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15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 reduces albumin-induced arthritis in temporomandibular joint of rats

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ABSTRACT

The aim of this study was to evaluate the peripheral effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) in albumin-induced arthritis in temporomandibular joint (TMJ) of rats. Antigen-induced arthritis (AIA) was generated in rats with methylated bovine serum albumin (mBSA) diluted in complete Freund's adjuvant. Pretreatment with an intra-articular injection of 15d-PGJ_2 (100 ng/TMJ) before mBSA intra-articular injection (10 μg/TMJ) (challenge) in immunized rats significantly reduced the albumin-induced arthritis inflammation. The results demonstrated that 15d-PGJ_2 was able to inhibit plasma extravasation, leukocyte migration and the release of inflammatory cytokines IL-6, IL-12, IL-18 and the chemokine CINC-1 in the TMJ tissues. In addition, 15d-PGJ_2 was able to increase the expression of the anti-adhesive molecule CD55 and the anti-inflammatory cytokine IL-10. Taken together, it is possible to suggest that 15d-PGJ_2 inhibit leukocyte infiltration and subsequently inflammatory process, through a shift in the balance of the pro- and anti-adhesive properties. Thus, 15d-PGJ_2 might be used as a potential anti-inflammatory drug to treat arthritis-induced inflammation of the temporomandibular joint.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune polyarthritis with synovial hyperplasia and joint destruction, leading to pain, loss of joint function and concomitant reduction in the quality of life (Németh and Mócsai, 2012). Being a synovial joint, the temporomandibular joint (TMJ) is subject to the same disorders affecting other synovial joints, including RA (Aliko et al., 2011). TMJ involvement in RA has high prevalence, from 65 to 92.9% (Aliko et al., 2011; Lin et al., 2007) and the most commonly reported symptoms include pain in the TMJ area, tenderness of the masticatory muscles, joint sounds and limited joint function (Goupille et al., 1993; Lin et al., 2007).

In the RA, leukocyte migration is enabled by endothelial activation in synovial microvessels, which increases the expression of adhesion molecules and chemokines. A variety of innate effector cells, including macrophages, mast cells and natural killer cells, are found in the synovial membrane, whereas neutrophils reside mainly in synovial fluid. Macrophages are central effectors of synovitis acting through released cytokines, such as TNF-α and interleukin-1, 6, 12, 15, 18, and 23, production of prostanoids and matrix-degrading enzymes, phagocytosis, and antigen presentation (McInnes and Schett, 2011). This interdependent network of cytokines, particularly TNF-α and IL-1β, prostanoids and proteolytic enzymes mediates many of the immune processes associated with the pathogenesis of RA (Di Paola and Cuzzocrea, 2008; Sachs et al., 2011).

Currently, keystone of RA therapy includes biologics such as antibodies to TNF-α, IL-1β and IL-6 (Taylor and Feldmann, 2009), despite conventional Disease-Modifying Antirheumatic Drug (DMARDs) – comprising a group of agents such as methotrexate, sulphasalazine, hydroxychloroquine and azathioprine – still being used (O'Shea et al., 2013). The precise mechanisms of action of these compounds remain elusive and, importantly, their introduction was not directed by a rationalization of target biology related to RA pathogenesis. Moreover, conventional DMARDs do not specifically target immune cells (O'Shea et al., 2013). Given the complex molecular pathogenesis and highly heterogeneous clinical picture of RA, there is an urgent need to dissect its multifactorial nature and to propose new strategies for preventive, early and curative treatments.

15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), a cyclopentenone-type prostaglandin with a wide spectrum of physiological activities

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is one of the terminal products of the cyclooxygenase-2 (COX-2) pathway. 15d-PGJ_2 was initially discovered as a potent ligand for peroxisome proliferator-activated receptor γ (PPAR-γ), a member of the nuclear receptor superfamily and a ligand-activated transcription factor with pleiotropic effects on adipocyte differentiation, glucose homeostasis, lipid metabolism, growth, and inflammation (Surh et al., 2011). Our group has previously demonstrated that peripheral administration of 15d-PGJ_2 was able to prevent the nociceptor sensitization into the TMJ by two different mechanisms: direct mechanism that involves activation of κ/δ opioid receptors following the activation of the intracellular L-Arginine-NO/cGMP/K^+_{ATP} on primary nociceptive neurons from TMJ (Pena-dos-Santos et al., 2009) and indirect mechanism that involves inhibition of TNF-α-induced hypernociception intracellular cascade (Quinteiro et al. 2012). It is currently known that the temporomandibular disorder, including the RA-affected, has an important inflammatory component as well as painful state, thus the aim of this study was to evaluate the peripheral effect of 15d-PGJ_2 in albumin-induced arthritis in rat's TMJ as well its mechanisms.

2. Materials and methods

2.1. Animals

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), were housed in temperature-controlled rooms (23 ± 1 °C) with 12/12 h light–dark cycle (lights on at 06:00 a.m.), with access to water and food ad libitum. All experiments 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, 1983) and with the approval of the Ethics Committee on Animal Research of the State University of Campinas (CEUA/UNICAMP no. 2949-1). The animals suffering and number per group were kept at a minimum and each animal was used once.

2.2. Induction of experimental arthritis

The protocol used to induce the experimental arthritis was described previously (Quinteiro et al., 2012). 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 by subcutaneous injection 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. 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.

In the previous work (Quinteiro et al., 2012) we demonstrated that arthritis induced a higher nociceptive behavioral response 24 h after intra-TMJ injection of mBSA (challenge, 10 μg/TMJ) in immunized rats, and the intra-TMJ injection of 15d-PGJ_2, after this period, was able to inhibit the arthritis-induced hypernociception into TMJ. Considering these results, in the present work the development of arthritis-induced inflammation into TMJ in different inflammatory parameters and the ability of the 15d-PGJ_2 to prevent this process was evaluated. For that, it was necessary to modify the original experimental protocol (Quinteiro et al., 2012) for the present work, animals receive the intra-TMJ injection of 15d-PGJ_2 prior to the intra-TMJ injection of mBSA (challenge, 10 μg/TMJ) in immunized rats (Fig. 1),

2.3. Effect of the 15d-PGJ_2 in the hypernociception of albumin-induced arthritis in TMJ of rats

RA-induced TMJ inflammatory hypernociception was assessed by measuring behavioral nociceptive responses induced by intra-articular injection of a low dose of formalin (0.5%) into the TMJ 6, 12, 24 or 48 h after challenge in immunized rats. To test the effect of 15d-PGJ_2 on TMJ-arthritis hypernociception, the rats were pretreated (15 min) with an intra-TMJ injection of 15d-PGJ_2 or vehicle (Calbiochem, San Diego, CA, USA) (100 ng/ TMJ; Quinteiro et al., 2012) followed by a challenge (mBSA 10 μg/TMJ). After 6, 12, 24 or 48 h, an intra-TMJ injection of formalin (0.5%) was administered. Immediately after the formalin injection, the behavioral nociceptive response was evaluated for 45 min observation period (Fig. 1). As previously described (Clemente et al., 2004; Roveroni et al., 2001) to evaluate the behavioral nociceptive response the animals were briefly anaesthetized by inhalation of isoflurane to allow the TMJ injection, which was performed with 30-gauge needle connected to 50-μl Hamilton syringe (Roveroni et al., 2001). Each animal regained consciousness approximately 30 s after discontinuing the anesthesia 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 for 1 s, each flinch was expressed as 1 s. Results are expressed as the duration time of nociceptive behavior (Clemente et al., 2004; Roveroni et al., 2001). All experiments were conducted in a double-blind manner, in

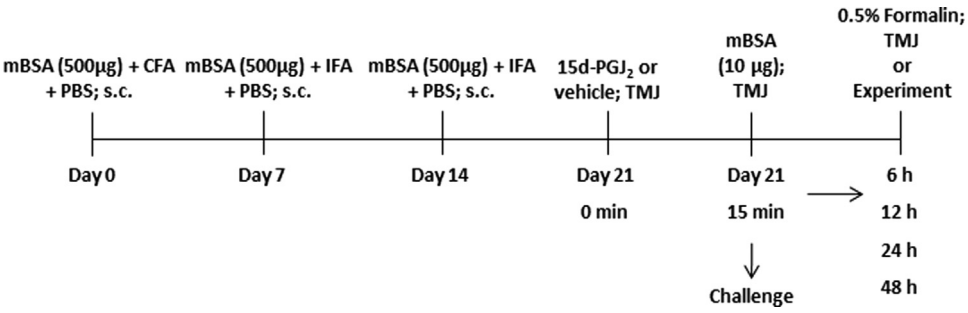

Fig. 1. Experimental design of the effect of 15d-PGJ_2 in the hypernociception of albumin-induced arthritis in TMJ.