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Research article

Blocking of TRPV-1 in the parodontium relieves orthodontic pain by inhibiting the expression of TRPV-1 in the trigeminal ganglion during experimental tooth movement in rats

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HIGHLIGHTS

- Experimental tooth movement increases the expression of TRPV1 in the parodontium.
- TRPV1 activation in the parodontium increases the expression of TRPV1 in the trigeminal ganglion.
- TRPV1 blockade in the parodontium decreases orthodontic pain by reducing the expression of TRPV1 in the trigeminal ganglion.

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ABSTRACT

Orthodontic pain has confused the orthodontics for a long time, and recent research demonstrated that transient receptor potential vanilloid type 1 (TRPV1) had crucial functions in transduction of painful stimuli. The present research investigated the analgesia effects of the blocking TRPV1 on orthodontic pain during experimental tooth movement. Under challenge with experimental tooth movement, the expression of TRPV1 in the parodontium was increased in a time-dependent and force-dependent manner. And treatment with selective TRPV1 antagonist AMG-9810 in the parodontium reduced the expression of TRPV1 in the trigeminal ganglion (TG) and decreased the secretion of IL-1β in the gingival crevicular fluid. Furthermore, AMG-9810 could relieve orthodontic pain arising from experimental tooth movement in rats. We suggest that TRPV1 both in the parodontium and trigeminal ganglion are involved in orthodontic pain, and TRPV1 in the parodontium influence on orthodontic pain through reducing the expression of TRPV1 in trigeminal ganglion. Our finding may help to develop strategies for relieving orthodontic pain after orthodontics.

1. Introduction

The majority of patients under clinical orthodontic treatment experience discomfort and pain during the process of tooth movement [1]. Orthodontic pain, the most cited negative effect arising from the application of orthodontic force has been rated, as a key deterrent to orthodontic therapy and a major reason for discontinuing treatment [2,3]. While many discoveries have been made recently that greatly facilitate the study of orthodontic pain, much about this topic remains to be elucidated, and further research may provide some clarification.

Transient receptor potential vanilloid type 1 (TRPV1) is part of a family of transient receptor potential (TRP) channels whose expression is associated with small diameter primary afferent fibers [4,5]. This family of nonselective cation channels is renowned for the abil-

Abbreviations: TRPV1, transient receptor potential vanilloid type 1; TG, trigeminal ganglion; TRP, transient receptor potential; PFA, paraformaldehyde; qRT-PCR, quantitative realtime PCR; DMSO, dimethylsulfoxide; GCF, gingival crevicular fluid; IL-1β, interleukin-1β.

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ity to respond to a wide variety of chemical and physical inputs [6]. Recent research has shown that TRPV1 is involved in the regulation of body temperature [7,8] and plays a crucial rule in the transduction of painful stimuli from the periphery towards the central nervous system [9,10]. Furthermore, our research group previously demonstrated that TRPV1 in the trigeminal ganglion (TG) is involved in orthodontic pain during experimental tooth movement in rats [11]; however, whether TRPV1 in the parodontium plays a role in orthodontic pain is still unknown.

In this study, we sought to identify the contribution of TRPV1 in the parodontium to orthodontic pain relief. We used a selective TRPV1 antagonist (E)-3-(4-t-butylphenyl)-*N*-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylamide (AMG-9810) [12], to observe the analgesic effects of the blockage of TRPV1 on orthodontic pain during experimental tooth movement. Our data showed that in the parodontium, TRPV1 expression is enhanced by experimental tooth movement, and blocking TRPV1 relieved orthodontic pain arising from experimental tooth movement through inhibiting the expression of TRPV1 in TG.

2. Materials and methods

2.1. Experimental tooth movement

The male Sprague-Dawley (SD) rats (250 g–300 g) used in these experiments were purchased from the Experimental Animal Center of Xi'an Jiaotong University Health Science Center (Certificate No. 22-9601018). A fixed, Ni-Ti alloy closed-coil spring appliance was constructed to carry out mesial movement of the left maxillary first molar. Rats were anesthetized by intraperitoneally injecting 10% chloral hydrate (350 mg/kg), and a 50 g force was then applied to the experimental animals for 4 h, 8 h, 12 h, 1 d, 3 d, 5 d, 7 d or 14 d. The sham group rats were subjected to the same procedures as the experimental rats, but the springs in their mouths were not activated, and the blank group received no treatment. To assess the expression of TRPV-1 after experimental tooth movement induced by different forces, 30, 50 and 80 cN forces were applied to the rats for 1 d after force application, and TRPV-1 expression was monitored according to a previously described procedure. At the end of trial, rats were extracted the left maxillary first molar. Then, scraped out the surface of tissues in the socket and the molar by using a curet. The collection tissues were used for western blot and qRT-PCR assay.

2.2. Immunohistochemistry staining

Rats were anesthetized and transcardially perfused with 400 ml normal saline followed by 400 ml of 4% paraformaldehyde (PFA). For parodontium, the left maxillary first molar decalcified in neutral 10% ethylene diamine tetra-acetic acid (EDTA) at room temperature for more than 28 days. The EDTA solution was changed daily. To determine whether calcification was complete, a methyl red-ammonia-ammonium oxalate solution test was carried out. For TG, the lateral restricted area of the maxillary division of the TG anterior to the rostral edge of bifurcation to the maxillary and mandibular nerves was collected as this portion has been identified as the specific TG tissue affected by force applied to the maxillary molar teeth [13]. The trigeminal ganglia tisues were dissected and postfixed in 4% PFA for 3 d at 4 °C. Then tisues were followed by dehydrated in 30% sucrose solution for 3 d at 4 °C. The frozen sections (20 μm thick) were used a microtome (Slee, Germany) and mounted onto glass slides. Immunostaining was performed previously described and with minor modification [14]. The following primary and secondary antibodies were used: Rabbit anti-TRPV-1 polyclonal (1:50, Santa Cruz, USA), mouse anti-IL1 receptor I (1:200, Abcam, UK). The secondary antibodies were as follows: Alexa Fluor 488 donkey anti-mouse IgG (1:500, Invitrogen), Alexa Fluor 594 goat anti-rabbit IgG (1:400, Invitrogen). Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, 1 μg/ml, Sigma-Aldrich) Immunostaining positive cells were observed using a BX51 fluorescent microscope equipped with a DP70 digital camera (both from Olympus, Japan). The quantification of the immunostaining was performed previously described, with minor modification [15].

2.3. Western blot analysis

Protein extraction of periodontal tissue was prepared using RIPA lysis buffer plus a protease inhibitor cocktail (Roche, Germany). The protein concentration was determined using the BCA (bicinchoninic acid) method (Pierce). Equal amounts of protein were used for immunoblotting. Western blotting was performed as previously described, with minormodification [16], and the following primary and secondary antibodies were used: polyclonal rabbit anti-TRPV-1 (1:200, Santa Cruz, USA), monoclonal mouse anti-β-Actin (1:10,000, Sigma-Aldrich), and horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:100,000, Sigma-Aldrich). The densities of the obtained protein blots were analyzed using LABWORKS Software (Ultra-Violet Products, Cambridge, UK). The housekeeping gene β-Actin was used as an internal control to normalize the levels of the target protein.

2.4. Real time reverse transcriptase PCR

Total RNA from the parodontium and TG was extracted using the TRIzol reagent (Invitrogen) following the manufacturer's instructions and then reverse transcribed into cDNA using a mixture of OligodT and random primers (RevertAid first-strand cDNA synthesis kit, Fermentas, Canada). Quantitative realtime PCR (qRT-PCR) was performed with GoTaq qPCR Master Mix (Roche, Germany) using the iQ5 Real-Time PCR Detection System (BioRad). The primer sets for these assays were synthesized by the TaKaRa company (TaKaRa, Japan), as follows:

TRPV1-F: 5′-GAGTTTCAGGCAGACACTGGAA3′,
TRPV1-R: 5′-CTATCTCGAGCACTTGCCTCTCT3′;
GAPDH-F: 5′-GACAACTTTGGCATCGTGGA-3′,
GAPDH-R: 5′-ATGCAGGGATGATGTTCTGG-3′.

The results were analyzed using the comparative ΔΔCT method, and GAPDH served as the reference gene.

2.5. Injection of AMG-9810 in the parodontium or trigeminal ganglion

One day after the application of 50 cN of force, the rats were anesthetized byintraperitoneally injecting 10% chloral hydrate (350 mg/kg) to enable the injection of a solution or the TRPV-1 antagonist AMG-9810 (Tocris Bioscience, USA). The rats in the experimental group received an injection of AMG-9810 (10 nmol), which was dissolved in 5% dimethylsulfoxide (DMSO), 95% PBS. The sham group received a solution of 5% DMSO and 95% PBS, which was administered locally with the AMG-9810 injection, and the control group did not receive any injection. For the parodontium, the rats in the experimental group received an injection of AMG-9810 into the periodontal tissues of the upper incisors and the left maxillary first molar (localadministration, 10 nmol) with a 26-gauge needle. For the TG, the rats were anesthetized as described previously and placed in a stereotactic frame (Narishige, Japan). A longitudinal incision was made in the midline to expose the bregma, and two burr holes were drilled at the designated coordinates (Bregma: anteroposterior, −3.2 mm; mediolateral, L3.0 mm; dorsoventral from dura, 10.0 mm). The animals received injections