Original article

Anti-inflammatory and anti-nociceptive effects of strontium ranelate on the zymosan-induced temporomandibular joint inflammatory hypernociception in rats depend on TNF-α inhibition

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ARTICLE INFO

ABSTRACT

Background: Temporomandibular joint (TMJ) disorders show inflammatory components, heavily impacting on quality of life. Strontium ranelate has previously shown anti-inflammatory and antinociceptive effects on other experimental inflammatory pain models. Thus, we aim to investigate the strontium ranelate efficacy in reducing the zymosan-induced inflammatory hypernociception in the TMJ of rats by evaluating the TNF-α, IL-1β, and hemeoxygenase-1 (HO-1) involvement.
Methods: Wistar rats were treated with strontium ranelate (0.5, 5 or 50 mg/kg, per os) 1 h before zymosan injection (iart). Mechanical threshold was assessed by Von Frey test and synovial lavage was collected for leukocyte counting and myeloperoxidase measurement, joint tissue and trigeminal ganglion were excised for histopathological analysis (H&E) and TNF-α/IL-1β levels dosage (ELISA). Moreover, rats were pre-treated with ZnPP-IX (3 mg/kg, sc), a specific HO-1 inhibitor, before strontium ranelate administration (0.5 mg/kg, per os), and Evans Blue (5 mg/kg, iv) was administered to assess plasma extravasation. Pre-treatment with indomethacin (5 mg/kg, sc) was used as positive control while the sham group received 0.9% sterile saline (per os and iart).
Results: Strontium ranelate did not reduce leukocyte counting, myeloperoxidase activity, Evans Blue extravasation, IL-1β levels, and TNF-α/IL-1β immunolabeling; but it increased the nociceptive threshold and reduced TNF-α levels. Additionally, HO-1 inhibition did not change the strontium ranelate effects.
Conclusion: Strontium ranelate may achieve its antinociceptive effects through the reduction of TNF-α levels in the trigeminal ganglion, but not suppressing IL-1β expression nor inducing the HO-1 pathway.

Introduction

The pathogenesis of temporomandibular joint (TMJ) disorders still remains unclear even with the remarkable progress already made towards the elucidation of their pathogenesis. Experimental models that allow the investigation of the inflammatory pain related to these disorders are of great clinical relevance and our group developed an experimental model of zymosan-induced arthritis in rats. Zymosan is a yeast cell-derived polysaccharide that

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produces a severe and erosive synovitis along with inflammatory pain in animal models of knee arthritis [1–3].

Inflammatory stimuli cause mechanical hypernociception by a defined sequential release of cytokines within inflamed joints by many cells such as tumor necrosis factor-α (TNF-α) and interleukin-1β [4]. TNF-α and IL-1β are highly expressed in the joints affected by TMJ disorders. Many studies showed considerable levels of both in the synovial fluid of patients suffering from this condition [5,6]. Furthermore, studies demonstrated that heme oxygenase – 1 (HO-1) exerts an antioxidant role and its induction would lead to negative feedback for cell activation and production of inflammatory mediators [7–9].

Strontium ranelate (Sran) {~5-[bis (carboxy-methyl) amino]-2-carboxy-4-cyano-3-thiophen-acetic acid distrontium salt} is a compound with two stable strontium atoms and ranelic acid. It affects the bone turnover and it is an orally active treatment which decreases the risk of vertebral and hip fractures in osteoporotic women [10,11]. Although its mechanism of action is not fully understood, this drug may possess analgesic effects [12]. Recent studies showed that strontium ranelate had a protective effect in experimental osteoarthritis [13] and it promoted analgesia in arthritic rats, which was associated to inhibition of the release of inflammatory cytokines into inflamed joints [14].

Thus, the present study attempts to investigate the unexplored anti-nociceptive and anti-inflammatory effects of strontium ranelate on the zymosan-induced inflammatory hypernociception in the TMJ of rats by evaluating the IL-1β and TNF-α levels after strontium ranelate treatment. Further, we aim to determine whether strontium ranelate effects on this experimental model of arthritis would depend on HO-1 pathway integrity as our previous results showed that HO-1 pathway inhibition is associated with increased inflammatory responses [8].
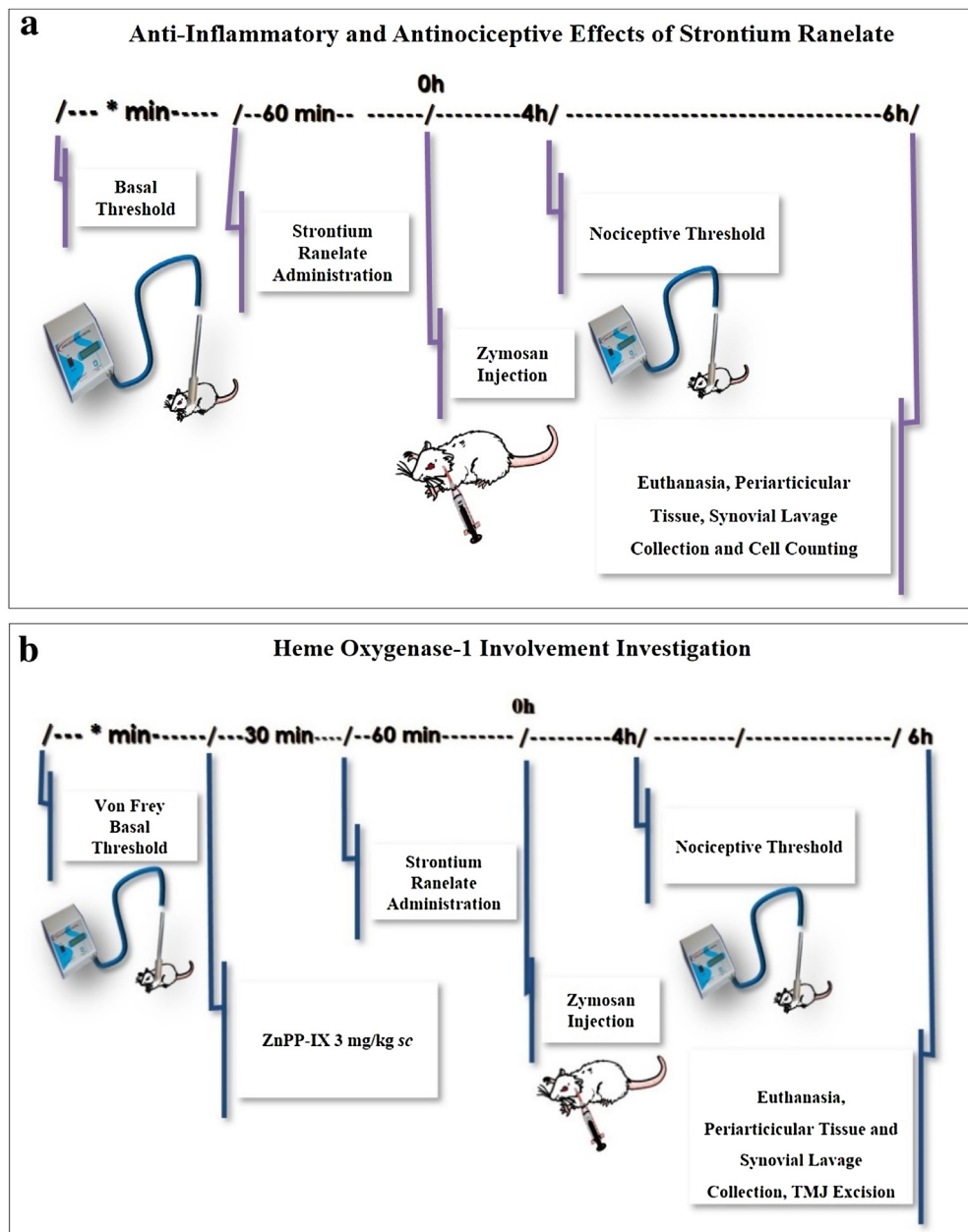

Fig. 1. Experimental design to assess strontium ranelate effects on the TMJ arthritis. (a) Evaluation of the anti-inflammatory and antinociceptive effects of strontium ranelate on the zymosan-induced mechanical allodynia in the rat temporomandibular joint. (b) Investigation of the involvement of heme oxygenase-1 in the anti-inflammatory and antinociceptive effects of strontium ranelate on the zymosan-induced mechanical allodynia in the rat temporomandibular joint.

Materials and methods

Animals

Male *Wistar* rats (n = 6 per group; 160–220 g) were housed in standard plastic cages, they had access to food and water *ad libitum* and were maintained in a temperature-controlled room ($23 \pm 2^\circ$C) with a 12/12-h light-dark cycle. This study was conducted in accordance with the local Institutional Animal Care and with the approval of the local ethical committee (registration number 54/12) and we designed it to reduce animal suffering and the number of animals.

Zymosan-induced inflammatory hypernociception

To induce the inflammatory hypernociception, rats were anesthetized with inhaled isoflurane (4%) and received intra-articular (*iart*) injection of zymosan (2 mg; 40 μL) dissolved in sterile saline into the left TMJ using a 30-gauge needle. Sham animals received saline solution (*per os*) before zymosan or saline solution injections. The TMJ skin was shaved, the postero-inferior border of the zygomatic arch was located, and the needle was inserted in a position inferior to this point until the needle contacted the condyle, which was verified by the movement of the mandible and the puncture of the needle into the joint space was confirmed by the loss of resistance. Gentle aspiration ruled out intravascular injection, after which zymosan or saline solution were injected. As previously shown by our group [1] the zymosan-induced inflammatory hypernociception is maximal at 4 h of arthritis while polymorphonuclear cell influx peaks after 6 h. Thus, we used these time points to assess the following parameters: head withdrawal threshold, total cell counting, and myeloperoxidase activity.

Mechanical threshold evaluation

Inflammatory hypernociception in the TMJ was evaluated by measuring the threshold of force needed to be applied to the TMJ region until the head withdrawal occurred. The measurements were performed by a blinded examiner who used a digital device (Insight, Brazil) that consisted of a rigid filament linked to an electronic device – automatic Von Frey anesthesiometer, which in turn measures the response threshold in grams (g) when the filament is applied to the surface of the tested region [15]. The facial areas to be tested around the TMJ were shaved before the experimental procedure and the animals were placed in individual plastic cages 45 min before the tests. The animals underwent conditioning sessions in the testing room for 4 consecutive days. On day five, the basal force threshold value was recorded three times before and 4 h after the intra-articular injections of either zymosan or vehicle. Then, we measured the inflammatory hypernociception of the zymosan-group animals and treated controls. The applied mechanical stimuli were innocuous in control animals.

Pharmacological modulation

Strontium ranelate (PROTOS® 2 g, Les Laboratoires Servier Industry, 45 520 Gidy, France) (0.5, 5 or 50 mg/kg, *per os*) was administered one hour prior to zymosan injection (*iart*). Food was removed 1 h before the treatment in order to avoid any changes in the pharmacokinetic profile of strontium ranelate (Fig. 1a). Data validation was achieved by using a positive control group that was pre-treated with indomethacin (5 mg/kg, *sc*) 1 h before zymosan injection. Sham group received (*per os* and *iart*) 0.9% sterile saline.

To analyze the possible effect of HO-1 pathway on anti-nociceptive and anti-inflammatory efficacy of strontium ranelate, animals were pre-treated (*sc*) with ZnPP IX (3 mg/kg), a specific HO-1 inhibitor, followed by an injection (*per os*) of strontium ranelate (0.5 mg/kg) 30 min later (Fig. 1b). After 1 h, intra-articular zymosan-injection was performed and at the 4th hour, inflammatory hypernociception in the TMJ was evaluated.

Synovial Lavage Collection, Cell Counting and Myeloperoxidase Activity Assessment

Six hours after zymosan injections, the rats were sacrificed under anesthesia and exsanguinated. The superficial tissues were dissected and the TMJ cavity was washed two times to collect the synovial fluid by the pumping and aspiration technique using 0.05 mL of EDTA (1.77 mg EDTA/1 mL PBS). The total number of white cells in the synovial lavage was counted using a Neubauer chamber. MPO activity assay measurement was described by Bradley et al. and it was conducted on the collected synovial lavage which was centrifuged at 4500 rpm for 12 min at 4°C. MPO activity was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride and 1% hydrogen peroxide. The results are reported as the MPO units/joint fluid and a unit of MPO activity was defined as the conversion of a μmol of hydrogen peroxide to water in 1 min at 22°C.

Evans blue extravasation assay

Strontium ranelate (0.5 mg/kg, *per os*) was administered 1 h prior to zymosan injection. Thirty minutes before euthanasia, Evans Blue (5 mg/kg, *iv*) was then injected to assess plasma extravasation. After its excision, the periarticular tissue was weighed, immersed into formamide solution (1 mL), and placed into water bath (60°C) overnight. The resulting supernatant was collected and the absorbance was read at 620 nm using a spectrophotometer. The concentration was determined by comparison with a standard curve of Evans blue dye in the extraction solution and the amount of Evans blue dye (μg) was then calculated per mL of exudate [16].

Histopathological analysis

The TMJ was excised six hours after the induction of the inflammatory hypernociception. The specimens were fixed in 10% neutral buffered formalin for 24 h, demineralized in 10% EDTA for 7 days, embedded in paraffin, and sectioned along the long axis of the TMJ. Sections of 5 μm, including the condyle, the articular cartilage, the articular disc, the synovial membrane, the peri-articular tissue, and the skeletal muscle were evaluated under light microscopy (400×). The specimens were prepared for routine hematoxylin-eosin (H&E) staining and histological analysis considered a 0–4 score based on the following parameters: cell influx into the synovial membrane, cell influx into the connective tissue and the skeletal muscle of the periarticular tissue, and synovial membrane thickness.

Immunohistochemistry

Immunohistochemistry for TNF-α and IL-1β was performed using the streptavidin-biotin (*Labeled Streptavidin Biotin* – LSAB) method in formalin-fixed, paraffin-embedded tissue sections (5 μm thickness), mounted on glass slides prepared with an organosilane-based adhesive (3-aminopropyltriethoxysilane, Sigma Chemical Co®, St Louis, MO, USA). The sections underwent 2 baths in xylol for ten minutes each one. They were after immersed

three times into alcohol at 100% concentration, and washed in distilled water.

Antigen recovery was performed with citrate (pH 6.0; 30 min; 99° C). After returning to ambient temperature, the sections were immersed into a 3% hydrogen peroxide blocking solution for 10 min. The sections were then incubated overnight (4° C) with a primary rabbit anti-TNF-α and anti- IL-1β antibody (ABCAM®, England, UK), at the dilution of 1:200, and washed with PBS solution.

The samples were incubated with the secondary antibody LSAB Kit for 10 min at ambient temperature. Next, incubation was performed in a chromogen solution prepared with 3,3′ diamino-benzidine (DAB) (DAKO®, Carpentaria, CA, USA), for 10 min in a dark chamber. Afterwards, the specimens were washed into running water and then into distilled water. Counter-staining was performed with hematoxylin, and afterwards the specimens were dehydrated in alcohol and diaphanized in xylol. Finally, they were mounted on glass slides. The negative control sections were performed excluding the application of the primary antibody. The parameter of positivity for the immunohistochemical marking of the antigen in all the specimens included in the sample consisted of the cells that exhibited brown staining in their cytoplasm irrespective of the intensity of the immunomarking.

TNF-α and IL-1β ELISA assays

The TMJ tissue and the trigeminal ganglion were excised 6 h after the zymosan-injection in rats and were homogenized in a solution of RIPA Lysis Buffer System (Santa Cruz Biotechnology, USA). The samples were centrifuged at 10000 rpm for 15 min at 4 °C. The supernatants were stored at −80C for posterior analysis to evaluate the protein levels of TNF-α and IL-1β in the TMJ tissue and the trigeminal ganglion. The cytokine levels were quantified by the following kits: TNF-α–Rat TNF-alpha/TNFSF1A Quantikine ELISA Kit (R&D Systems, catalog number RTA00); and IL-1β–Rat IL-1 beta/IL-1F2 Quantikine ELISA Kit (R&D Systems, catalog number DY501). The absorbance was measured at 450 nm. IL-1β and TNF-α concentrations were expressed as pg/mL.

Statistical analysis

The data are presented as the mean ± SEM or medians where appropriate. Differences between means were compared using one-way ANOVA followed by the Bonferroni test. The Kruskal-Wallis test followed by Dunn's test was used to compare medians. A value of $p < 0.05$ indicated significant differences.
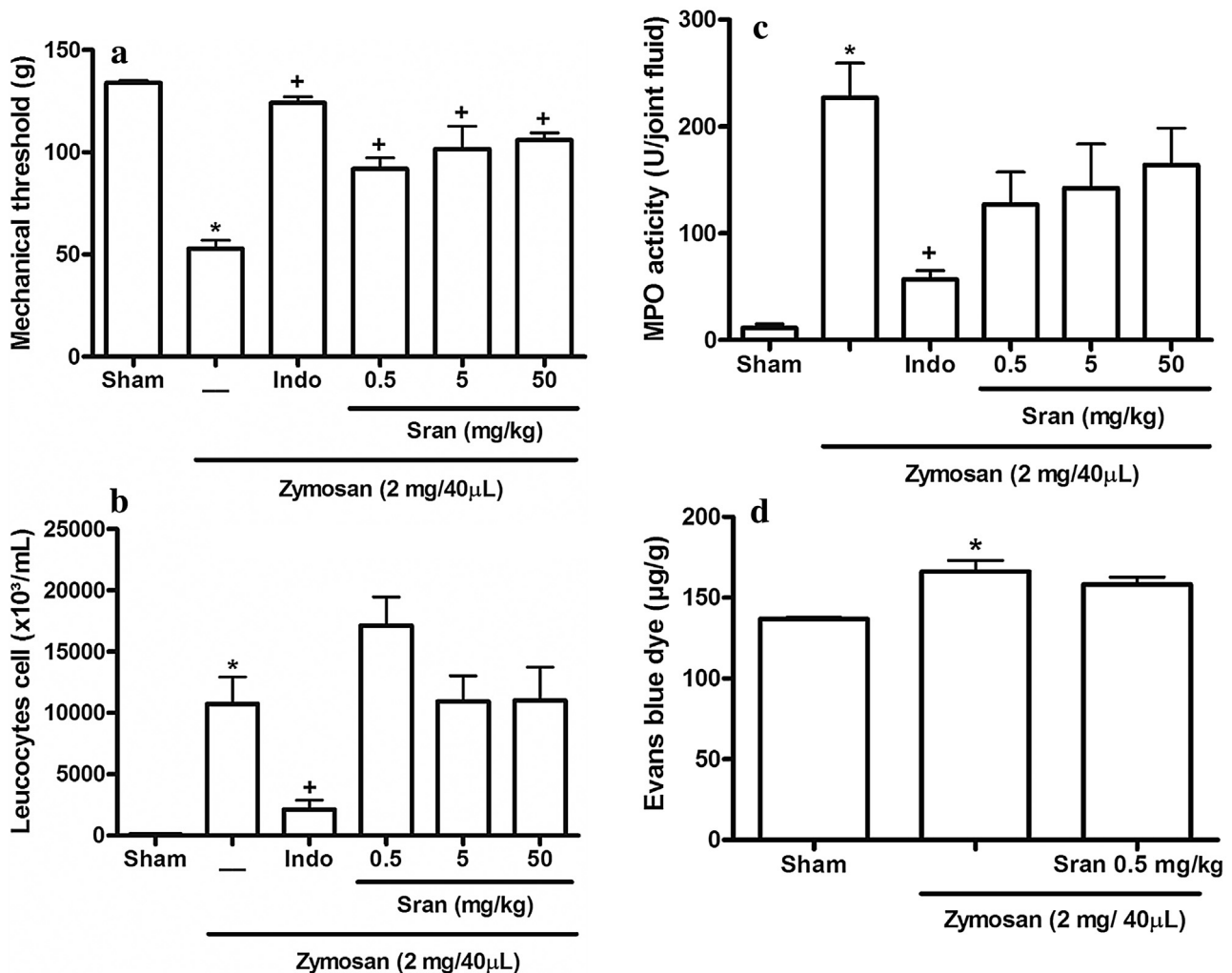

Fig. 2. Effects of strontium ranelate on the zymosan-induced TMJ inflammatory hypernociception. (a) Head withdrawal threshold in strontium ranelate-treated rats (b) Leukocyte counting in strontium ranelate-treated rats (c) MPO activity from TMJ synovial lavage in strontium ranelate-treated rats. (d) Plasma extravasation in strontium ranelate-treated rats. Data are expressed as the mean ± SEM of 6 mice for each group; *$p < 0.05$ indicates a significant difference from the sham group, +$p < 0.05$ indicates a significant difference from the zymosan group (ANOVA, Bonferroni).

Results

Effects of strontium ranelate on the zymosan-induced inflammatory hypernociception (Fig. 2)

The intra-articular injection of zymosan caused inflammatory hypernociception that is observed as a decrease in the mechanical threshold of head withdrawal (Fig. 2a). It resulted in a significant increase in the number of polymorphonuclear cells (Fig. 2b) which in turn was certified by the increase of MPO activity in the TMJ synovial lavage after zymosan injection (Fig. 2c). These changes were followed by plasma extravasation into the TMJ after 6 h (Fig. 2d). Sham animals showed no significant changes in withdrawal threshold, polymorphonuclear cells count, and MPO activity (Fig. 2 a, b, c). Strontium ranelate (0.5, 5 or 50 mg/kg) injected (*per os*) 1 h prior to zymosan injection significantly ($p < 0.05$) increased the nociceptive threshold (Fig. 2a). However, strontium ranelate failed to decrease the number of polymorphonuclear cells (Fig. 2b), MPO activity (Fig. 2c), and Evans blue dye extravasation in the synovial lavage (Fig. 2d).

Effects of zinc protoporphyrin IX (ZnPP IX) on the strontium ranelate efficacy (Fig. 3)

To investigate the role of HO-1 activity in the antinociceptive effect of strontium ranelate, the animals were pre-treated with ZnPP IX (3 mg/kg; sc), a specific HO-1 inhibitor. The effects of strontium ranelate (0.5 mg/kg) on the zymosan-induced inflammatory hypernociception (Fig. 3) were not changed in the presence of ZnPP-IX (3 mg/kg).

Joint tissue and trigeminal ganglion TNF-α and IL-1β ELISA assays (Fig. 4)

The intra-articular injection of zymosan resulted in a significant increase in TNF-α (Fig. 4a and 4b) and IL-1β (Fig. 4c and d) levels in both joint tissue and trigeminal ganglion after. Albeit strontium ranelate treatment was not able to significantly reduce IL-1β levels when compared with the zymosan group (Fig. 4c and 4d),
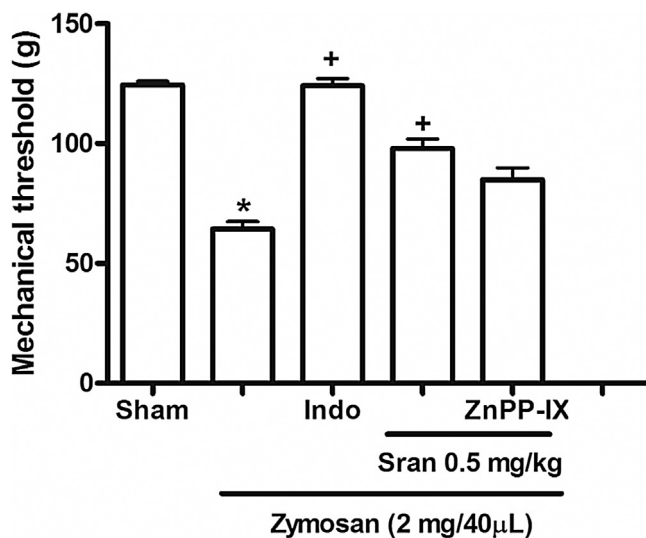
strontium ranelate reduced TNF-α levels in both joint tissue and trigeminal ganglion (Fig. 4a and 4b).

Histopathological analysis (Fig. 5)

Inflammatory cell influx was observed into the synovial membrane (Fig. 5b) 6 h after zymosan-injection compared with the sham group (Fig. 5a). The predominant cell types were neutrophils, which characterized acute inflammation. Edema was also observed in the synovium (Fig. 5b). Table 1 shows the scores attributed to TMJ histopathological analysis and compares the values between the sham and zymosan groups. A significant ($p < 0.05$) increase in the inflammatory parameters was observed in the zymosan group. Table 1 also shows the scores attributed to the TMJ histopathological analysis and compares the values between the zymosan and strontium ranelate (0.5, 5 or 50 mg/kg) groups. Strontium ranelate (0.5, 5 or 50 mg/kg) did not reduce the inflammatory parameters. Figs. 5c and 5d show the TMJ of rats pre-treated with strontium ranelate (0.5 mg/kg, *per os*).

Immunohistochemical analysis (Fig. 6)

The immunohistochemical analysis of TNF-α and IL-1β showed increased immunolabeling for both TNF-α and IL-1β in synvio-cytes and neutrophlis after zymosan challenge that was characterized by brown-colored cells in the synovial membrane (Fig. 6). The synovial cells in the synovial membrane of the zymosan and strontium ranelate-treated animals also showed both TNF-α and IL-1β expression (Fig. 6). However, in the conjunctive tissue, strontium ranelate (0.5 mg/kg) treatment reduced TNF-α expression. The negative control group sections consisted of zymosan-induced TMJ inflammatory hypernociception that were not treated with anti-TNF-α or anti-IL-1β antibody. None of the negative controls showed TNF-α or IL-1β immunoreactivity.

Discussion

We demonstrated that the effects of strontium ranelate on the zymosan-induced TMJ inflammatory hypernociception in rats may occur *via* TNF-α suppression as well as its mechanism of action in this disease model is IL-1β/HO-1 independent. Experimental animal models of TMJ inflammatory hypernociception have been used to study inflammatory conditions and we performed the first demonstration of TMJ arthritis induced by zymosan, through which we showed that zymosan caused a time-dependent leucocyte migration, plasma extravasation, mechanical hyper-nociception, and neutrophil accumulation [1]. This shows that the zymosan-induced TMJ arthritis is a reproducible experimental model that can be used to explore the mechanisms underlying TMJ inflammation and potential therapies.

Strontium ranelate was originally designed to treat osteoporo-sis [17–21], but it may exert effects on osteoarthritis and it has led to positive outcomes in a phase III clinical study [13,22]. Albeit the mechanism of action of strontium ranelate is not fully understood, it appears to stimulate the differentiation of osteoblasts by eliciting the calcium sensor receptor, inhibiting osteoclast differentiation by inhibiting RANKL production, and increasing osteoprotegerin (OPG) activity [23,24].

In addition, patients treated with strontium ranelate had a greater reduction in the total score and pain subscore compared with the placebo group [25]. Since osteoporosis and osteoarthritis are associated with a variety of symptoms, including pain, it could be hypothesized that strontium ranelate may also be effective in reducing the temporomandibular joint inflammatory hypernoci-ception. Our results demonstrated that the intra-articular zymosan injection diminished the mechanical nociceptive threshold, which
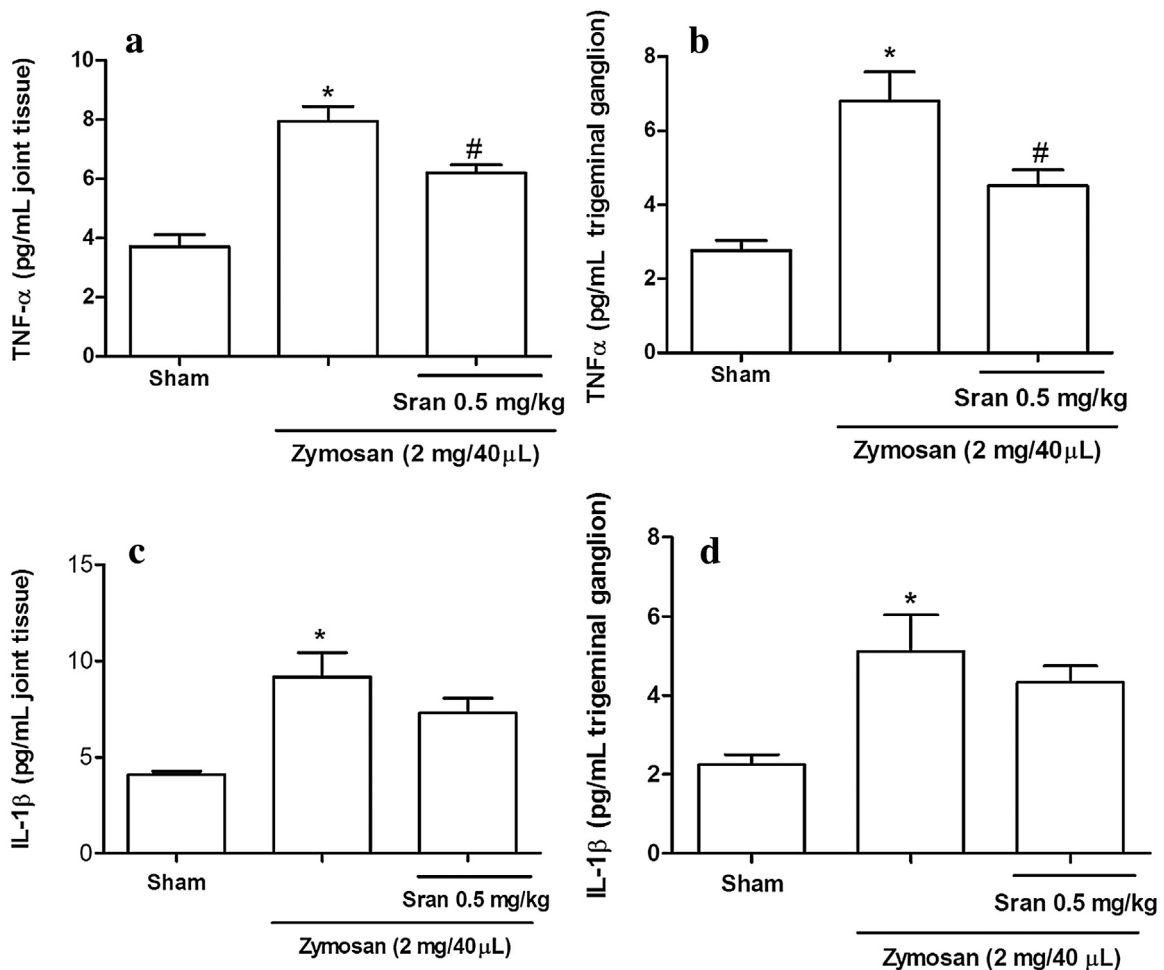

Fig. 3. Effect of zinc protoporphyrin IX (ZnPP IX), a specific HO-1 inhibitor, on the strontium ranelate efficacy on zymosan-induced TMJ inflammatory hypernocicep-tion. Data are expressed as the mean ± SEM of 6 rats for each group; $^*p < 0.05$ indicates a significant difference from the sham group,$^+p < 0.05$ indicates a significant difference from the zymosan group (ANOVA, Bonferroni).

Fig. 4. Joint tissue and trigeminal ganglion TNF-α (a/b) and IL-1β (c/d) levels from study rats either subjected or not to on zymosan-induced TMJ inflammatory hypernociception and assayed on the 6th hour post challenge. Data are expressed as the mean ± SEM of 6 rats for each group; *p < 0.05 indicates a significant difference from the sham group (ANOVA, Bonferroni).

in turn was increased by the strontium ranelate treatment. However, the inflammatory parameters – cell influx and MPO activity – were not reduced after strontium ranelate treatment. Evans blue extravasation measurement into the synovial lavage did not change following the drug administration. The TMJ histopathological analysis after zymosan injection showed inflammatory cell influx into the synovial membrane, periarticular tissue, and musculoskeletal tissue associated with thickness of synovial membrane, being the drug treatment unable to reverse these findings to a normal status. Likewise, it was demonstrated that strontium ranelate treatment had no particular effect on synovitis in dogs [13].

Studies correlate the HO-1 activity with oxidative damage inhibition and reduction in proinflammatory cytokines production [7]. It was reported that this enzyme had antinociceptive effects on acetic acid-evoked nociception and positive outcomes after induction of HO-1 in a zymosan-induced air pouch inflammation model [8,26]. Considering these data, we evaluated the involvement of HO-1 in the strontium ranelate antinociceptive effects and we observed that they were not changed after the pre-treatment with ZnPP-IX, suggesting that HO-1 activity is not involved in its antinociceptive effects.

Many cell types produce cytokines in response to a variety of stimuli, which is a link between cellular injury and the development of local signs and symptoms of inflammation. There is a cascade of release of cytokines linking injuries and the release of

nociceptive mediators in rats: a concept that allows us to understand why the inhibition of cytokines causes analgesia [27]. Many studies have demonstrated the contribution of TNF-α to inflammatory hyperalgesia and the clinical success of the anti-TNF-α therapy of rheumatoid arthritis also exemplifies this concept [4,28]. During the inflammatory response, TNF-α is the first released cytokine and IL-1β is a potent pleiotropic mediator involved in inflammatory responses [29]. Hence, TNF-α and IL-1β are recognized contributors to the pathogenesis of joint diseases, leading to synovial fibroblast hyperplasia and to the destruction of the extracellular matrix [30,31].

In the present study, the zymosan injection resulted in a significant increase in both TNF-α and IL-1β levels so that our findings are in accordance with other ones, suggesting that TNF-α is as driving cytokine of the nociceptive process. TNF-α plays a crucial role in the development of inflammatory hyperalgesia during the inflammatory response in rats, being highly expressed in the synovial fluid of patients with TMJ disorders [5–7]. Additionally, we also demonstrated that treatment with strontium ranelate reduced TNF-α levels in both joint tissue and trigeminal ganglion.

TNF-α is the first cytokine released during an inflammatory response, triggering the release of IL-1β known to activate inflammatory and degradative pathways in synovial cells. Studies suggested high IL-1β levels in the synovial fluid of patients suffering from TMJ disorders [5,29,32]. Nunes et al. [14]
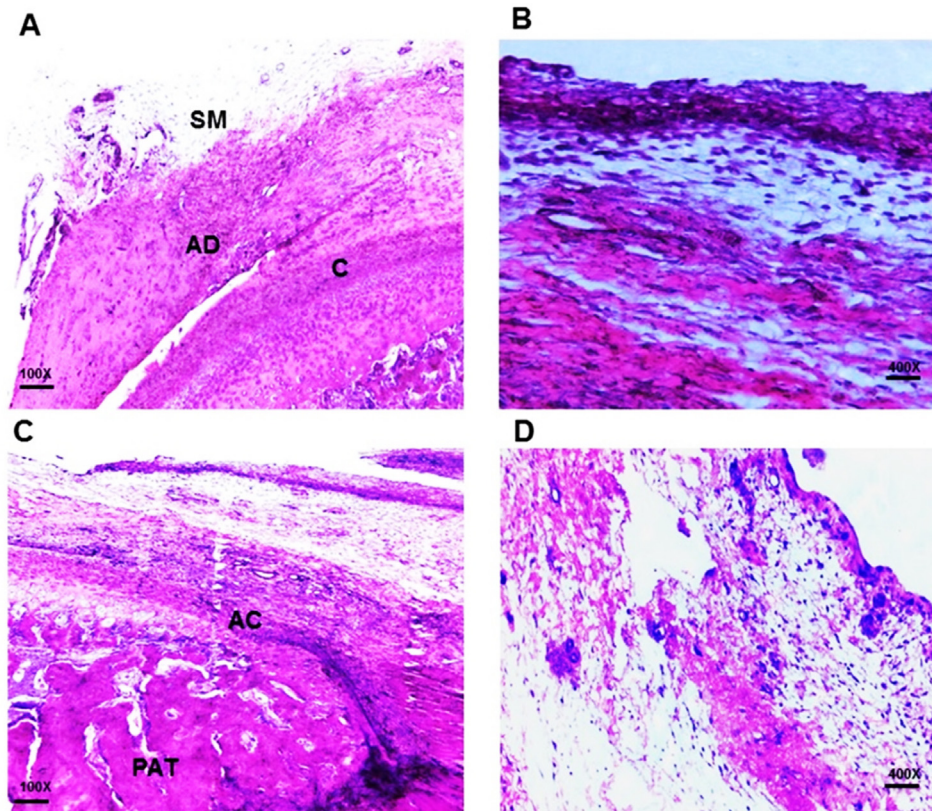
Fig. 5. Photomicrographs of the histopathological analysis of temporomandibular joints (TMJ). (a) sham group TMJ (100×); (b) zymosan 2 mg group (400×) showing inflammatory cell influx in the synovial membrane; (c) and (d) TMJ of rats pretreated (*per os*) with strontium ranelate (0.5 mg/kg) and injected (i.art.) with zymosan 2 mg (100 and 400 x, respectively). C: condyle; AC: articular cartilage; AD: articular disc; SM: synovial membrane; PAT: periarticular tissue. Hematoxylin and eosin (H&E) staining.

Table 1
Histopathological analysis by hematoxylin-eosin staining (HE) of the temporomandibular joint of rats after intra-articular zymosan injection and strontium ranelate (Sran) treatment.

Groups	Cell influx in the synovial membrane	Periarticular cell influx	Cell influx in the Muscular tissue
Sham	1 (0–1)	1 (0–2)	0 (0–0)
Zy	4 (2–4)[*]	3 (3–4)[*]	2.5 (2–4)[*]
Indo	2 (1–2)[**]	2 (1–2)[**]	1.5 (1–2)[**]
Sran 0.5	3.5 (2–4)	3.5 (3–4)	3.5 (2–4)
Sran 5	3 (2–3)	3.5 (3–4)	3.5 (2–4)
Sran 50	3 (2–4)	4 (2–4)	4 (2–4)

[*]$p < 0.05$ versus Sham; [**]$p < 0.05$ versus Zymosan (Kruskal-Wallis, Dunn's).

investigated the anti-inflammatory activity of strontium ranelate in the articular incapacitation test, in the paw-pressure test, and in the anterior cruciate ligament transection model. Unlike our protocol, the animals received strontium ranelate at higher doses: 30–300 mg/kg *per os*. They find that strontium ranelate dose-dependently inhibited joint pain in both types of arthritis models, but it did not alter cell influx which is a similar result obtained here. Contrary to them, our findings suggest that the strontium ranelate treatment is not capable of reducing IL-1β levels compared with the zymosan group.

This might be related to the differences in the design performed by Nunes et al. [14]. The strontium ranelate dose administered in their protocol is far higher than the one we used, which could potentiate the analgesic effects of this drug. Other essential difference is the experimental model of diseases that could

culminate in distinct results as different disease models may imply in contrasting pathogenesis mechanisms, thus, leading to different results. As pointed out by Nunes et al. [14], naloxone abolished the strontium ranelate analgesic effect which is valuable information on the strontium ranelate unspecific mechanism of action.

Furthermore, the immunohistochemical analysis showed increased TNF-α and IL-1β immunolabeling in the synovial cells after the intra-articular zymosan injection. Albeit strontium ranelate slightly reduced the TNF-α immunolabeling in the conjunctive tissue, it was not able to diminish the IL-1β immunolabeling one. Contrary to this result, in the synovial membrane of dogs undergoing sectioning of the anterior cruciate ligament, the genetic expression of IL-1β was significantly reduced by strontium ranelate treatment at the doses of 50 or 75 mg/kg per day for 16 weeks [13]. A possible explanation for these
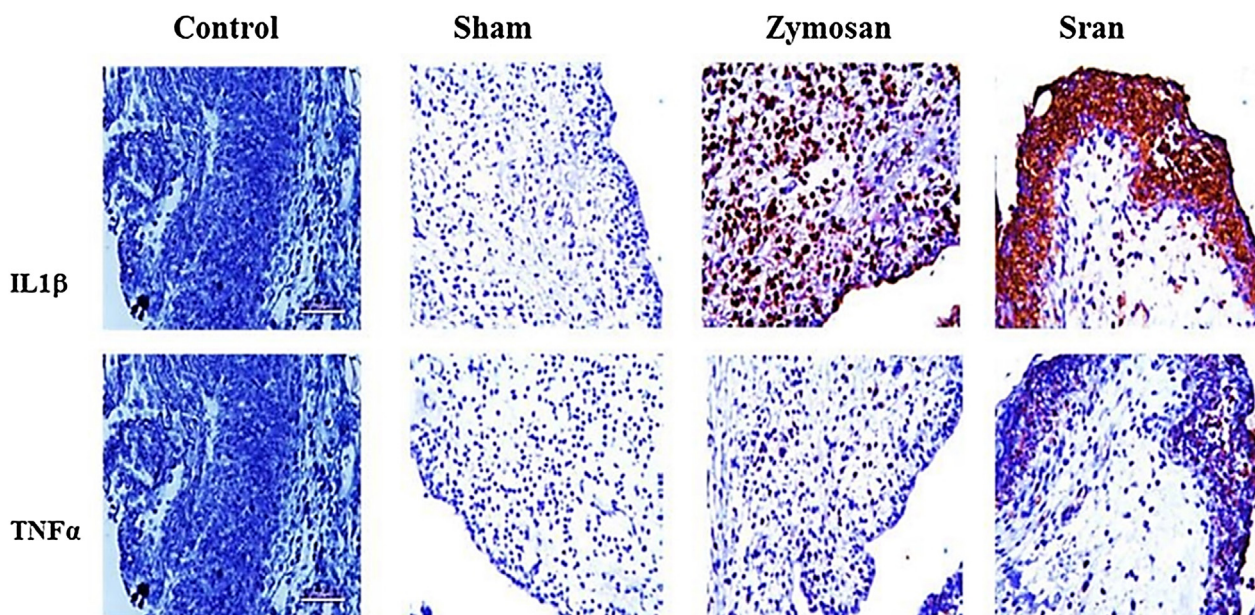
Fig. 6. Representative immunohistochemistry of TMJ tissues for IL-1β (upper panel), and TNF-α (lower panel) from rats at the sixth hour after zymosan injection. The upper panel shows increased IL-1β immunolabeling of synoviocytes and neutrophlis after zymosan-challenge (400×). The bottom panel shows increased TNF-α immunolabeling of synoviocytes and neutrophlis after zymosan-challenge (400×). Control: negative control (sections in the absence of anti-IL-1β and anti-TNF-α antibody); Sham: unchallenged rats; Zymosan: zymosan-challenged rats receiving 0.9% saline solution; Sran 0.5: zymosan-challenged rats receiving strontium ranelate (0.5 mg/kg).

contradictory results could be the different animal model of disease along with the adopted posology, leading to contradictory results from what we obtained.

Therefore, albeit the mechanisms of action through which strontium ranelate exerts antinociceptive effects remain relatively elusive, this study provides novel information on its effects on the zymosan-induced TMJ inflammatory hypernociception as strontium ranelate primarily suppressed TNF-α levels and reduced the nociceptive threshold. The inflammatory stimuli or tissue injuries stimulate the release of characteristic cytokine cascades, which ultimately trigger the release of final mediators responsible for inflammatory pain. These final mediators, such as prostanoids or sympathetic amines, act directly on the nociceptors to cause hypernociception, which results from the lowering of threshold due to modulation of specific voltage-dependent sodium channels.

As reported here, strontium ranelate could decrease hypernociception thresholds by reducing TNF-α levels in the periarticular tissues and trigeminal ganglion. Given the importance of these structures to the temporomandibular joint pain onset and progression, we suggest that TNF-α functions as a target point for strontium ranelate. This suggests that strontium ranelate might be a potential candidate for the treatment of TMJ pain through TNF-α inhibition and more studies of longer duration are necessary to validate the use of strontium ranelate in the pain management.

Conflict of interests

The authors declare that they have no conflict of interest regarding the publication of this study.

Funding

Acknowledgment

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