in Figure 1). 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., Pain. 94:185-91, 2001; Clemente et al., Neurosci. Lett. 372:250-5 2004).

Formalin solution was prepared from commercially stock formalin (an aqueous solution of 37 % of formaldehyde - Sigma Aldrich, St. Louis, MO, USA) and further diluted in 0.9 % NaCl.

To evaluate how long the anti-nociceptive effect of the composition PL-PGJ2 lasts, animals was administered with a single injection of 1.0 ng/TMJ of PL-PGJ2 and the nociceptive behavior was evaluated after injection of formalin solution. Animals received the formalin injection 15 minutes, 24 h, 48 h, 72 h, 7 days, 10 days and 14 days after the injection of PL-PGJ2 (Fig. 3).

Example 5: Plasma extravasation

At the conclusion of the experiment of nociceptive behavior, animals were anesthetized by an intraperitoneal injection of a mixture of urethane (1 g/kg) and α-chloralose (50 mg/kg), followed by i.v. administration of Evan's blue dye (1%; 5 mg/kg), to visualize formalin-induced plasma extravasation upon *post-mortem* examination of injected TMJs (Haas et al., Arch. Oral Biol. 37:417-422, 1992). This procedure also confirmed that the plasma extravasation induced by TMJ injection at the correct site was restricted to the immediate TMJ region (Fig. 4). A different investigator performed each test, prepared the solution, and administered the TMJ injections. All animals received a final volume of 45 µL into TMJ as previously described (Clemente et al., Neurosci. Lett. 372:250-5, 2004). All experiments were conducted in a double blind fashion in which the person who injected the solutions was different of the one who made the behavioral assessment.

Example 6: Leucocyte Migration to TMJ Periarticular Tissues

After behavioral experiments, the rats were euthanized and the articular cavity was washed with 10 µL with phosphate buffer saline (PBS) containing 1 mM EDTA for leukocytes migration analysis. The TMJ periarticular tissues were also removed and homogenized in 500 µL of the appropriate buffer containing protease inhibitors (Ripa Lysis Buffer, Santa Cruz, Biotechnology, Dallas, Texas, USA) followed by a centrifugation of 10 min/10,000 rpm/4°C.

Total leukocyte counts were performed in a Neubauer chamber diluting the exudate in Türk solution (1:2) and expressed as number of cells x 10^4/cavity. The differential leukocyte counts, was performed by preparing smears in a cytocentrifuge, which were stained with fast Panotic kit, and for differentiated cells (100 cells total), an optical microscope (under oil immersion at 1000 x magnification) was utilized. The result of each cell type was calculated using the percentage of those cells and the total number of cells obtained in the total count (as shown in Fig. 5).

Example 7: Induction of experimental arthritis:

The protocol used to induce the experimental arthritis was described previously (Quinteiro et al., Eur. J. Pain. 16:1106-15, 2012) and is depicted in Fig. 6. Briefly, male Wistar rats were sensitized with 500 µg of methylated bovine serum albumin (mBSA) (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 200 µL of an emulsion containing 100 µL phosphate buffered saline (PBS) and 100 µL Freund's complete adjuvant (CFA) (Sigma-Aldrich, St. Louis, MO, USA) administered subcutaneously in the back. Booster injections of mBSA dissolved in Freund's incomplete adjuvant (IFA) (Sigma-Aldrich, St. Louis, MO, USA) were given 7 and 14 days after the first immunization in different sites in the back of the rat. To evaluate the acute pain, twenty-one days after the initial injection, TMJ-arthritis was induced in the immunized animals by intra-articular injection of mBSA (10 µg/ TMJ)

dissolved in 15 µL of PBS (challenge). Non-immunized rats (control group) were treated by an intra-articular injection of mBSA. With another group of animals, besides the administration of mBSA on day 21, novel challenges with mBSA were done on days 28 and 35. Again, control group (non-immunized rats) were treated with an intra-articular injection of mBSA.

Example 8: Effect of the PL-PGJ2 in the hypernociception of on arthritis-induced acute and chronic pain in TMJ of rats:

To test the effect of PL-PGJ2 on acute and chronic TMJ-arthritis hypernociception it was used the measurement of the behavioral nociceptive response induced by formalin injections (as described on Example 4, above). The rats were pretreated with an intra-TMJ injection of PL-PGJ2 (1.0 ng/ TMJ) or vehicle 24 hours after the last mBSA challenge (day 22 for arthritis acute phase pain evaluation, and day 36 for arthritis chronic pain evaluation). After 15 minutes an intra-TMJ injection of formalin (0.5%) was administered. Immediately after the formalin injection, the behavioral nociceptive response was evaluated for a 45 min observation period (Fig. 7).

To compare the effect of PL-PGJ2 with plain PGJ2 in arthritis induced acute pain, immunized (arthritic) and non-immunized (non-arthritic) animals received intra-TMJ injection 100 ng plain PGJ2 or 1 ng PL-PGJ2 24h after the mBSA challenge (Fig. 8). After 15 minutes an injection of 0.5% formalin was administered to the animals and the painful behavior was evaluated. As the controls, non-immunized animals received only injections of PBS or formalin or mBSA+formalin, and immunized animals received PBS injection.

CLAIMS

1. A pharmaceutical composition characterized by comprising a poloxamer-based micellar system, PGJ2 as the active ingredient, and an aqueous carrier.

2. The composition according to claim 1, characterized by the fact that the poloxamer-based micellar system comprises the poloxamer P188, P407 or associations thereof.

3. The composition according to claim 2, characterized by the fact that the poloxamer-based micellar system comprises an association of poloxamers P407 and P188.

4. The composition according to claim 3, characterized by the fact that the ratio between the poloxamers P407 and P188 is 1:1.

5. The composition according to claim 1, characterized by the fact that the total amount of poloxamer ranges between 7.5 and 15% w/v of the total composition.

6. The composition according to claim 5, characterized by the fact that the total amount of poloxamer is 15% w/v of the total composition.

7. The composition according to claim 1, characterized by the fact that the aqueous carrier is water for injection, saline physiological solution or an appropriated buffer.

8. The composition according to claim 7, characterized by the fact that the aqueous carrier is water for injection.

9. The composition according to claims 1 to 8, characterized by being a thermoreversible liquid or viscous-liquid.

10. The composition of claims 1 to 9, characterized by being suitable for injection.

11. The composition according to claim 10, characterized by being suitable for systemic or local administration.

12. The composition according to claims 1 to 11, characterized by providing prolonged residence of PGJ2 at the

site of injection and enhanced PGJ2 analgesic and anti-inflammatory effect.

13. The composition according to claims 1 to 12, for use in the treatment or prophylaxis of acute or chronic inflammatory conditions of TMJ, arthritis or other conditions mediated by inflammatory intermediates.

14. Use of the composition according to claim 1 for the manufacture of a medicament for treatment or prophylaxis of acute or chronic inflammatory conditions of temporomandibular joint (TMJ), arthritis or other conditions mediated by inflammatory intermediates.

15. A method for the treatment or prophylaxis of acute or chronic inflammatory conditions of temporomandibular joint (TMJ), arthritis or other conditions mediated by inflammatory intermediates, characterized by comprising the administration of an effective amount of the composition of claim 1 to a subject in need thereof.

Figure 1

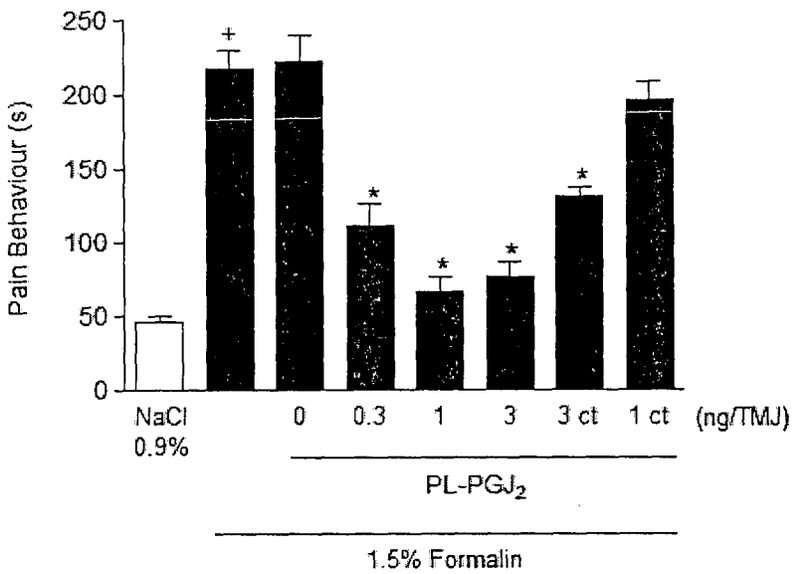


Figure 2

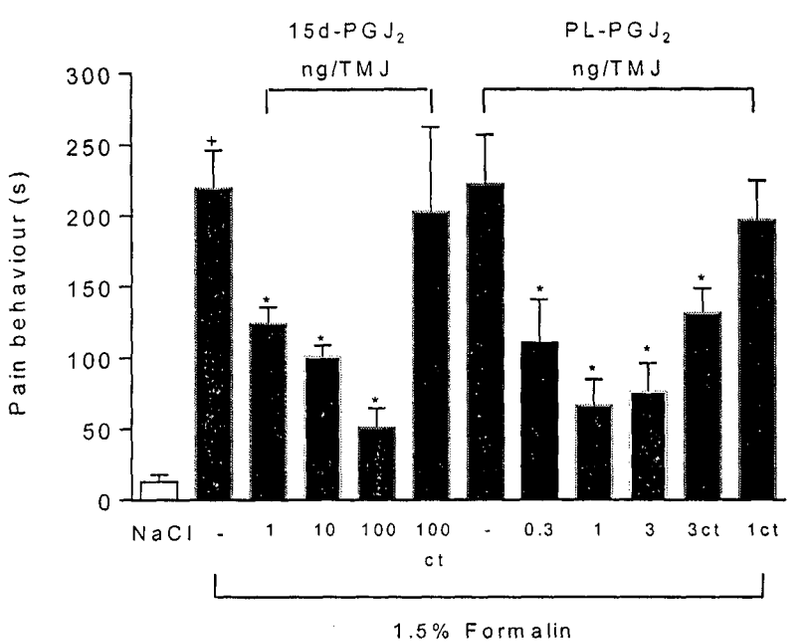


Figure 3

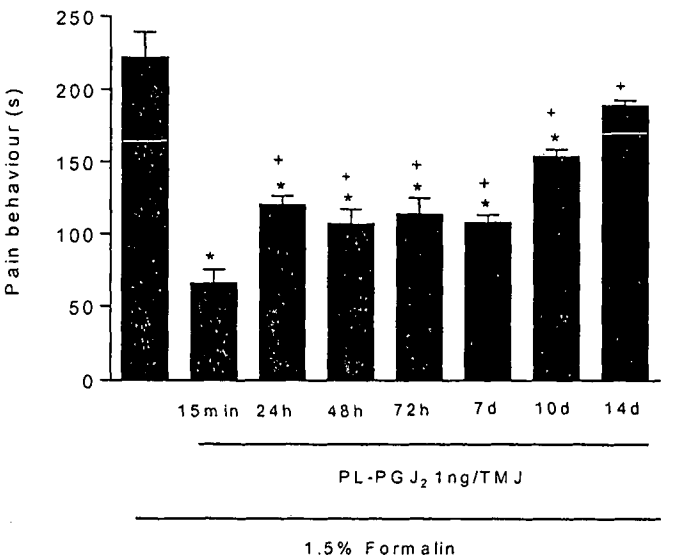


Figure 4

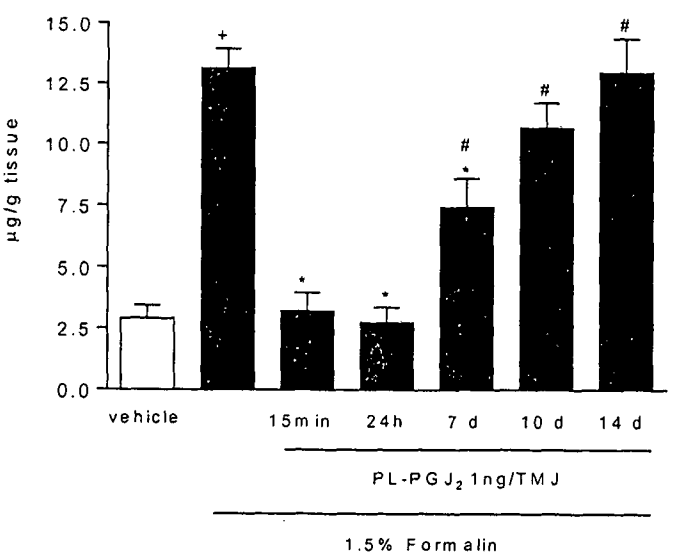


Figure 5

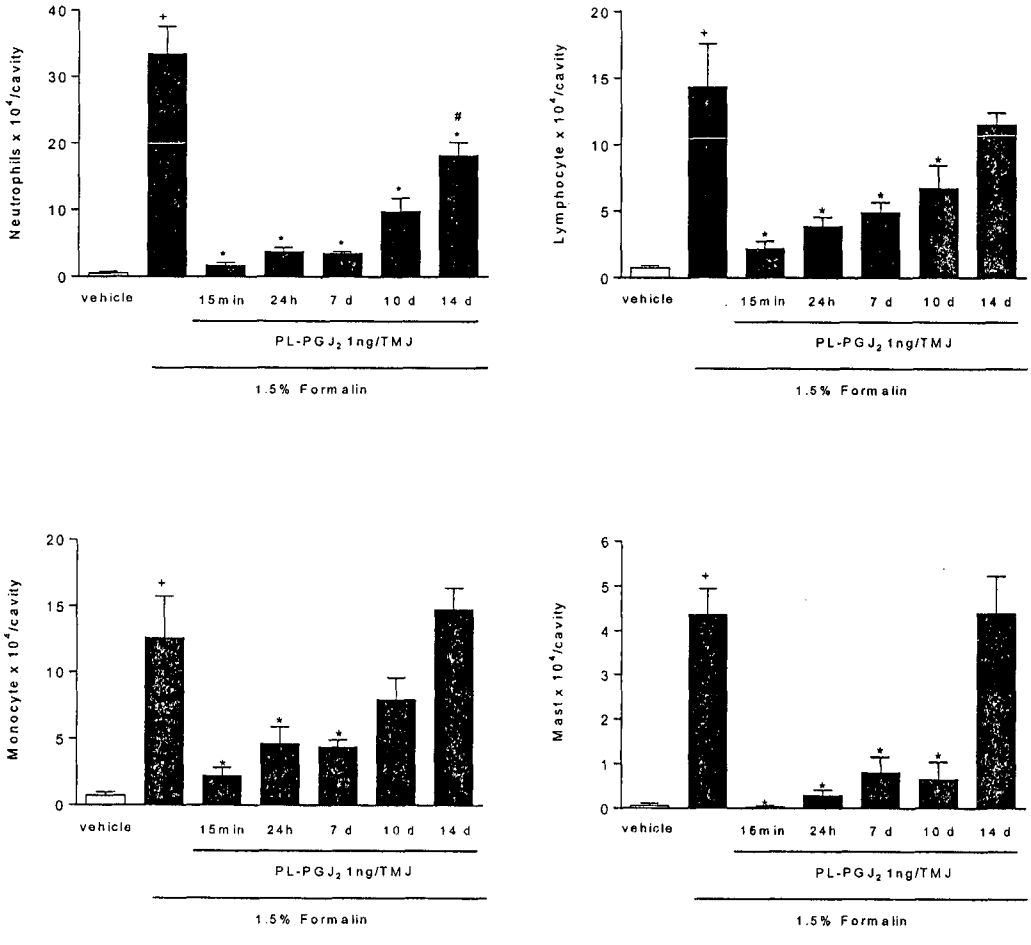

Neutrophils x 10⁴/cavity
Lymphocyte x 10⁴/cavity
Monocyte x 10⁴/cavity
Mast x 10⁴/cavity
vehicle
15min
24h
7 d
10 d
14 d
PL-PGJ2 1ng/TMJ
1.5% Formalin

Figure 6

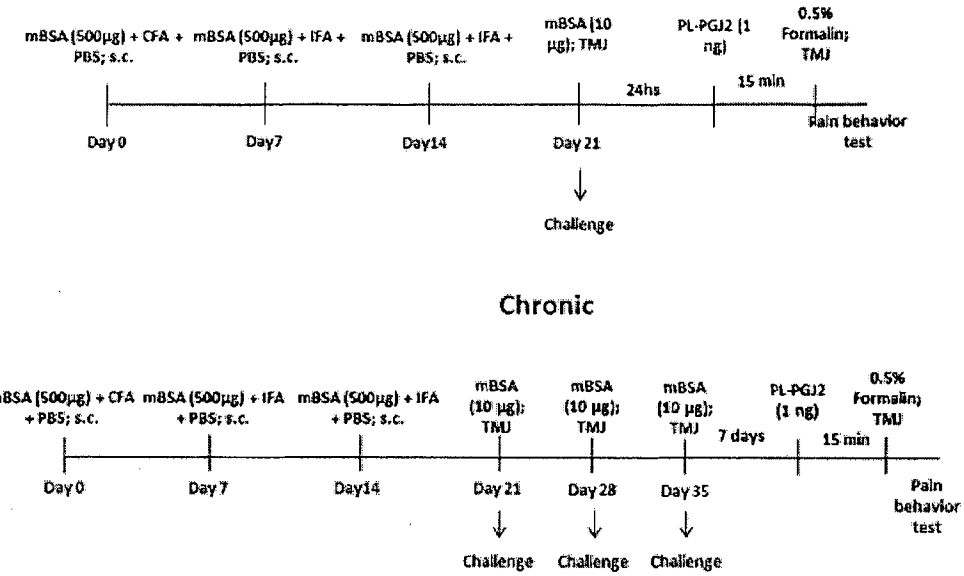


Figure 7

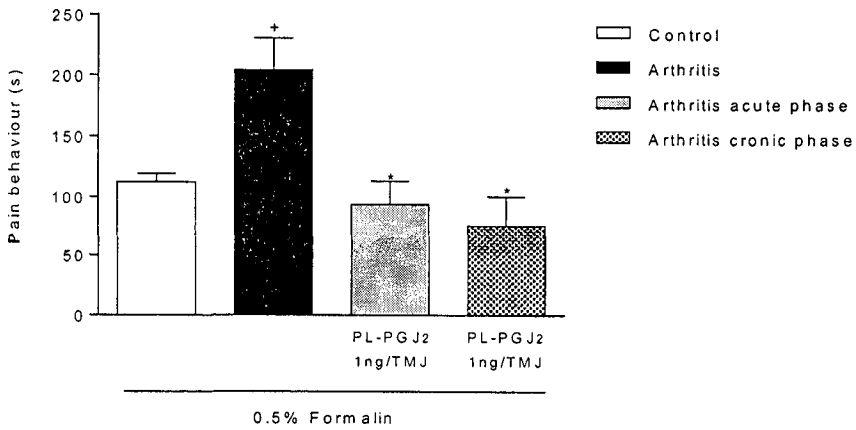
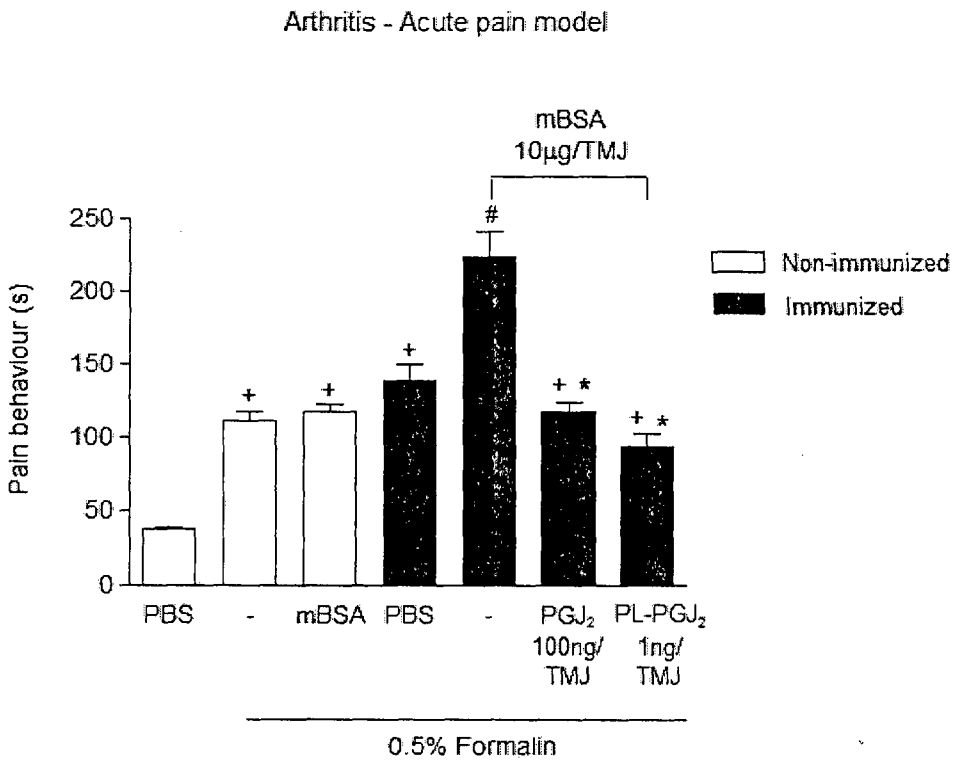


Figure 8


Arthritis - Acute pain model
mBSA
10µg/TMJ
Pain behaviour (s)
250
200
150
100
50
0
#
+
+
+
+ *
+ *
Non-immunized
Immunized
PBS
-
mBSA
PBS
-
PGJ2
100ng/
TMJ
PL-PGJ2
1ng/
TMJ
0.5% Formalin

INTERNATIONAL SEARCH REPORT

International application Nº
PCT/BR2014/000349

A. CLASSIFICATION OF SUBJECT MATTER

A61K31/5575 (2006.01), A61K9/10 (2006.01), A61K9/14 (2006.01), A61K9/66 (2006.01), A61K47/34 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SINPI- Database INPI-BR; Periódicos Capes

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC; ProQuest-Dialog (all databases); STN (Registry, Caplus); Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claims Nº
A	IADR/AADR/CADR General Session & Exhibition, Seattle, USA, vol. 92, march 22, 2013, p.161-161. Abdalla, H.B. *et al.* [Nanotechnology a great option for drug delivery into TMJ].	1-14
A	Life Sciences, vol. 90, 2012, p. 944 – 949. Clemente-Napimoga, J.T. *et al.* [15d-PGJ2-loaded in nanocapsules enhance the antinociceptive properties into rat temporomandibular hypernociception].	1-14
A	Int. J. Pharm., vol. 33, 1986, p. 65-69. Law, T.K. et al. [Some chemically modified poloxamer hydrogels: controlled release of prostaglandin-E2 and testosterone].	1-14
A	WO 0193836 A2 (BOULIKAS TENI [US]) 13 December 2001 (2001-12-13)	1-14
A	WO 2009120566 A1 (BAUSCH & LOMB INCORPORATED [US]) 01 October 2009 (2009-10-01)	1-14

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to stablish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority claimed

"T" later document published after the internationa filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvius to a person skilled in the art

"&" document member ot the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
october 10. 2014	**07/01/2015**

Name and mailing address of the ISA/**BR**

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claims Nº
A	US 8460644 B2 (IS PHARMACEUTICALS LIMITED [GB]) 11 June 2013 (2013-06-11)	1-14
A	Pharmaceutical Research, vol. 23 (12), december 2006, p. 2709-2728. Dumortier, G. *et al.* [A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics].	1-14
A	J. Chil. Chem. Soc., vol. 59 (2), jul 2014, p. 2451-2454. Olea, A. F. *et al*. [Solubilization of p-alkylphenols in pluronics F-68 and F-127 micelles: partition coefficients and effect of solute on the aggregate structure].	1-14

INTERNATIONAL SEARCH REPORT

International application N°
PCT/BR2014/000349

Box No II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 15

because they relate to subject matter not required to be searched by this Authority, namely:

Claim 15 is directed to a method for treatment of the human or animal body by surgery or therapy, which the International Search Authority is not required to search under PCT Rule 39.1 (iv).

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application Nº
PCT/BR2014/000349

Patent documents cited in search report	Publication date	Patent family members	Publication date
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