Capsazepine antagonizes TRPV1 activation induced by thermal and osmotic stimuli in human odontoblast-like cells

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ABSTRACT

Objectives: Dental pain, which is the main reason for patients consulting dentists, is classified as a public health concern. The study of cellular and molecular mechanisms contributing to pain is a fundamental element for developing new analgesics. By using a selective antagonist in an in vitro model, this study aimed to establish the role of TRPV-1 in human odontoblast-like cells (OLCs) as a therapeutic target for dental pain mediated by noxious thermal and osmotic stimuli.

Methods: OLCs were differentiated from dental pulp mesenchymal cells and TRPV1 expression was evaluated. Activation of TRPV-1 was determined by evaluating changes in calcium concentration after stimulation with mannitol and xylitol hyperosmotic solutions or DMEM heated at 45 °C, using the fluorescent calcium probe Fluo-4 AM. In addition, changes in fluorescence (F/F0) due to calcium flux were evaluated using fluorometry and flow cytometry. Simultaneously, the cells were co-stimulated with the selective antagonist capsazepine (CZP).

Results: OLCs expressed DSPP and DMP-1, confirming their cellular phenotype. TRPV1 was expressed, and its activation by different stimuli produced an increase in cytosolic Ca2+ which was reduced by the antagonist. Both methods used to evaluate TRPV1 activation through the measurement of calcium probe fluorescence showed similar patterns.

Conclusions: These results suggest that TRPV-1 modulation using an antagonist can be implemented as a pharmacological strategy for managing dental pain mediated by hyperosmotic and thermal stimuli.

1. Introduction

Pain is an unpleasant sensory and emotional experience associated with, or resembling, actual or potential tissue damage.1 It is the main cause of medical and dental consultations, which is why it is a public health problem in several countries. In the dental field, various injuries and aggressions can induce sensitivity and pain in teeth with altered structures, such as the intake of sweet foods, hot or cold drinks, chewing, among others.

The pathophysiology of dental pain is not entirely understood, although several theories have been described, such as the hydrodynamic, odontoblastic transduction, and neural theories; odontoblasts play an important role in each of them. Odontoblasts are highly differentiated postmitotic cells unique to dental tissue, which differentiate from the ecto-mesenchymal cells of the dental papilla. These cells are a part of the dental pulp and participate in dentinogenesis and proprioception. However, due to the postmitotic characteristics of primary odontoblasts, their cell culture is difficult, and their acquisition and maintenance in vitro is achieved for a few hours; therefore, odontoblast-like cells differentiated from dental pulp stem cells are used to perform physiological experiments. In recent years, odontoblasts have been identified as essential cells for the transduction process through transmembrane receptors, among which transient receptor potential channels (TRP) have been identified.2

In mammals, six TRP subfamilies have been described and grouped according to their structure and stimuli that generate their activation. The functional structure of TRP channels consists of four subunits that form homo- or heterotetramers arranged symmetrically around a central pore, and each subunit is made up of six transmembrane segments that allow the influx of ions through the pore.3 Some of these channels are expressed in primary afferent neurons and involved in nociceptive pain.4 In addition, evidence exists that they act as transducers of thermal, mechanical, and chemical stimuli. Due to their characteristic polymodality, TRPV1, TRPV3, TRPM8, and TRPA1...
act as chemoreceptors, stimulated by capsaicin and endocannabinoids, camphor, menthol, mustard, and cinnamon oil, respectively. For this reason, they have been considered potential therapeutic targets for managing various pathologies, including different types of pain. TRPV1 channels have been extensively studied and their participation in the transduction of multiple stimuli in tissues of different origins including craniofacial and dental tissues have been described. Additionally, this channel has been also involved in energy homeostasis, modulation of autophagy and proteasome activity, and crosstalk between the sensory nervous and immune systems. TRPV1 alterations are related to pathologies framed within channelopathies associated with obesity, pain, cancer, severe bronchial asthma, psoriasis, itch, inflammation, among others. Biological and pharmacological studies of TRPV1 have increased since its discovery, which was awarded the 2021 Nobel Prize in Physiology or Medicine.

TRPV1 is activated by stimuli such as thermal and osmotic stimuli, pH changes, endovanilloid molecules, and multiple exogenous molecules. Additionally, thermal, and osmotic stimuli (e.g., sweet foods) are known to induce a painful process in teeth with structural alterations, a response that can be related to channel activation.

Capsaicin (CAP; 8-Methyl-N-vanillyl-trans-6-nonenamide), a compound isolated from chilli pepper, is responsible for its characteristic taste and pungency. On the one hand, it is an important TRPV1 agonist used clinically for the management of pathologies or symptoms, such as neuropathic pain and muscularkeletal pain, among others. Capsaicin has also been studied in the management of cancer, mucositis, cardiovascular diseases, anti-obesity, anti-pruritic, anti-inflammatory, anti-apoptotic, antioxidant, and neuroprotective functions.

On the other hand, the antagonism of TRPV1 has been proposed as a therapeutic target for the management of acute and chronic pain, and several molecules with this pharmacological activity have been studied for the past few years. Capsazepine (CZP; N-[2-(4-Chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide), the best-studied TRPV1 antagonist, is a synthetic analog of capsaicin that acts as a competitive antagonist.

Dental pain is controlled by using traditional and selective non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, and opioids; however, their chronic use is related to adverse effects on the gastrointestinal, renal, and cardiovascular systems, among others, and significantly the abuse potential in the case of opioids. The pathophysiology of pain involves the participation of a variety of endogenous chemical mediators (leukotrienes, substance P (SP), histamine, bradykinin, 5-hydroxytryptamine, prostaglandins, among others), some receptors and cells that limit the correct clinical management of this symptom. Furthermore, some patients are refractory to the proper analgesic treatment, for which the development of novel strategies for the treatment of dental pulp-derived pain is required.

The objective of this study was to analyze the activation of TRPV1 in human odontoblast-like cells (OLCs) in response to noxious stimuli and the modulation of the response using an antagonist. CZP reduces OLCs activation mediated by osmotic stimuli and noxious temperatures. Therefore, TRPV1 antagonism could be a potential pharmacological target for managing dental pain.

2. Materials and methods

2.1. Human OLCs culture and confirmation of cell phenotype

A previous ethics endorsement (CIEFO-008-2021) and dental pulp stem cells (DPSCs) were obtained from healthy teeth extracted for orthodontic reasons and cultured in Dulbecco’s modified Eagle’s modified (DMEM) supplemented with 10% fetal bovine serum (FBS; Biowest). Subsequently, differentiation of DPSCs to OLCs was performed as described in a previous study. Briefly, the cells were cultured with DMEM supplemented with 10% FBS, dexamethasone (0.1 μM; Sigma-Aldrich), β-glycerophosphate (5 mM; Santa Cruz-Biotechnology), ascorbic acid 50 μg/mL (Sigma-Aldrich), TGF-β1 10 ng/mL (Abcam), penicillin 100 U/mL, streptomycin 100 μg/mL, amphotericin 0.25 μg/mL (Biowest), for 21 days at 37 °C in a 5% CO₂ incubator. At 21 days, the cells were detached using trypsin (0.25%)/EDTA (0.5 mM) and cultured to develop different experiments. The cell phenotype was confirmed using RT-PCR, immunohistochemistry, and mineralization profile (calcium deposits and mineralization nodules) during the differentiation process.

Total RNA was extracted from the OLCs monolayer using TRIzol (Invitrogen) and quantified using spectrophotometry (IMPLEN) for RT-PCR. The reverse transcription process was conducted at 37 °C for 1 h using the MMLV enzyme (Invitrogen). Taq polymerase (Promega) and specific primers for dentin sialophosphoprotein (DSPP) and dentin matrix acidic phosphoprotein (DMP-1) were used. β-actin was used as normalizing gene. The amplification conditions were 95 °C for 3 min, 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. The amplicons were separated and visualized on a 2% agarose gel stained with ethidium bromide.

Simultaneously, OLCs were seeded on round 12 mm cover slips for 24 h and fixed with 4% paraformaldehyde (PFA). Immunofluorescence for DSPP and DMP-1 was performed using anti-DSPP (Abcam) and anti-DMP-1 (Invitrogen) polyclonal antibodies at a dilution of 1:500. The antibodies were washed, and the cells were incubated with Alexa 549-coupled secondary anti-rabbit IgG (2 μg/mL; Invitrogen) for 45 min, and then the DNA was counterstained with DAPI (4′,6-diamidino-2-phenylindole). Slides were mounted with ProLong Gold (Cell Signaling Technology) and observed and recorded using a Zeiss Axio Imager M2 using the X-Gite 120Q light system and Axios Vision software.

2.2. Evaluation of TRPV1 presence in OLCs

TRPV1 expression in OLCs was determined using RT-qPCR and immunofluorescence. The previously extracted RNA was used to evaluate the presence of TRPV1 transcripts using SYBR green (Luna®, New England Biolabs) and specific primers (Table 1). The amplification conditions were 55 °C for 10 min, 95 °C for 3 min, 40 cycles of 95 °C for 15 s and 60 °C for 30 s. Relative quantification was performed using Schefé’s formula, taking into account the amplification cycles and the efficiencies obtained through the LinRegPCR software and taking DMP-1 and β-actin as reference genes. RNA from DPSCs was used as positive control.

OLCs seeded on glass coverslips and fixed with PFA were used to perform indirect immunofluorescence using an anti-TRPV1 polyclonal antibody (ab3487) at a 1:1000 dilution. The antibody was washed, and the cells were incubated with Alexa 549-coupled secondary anti-rabbit IgG (2 μg/mL; Invitrogen) for 45 min, and then the DNA was counterstained with DAPI. Coverslips were mounted on slides for microscopic observation.

2.3. Cytotoxic concentration 50 (CC50) and inhibitory concentration 50 (IC50) for CZP

The CC50 was calculated from the viability test obtained using the resazurin assay. Briefly, OLCs were seeded in 96-well plates at a density

| Table 1 | Oligonucleotide probes used for RT-PCR and RT-qPCR. |
|---------|---------------------------------------------------|
| Gene    | Primer sequences                                  | Fragment size |
| DSPP    | 5′-AGAAGGACCTGGCAGAAAAAT-3′ 5′-CTCTCCTGGCATCTGTT-3′ | (201 bp)      |
| DMP-1   | 5′-GAAAGTAGGGAGGATGAACCTC-3′ 5′-CTGAGATGAGGAGCCTTCATTA-3′ | (128 bp)     |
| β-actin | 5′-GGGCGGGACTCCTACAT-3′ 5′-CCACCTGGAAGGAAAGG-3′ | (120 bp)     |
| TRPV1   | 5′-GCTGCTTCATCCATCCCTGCTG-3′ 5′-GGTCTGCTCCCTGCGATCTGT-3′ | (118 bp)     |

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of 20,000 cells/well and treated with different concentrations of CZP (100 nM-500 μM), as described on previous reports for matrix-forming cells. At 48 h, the stimulus was removed, and the cells were incubated with 4.4 μM resazurin in FBS-free DMEM for 3 h. Fluorescence was measured at 530/590 nm wavelengths (CLARIOstar-BMG), and the CC50 was calculated using GraphPad-Prism 8.

To determine the IC50 (concentration of drug necessary to reduce the response of capsaicin by 50%), the cells were loaded with 2 μM of the fluorescent probe Fluo-4-AM (Invitrogen) diluted in PBS for 45 min. At the end of the incubation period, the medium was removed, and fresh PBS was added for 30 min to promote probe de-esterification. Next, the cells were pretreated with different concentrations of CZP (50 μM-1nM), stimulated with 100 μM capsaicin in phenol red-free DMEM, and fluorescence was measured at Ex/Em wavelengths of 485/520. Finally, the IC50 of CZP was calculated using GraphPad-Prism 8.

2.4. Cellular stimulation and TRPV1 activation

To determine the functionality of TRPV-1 channels, OLCs were seeded on 384 black plates (SPL) at a density of 3000 cells/well, allowed to adhere for 24 h to obtain 80% confluence, and loaded with 2 μM Fluo-4-AM for 45 min. After incubation, the medium was removed, and fresh PBS was added for 30 min.

For hyperosmotic stimulation, the solutions were prepared by supplementing phenol red-free DMEM with mannitol or xylitol (370 mOsm/L). The osmolarity of the solutions was confirmed using an FPOSM-V2.0 osmometer.

The loaded cells were subsequently stimulated with 100 μM capsaicin (Sigma Aldrich), 20 μM CZP (Tocris), phenol red-free-DMEM heated at 45 °C, hyperosmotic solutions of mannitol (1.5% Mannitol, Santa Cruz) or xylitol (Sigma) and immediately after the stimulus, the fluorescence reading was performed in a microplate reader (CLARIOstar, BMG), with Em/Ex 494/525 wavelengths. In addition, OLCs loaded with

![Fig. 1. Phase-contrast microscopy of OLCs phenotype during the differentiation process and confirmation by RT-PCR](image-url)

A. The morphological changes of the cells during the differentiation process of the DPSCs at 7, 14 and 21 days are shown. At 21 days, the OLCs were enzymatically dissociated and used for the different experiments. The typical fusiform morphology of these cells is observed. Scale bar = 100 μm. All data are representative of the mean of three independent experiments. B. Electrophoresis on a 2% agarose gel. Amplicons for DMP-1 (128 bp), DSPP (201 bp) and β-actin (120 bp) in OLCs. As a positive control (Ctrl +), RNA from DPSCs was used. As a negative control (Ctrl -), water was used. DNA ladder of 100 bp (Invitrogen).
The expression of DSPP and DMP-1 in OLCs was observed in the membrane, cytoplasm and nucleus of the cells. A detail of the labeling of each marker distributed
fluorescence was measured.

Calcium (Ca\(^{2+}\)) influx was also evaluated using flow cytometry (FC). OLCs were detached, and 5x10\(^5\) cells were loaded with 2 \(\mu\)M Fluo-4-AM in a 1.5 ml tube, in a final volume of 200 \(\mu\)L. After loading with the fluorescent probe, the cells were stimulated, and fluorescence was measured immediately using a 530/30 (FITC) filter (Accuri C6, BD). Simultaneously, OLCs were co-stimulated with CZP and treated with the agonists mentioned above. In both cases, calcium influx was measured using the ratio F/F0 corresponding to the fluorescence of the stimulated loaded cells/fluorescence of the unstimulated loaded cells.

2.5. Statistics

The experiments were performed four times in triplicate. Data are presented as the mean ± standard deviation (SD). A T-test was used to determine differences using GraphPad-Prism 8. P was set at p < 0.05.

3. Results

3.1. OLCs differentiated from DPSCs present odontoblastic phenotype

The 21-days differentiation process of DPSCs into OLCs allowed us to obtain cells with a typical fibroblastic-like shape and long cytoplasmic processes (Fig. 1A), consistent with the morphology of the expected cell model, that previously was obtained and described.\(^{11}\)

The cell phenotype was confirmed by visualizing the expected bands for DMP-1 and DSPP amplification products of the transcripts by RT-PCR on a 2% agarose gel (Fig. 1B) and visualizing protein expression in the cells. The quality of the RNA was confirmed by the amplification of \(\beta\)-actin (120 bp). Fluorescence microscopy revealed mainly cytoplasmic and membrane labeling of DSPP and DMP-1, although the antigen was also present at the nuclear level (Fig. 2). The mineral deposition of OLCs and membrane labeling of DSPP and DMP-1, although the antigen was evidence at the membrane, cytoplasmic, perinuclear, and nuclear levels (Fig. 3B), although the main labelling was observed as cytoplasmic aggregates.

3.2. Subcellular distribution of TRPV-1 in OLCs

From the RNA extracted from the culture of OLCs, the presence of transcripts for TRPV1 was determined, the amplicon of the expected size (118 bp) was visualized (data not shown), and the relative quantification showed a 266-fold upregulation in comparison to DPSCs (Fig. 3A). The protein was detected at the membrane, cytoplasmic, perinuclear, and nuclear levels (Fig. 3B), although the main labelling was observed as cytoplasmic aggregates.

3.3. Capsazepine decreases OLCs activation, mediated by osmotic and thermal stimuli

Cell viability in response to CZP in OLCs was determined using the resazurin technique at 48 h, and a CC50 of 45.28 \(\mu\)M was found (Fig. 4A). Higher concentrations induced morphological changes in the OLCs (Fig. 4B), such as detachment, rounding, cell grouping, and loss of the monolayer, compatible with cell death. However, untreated control cells retained the morphology described above. The IC50 of CZP in capsaicin-stimulated cells was determined by measuring fluorescence intensity, and a value of 20.95 \(\mu\)M was obtained.

The activation of OLCs with hypertonic solutions and thermal noxious stimulation was evidenced by increased fluorescence driven by the influx of Ca\(^{2+}\), which was evaluated using the Fluo-4-AM fluorophore. Measuring the fluorescence intensity using a microplate reader, in the cells treated with 45 °C heated culture medium an F/F0 of 4.28 ± 0.27 was found, and in those stimulated with xylitol and mannitol, the indexes were 4.51 ± 0.41 and 4.41 ± 0.42, respectively (Fig. 5A). The indices were comparable with those found through flow cytometry, since in the cells stimulated with heat, an index of 6.01 ± 0.17 was obtained, and in those treated with xylitol and mannitol, the values were 5.19 ± 0.40 and 5.53 ± 0.22, respectively (Fig. 5B-C).

Those cells co-treated with CZP for 15 min, and subsequently stimulated with different solutions, the antagonist reduced the Ca\(^{2+}\) influx to values of 0.84 ± 0.10, 0.85 ± 0.08, and 0.87 ± 0.07 (p < 0.0001) with mannitol, xylitol, and 45 °C DMEM treatment respectively, measured by microplate reader. Measurements performed by the cytometer showed a reduction to values of 1.1 ± 0.36, 1.23 ± 0.32 and 1.06 ± 0.29 for the stimuli with mannitol, xylitol, and noxious heat (p < 0.0001). Furthermore, the activation mediated by capsaicin was significantly blocked by CZP, reducing the ratio from 3.8 ± 0.34 to 1.1 ± 0.11 (p = 0.0001) by cytometry assessment (Fig. 5B).

Fig. 2. Confirmation of the OLCs phenotype by immunofluorescence. The expression of DSPP and DMP-1 in OLCs was observed in the membrane, cytoplasm and nucleus of the cells. A detail of the labeling of each marker distributed homogeneously in the cytoplasm (a) and in the nucleus (b) is shown. The intranuclear position was confirmed by a 3D reconstruction of the image (c). The specific antigen and the DAPI nuclear marker are shown in red and blue, respectively. Scale bar = 50 \(\mu\)m. All data are representative of the mean of three independent experiments.
4. Discussion

The present study describes the expression of TRPV1 in OLCs and its activation in response to various stimuli. The OLCs model was confirmed regarding the differentiation process and phenotype of cells, showing that this is a reliable culture that can be used in functional studies.

In TRP channels, several subfamilies have been related to the transduction of stimuli that lead to pain perception. Some of them, including TRPV1, has been previously reported in mouse and rat odontoblasts. Moreover, the expression of TRPV1, TRPA1 and TRPM8 also has been described in human OLCs differentiated from the dental pulp of healthy third molars.

TRPV1 has been found in cell membranes and organelles, such as the mitochondria, endoplasmic reticulum, and Golgi, among others. The role of TRPV1 at the intracellular level is related to the presence of the protein incorporated into vesicles to be exported or inserted into the cell membrane to maintain Ca\(^{2+}\) homeostasis in organelles. In this study, the channel was observed in different locations of human OLCs such as membrane and the cytoplasm, which coincides with other study that showed a low expression of TRPV1 at the cell membrane with the majority protein sequestered in cytoplasmic vesicles in rat nodose ganglion. This expression in OLCs may suggest that TRPV1 participates in multiple physiological processes of the odontoblast, for which further studies are required. Additionally, understanding how each channel responds to different activation stimuli is difficult because of its poly-modal properties. This activation induces changes in the membrane potential, allows the translocation of monovalent and divalent ions, modifies enzymatic activity, and initiates endocytosis/exocytosis processes, among others, for which these channels play a crucial role in many fundamental life processes, such as sensory transduction, cell survival and development. In addition, the increased Ca\(^{2+}\) concentration after TRPV1 activation drives the release of ligands such as adenosine triphosphate (ATP), SP, and calcitonin gene-related peptide (CGRP), which are essential to the odontoblast/nerve-endings communication and participating in the pain pathways. Therefore, measurement of calcium influx using various techniques may be a useful indicator of the activation of these receptors and additionally the evaluation of some of these ligands released in this model could be relevant to understand the dental pain pathophysiology.

TRPV1 antagonism has also been analyzed for the management of migraine, neuropathic pain associated with cancer and diabetes, urinary urge incontinence, chronic cough, irritable bowel syndrome, among others. Some of these antagonists are currently in clinical phases, and interestingly, a molecule for managing dental pain has concluded phase 2 of a clinical trial.

Furthermore, the present study showed how CZP, a synthetic antagonist of capsaicin, decreases TRPV1 activation mediated by thermal and osmotic stimuli. The inhibition of TRPV1 activation mediated by CZP in other odontoblast models has been previously reported; for example, El Karim et al., showed a decrease in the response of human OLCs treated with 10 \(\mu\)M CZP and subsequently stimulated with capsaicin or 45 °C heated culture medium.

![Fig. 3. Expression of TRPV1 in OLCs. A. Relative quantification of TRPV1 in OLCs, using Schefe’s formula. Bar plots and error bars denote mean ± SD. All data are representative of the mean of three independent experiments. B. TRPV1 was mainly detected in the cytoplasm and surrounding the nucleus of OLCs by immunofluorescence. Scale bar = 50 μm.](image1)

![Fig. 4. CC50 of CZP in OLCs. A. The CC50 was determined using the resazurin technique on OLCs stimulated with different concentrations of CZP. All data are representative of the mean of three independent experiments. B. Phase contrast microscopy shows the morphological changes in OLCs treated with 100 \(\mu\)M de CZP; loss of the cell monolayer and rounding of OLCs were observed. Control: cells not treated.](image2)
AMTB, a selective antagonist of TRPM8. A reduction in intracellular calcium was observed after pretreatment with an intracellular calcium in trigeminal ganglion neurons. A significant effect of hyperosmotic sucrose solution, as measured by the influx of calcium, has also been described. It has been shown that the calcium influx is enhanced by hyperosmotic stimuli. Pan et al., used a hyperosmotic sucrose solution (450 mOsm) in human corneal epithelial cells and achieved 2-fold Ca$^{2+}$ influx using fluorescence intensity measured by the microplate reader. Fluo-4-AM has been widely used to measure calcium probe fluorescence. The two methods used to evaluate TRPV1 activation through the use of CZP reduce the Ca$^{2+}$ influx mediated by different stimuli.

TRP channels have been studied as transducers of osmotic stimuli in mammals and invertebrates, probably through changes in the cell membrane tension. In the present work, we observed how mannitol and xylitol hyperosmotic solutions generate an increase in the cationic influx in OLCs, which is reflected in the activation of several channels, including TRPV1, a flux that CZP abrogates. Tokuda et al., used mouse OLCs and showed that xylitol-hyperosmotic solutions (500 mOsm) induced hypertonic stress at plasma membrane and that stimulus enhanced TRPV1 mRNA expression.

The use of antagonists, such as CZP, to block TRPV1 activation by hyperosmotic stimuli has been reported in other cell models. Pan et al., used a hyperosmotic sucrose solution (450 mOsm) in human corneal epithelial cells and achieved 2-fold Ca$^{2+}$ transients that were suppressed by the TRPV1-selective antagonists CZP and JYL 1421.

The responses of other TRP channels to osmotic stimulation have also been described. An in vivo study showed the activation of TRPM8 with a hyperosmotic sucrose solution, as measured by the influx of intracellular calcium in trigeminal ganglion neurons. A significant reduction in intracellular calcium was observed after pretreatment with AMTB, a selective antagonist of TRPM8. These results suggest that other channels should be analyzed, and that their blockade could participate in nociceptive responses.

Regarding temperature stimulus, some possible mechanisms have been proposed: (1) changes in temperature could lead to the production and binding of ligands; (2) the TRP channel may undergo a series of conformational changes of multiple subdomains that generate a rearrangement of the outer pore, leading to gate opening; (3) the TRP channel may be able to sense changes in membrane tension due to temperature-dependent lipid bilayer rearrangements; (4) voltage-dependent mechanism activation of TRPV1 and TRPM8 upon depolarization by different temperatures.

The two methods used to evaluate TRPV1 activation through the measurement of calcium probe fluorescence showed similar patterns; however, the F/F0 ratio obtained from FC was higher than that obtained from the microplate reader. Fluo-4-AM has been widely used to measure calcium influx using fluorescence intensity; however, few studies have reported the use of cytofluorometry to measure calcium fluxes. Wu et al., described that in Fluo-4 labelled cells, the ionomycin stimulus allows free entry of Ca$^{2+}$, showing that this fluorophore allows the study of the activity of some ion channels and G-protein coupled receptors (GPCRs), using confocal microscopy and FC. Our results suggest that the use of Fluoro-4-AM is a reliable and reproducible method for measuring cytosolic Ca$^{2+}$, which could also be used in other cell models.

Here, we did not evaluate the other TRP channels, which is a limitation of our study, as other types of cellular receptors may participate in OLCs activation due to the assessed stimuli. Additionally, CZP can inhibit other channels; thus, additional studies are required to clarify these aspects and use molecules with the highest selectivity for different channels.

5. Conclusion

The results presented here show that the TRPV1 channel is one of the receptors involved in the odontoblastic response to hyperosmotic and thermal noxious stimuli and, therefore, it could participate in pain perception and dental hypersensitivity. Based on this, the antagonism of TRV1 can be considered in future studies to establish the blockade of this channel for managing certain oral pathologies.

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Ethical approval

Present study has approved by School of Dentistry, Universidad Nacional de Colombia (CIEFO008-2021)

Declaration of competing interest

There are no conflicts of interest.

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