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Transient receptor potential vanilloid 4 (TRPV4) expression on the nerve fibers of human dental pulp is upregulated under inflammatory condition

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ABSTRACT

Objective: Transient receptor potential vanilloid 4 (TRPV4) has been considered as a mechano-, thermo- and osmo-receptor. Under inflammatory conditions in dental pulp, teeth can become sensitive upon exposure to a variety of innocuous stimuli. The objective of the present study was to investigate the expression of the TRPV4 channel on nerve fibers in human dental pulp of non-symptomatic and symptomatic teeth associated with inflammatory conditions.

Design: Dental pulp from extracted human permanent teeth was processed for fluorescence immunohistochemistry. Ten asymptomatic (normal) and 10 symptomatic (symptoms associated with pulpitis) teeth were used in this study. Nerve fibers were identified by immunostaining for a marker, protein gene product 9.5, and the cells were counterstained with 4′,6-diamidino-2-phenylindole. An anti-TRPV4 antibody was used to trace TRPV4 expression.

Results: TRPV4 expression was co-localized with the nerve fiber marker. Immunoreactivity for TRPV4 was more intense ($p < 0.05$) in the nerves of symptomatic teeth than those of normal teeth. The number of co-localization spots was increased significantly ($p < 0.05$) in the dental pulp of symptomatic teeth compared with that of asymptomatic (normal) teeth.

Conclusions: There is expression of TRPV4 channels on the nerve fibers of human dental pulp. Our findings suggest upregulation of TRPV4 expression under inflammatory conditions in the pulp. The upregulation of TRPV4 channels may be associated with the exaggerated response of dental pulp to innocuous mechanical, thermal and osmotic stimuli under inflammatory conditions.

1. Introduction

Transient receptor potential (TRP) channels comprise a group of nonselective calcium-permeable cationic channels that serve as polymodal sensors/receptors involved in detecting environmental stimuli such as thermal, chemical and mechanical stimuli (Clapham, 2003; Dhaka, Viswanath, & Patapoutian, 2006). For example, transient receptor potential vanilloid (TRPV) 1 and TRPV2 respond to noxious heat, while TRPM8 and TRPA1 respond to cool and noxious cold sensations, respectively (Dhaka, Viswanath, & Patapoutian, 2006). TRPV4 has been suggested to act as both a mechanoreceptor and osmoreceptor (Alessandri-Haber et al., 2003; Liedtke, 2006; White, Cibelli, Urban, Nilius, McGeown, & Nagy, 2016; Zhang, 2015). These receptors have been detected in afferent neurons of the rat trigeminal ganglion (Chen et al., 2013, 2014; Gibbs, Melnyk, & Basbaum, 2011; Park et al., 2006; Zakir et al., 2012). In animal models, TRP channels have been implicated in inflammatory and neuropathic facial pain (Chen et al., 2013, 2014; Hossain, Unno, Ando, Masuda, & Kitagawa, 2017b; Mickle, Shepherd, & Mohapatra, 2016; Zakir et al., 2012). These findings suggest that these channels are involved in the transduction of noxious stimuli when the stimulus is transduced into electrical signals in the nociceptive afferents of dental pulp; the information is then interpreted and processed by higher cortical areas, resulting in the experience of pain (Chung & Oh, 2013). Applying heat or cold stimuli to the tooth evokes pain by activating the nerves that innervate the dental pulp. Under inflammatory conditions involving dental pulp (pulpitis), the dental pulp becomes sensitive and may lead to thermal, mechanical, and osmotic hypersensitivity, i.e., exaggerated sensitivity to cold, heat, mechanical and osmotic stimuli in the tooth (Renton, 2011). The biological mechanisms involved in hypersensitivity remain unclear, and it

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is possible that TRP channels are involved in the hypersensitive condition of the pulp. Expression of TRPV1 (Kim et al., 2009) and transient receptor potential ankyrin 1 (TRPA1) (Kim et al., 2012) has been reported in human dental pulp, which are suggested to be involved in noxious heat and cold sensations of dental pulp. However, until the present study, the expression of TRPV4 in human dental pulp had not been examined. It is the objective of this study to determine the expression of TRPV4 channels on nerve fibers of human dental pulp of non-symptomatic and symptomatic teeth associated with inflammatory conditions.

2. Materials and methods

The teeth included in this study were 10 asymptomatic teeth (normal) and 10 symptomatic teeth (symptoms associated with pulpitis) collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Malaya. All patients involved in this study had complete dental records and gave consent for their teeth to be extracted and used for the present study. This study was approved by the Ethical Committee of the Faculty of Dentistry, University of Malaya (DF OB1414/0066(L). Symptomatic teeth were obtained from patients whose teeth were diagnosed by a dentist as having chronic pulpitis and associated with any two of the following symptoms: spontaneous pain (dull aching), pain during thermal stimulation (during consumption of hot or cold drinks), prolonged pain following removal of the stimulus, and pain during eating. Normal asymptomatic teeth without caries were obtained from patients who underwent elective third molar extraction or orthodontic treatments. All teeth used in this study were permanent teeth with completed roots. Following extraction, the teeth were rinsed with 0.01 M phosphate buffered saline (PBS), stored in separate bottles containing PBS, and immediately transferred to the laboratory. The apical third of the root was removed before placing the teeth in 10% neutral buffered formalin for 24 h at 4 °C (Daud, Nambiar, Hossain, Rahman, & Bakri, 2016a; Daud, Nambiar, Hossain, Saub et al., 2016b; Hossain, Daud et al., 2017a). The teeth were then split longitudinally, and the dental pulp was removed and placed in 10% neutral buffered formalin for 1 h at room temperature. Although removal of the pulp tissue was successful, it was later discovered that the odontoblast layer had been destroyed, possibly during the process of removing the pulp tissue. The dental pulp was then embedded in Tissue-Tek (Fisher Scientific, Waltham, MA, USA), and 20 µm-thick sections were prepared in a cryostat. Every third section was placed on a SuperFrost Plus glass slide (Fisher Scientific, Waltham, MA, USA). Some sections were used for hematoxylin and eosin staining.

2.1. Immunohistochemistry

The sections were washed (three times for 5 min each in PBS) and incubated at room temperature with 3% normal goat serum (NGS) in 0.01 M PBS containing 0.3% Triton X-100 for 90 min. They were then incubated for 24 h at 4 °C with both a rabbit anti-TRPV4 antibody (1:100; Alomone Labs Ltd., Jerusalem, Israel) and mouse monoclonal anti-protein gene product 9.5 (PGP9.5) antibody (1:500; Alomone Labs Ltd., Jerusalem, Israel) diluted with 3% NGS in 0.01 M PBS containing 0.3% Triton X-100. The sections were washed three times with PBS and then incubated with goat anti-rabbit IgG (Alexa Fluor 488, 1:1000; Invitrogen, Waltham, MA, USA) and goat anti-mouse IgG (Alexa Fluor 568, 1:1000; Invitrogen, Waltham, MA, USA) for 2 h at room temperature. After washing with PBS, the sections were mounted with Vectashield mounting medium with 4′, 6-diamidino-2-phenylindole counterstain (Vector Laboratories, Inc., Burlingame, CA, USA). The stained sections were viewed, and images were captured using a camera attached to a BX51 fluorescence microscope (Olympus, Tokyo, Japan). The images were viewed and captured by an investigator blinded to the grouping of the teeth. Three sections (one with the largest area of TRPV4 immunoreactivity on dental pulp nerves and the following two sections) were selected for analysis. In each section, images of three areas with the highest expression of TRPV4 were captured with a 20 × lens using the same settings for gain, gamma, and exposure time. In each image, two 100 μm^2 areas were selected to measure the corrected total fluorescence intensity (CTFI) using the following formula with the aid of ImageJ software (NIH Image, USA): CTFI = integrated intensity – (selected area × mean fluorescence of background readings in the selected area).

Mean CTFI values were used for statistical comparison between the sections of dental pulp from symptomatic (pulpitis) and asymptomatic (normal) teeth. Co-localization of TRPV4 and nerve fibers was investigated and compared between symptomatic and asymptomatic teeth using an automatic co-localization calculation plugin named Co-localization Color-map (Jaskolski, Mulle, & Manzoni, 2005) in ImageJ software. The plugin provides normalized mean deviation product (nMDP) values (−1 to 1) that indicate the spatial distribution of co-localization with the degree of co-localization. It also provides a color-scaled image of co-localization. In the color-scaled image, negative indexes are represented by cold colors (exclusion). Indexes above 0 are represented by hot colors (co-localization). The total number of spots that had nMDP values of 0.3 to 1 was recorded for each of the three sections, and mean values were compared between symptomatic and asymptomatic teeth.

2.2. Statistical analysis

CTFI and nMDP values were compared between symptomatic and asymptomatic teeth using a *t*-test. P-values of less than 0.05 were considered as statistically significant.

3. Results

The symptomatic group of teeth included 10 teeth (nine third molars and one premolar tooth), whereas the teeth in the asymptomatic group included eight third molars and two premolars. Inflammatory cells were observed in the dental pulp of symptomatic teeth (Fig. 1). The nerve fibers were immunoreactive for PGP9.5. Nerve fibers were scattered in the periphery of the crown part of the dental pulp, whereas bundles of nerve fibers were observed at the center of the dental pulp and in the root area. TRPV4 expression was co-localized with PGP9.5-

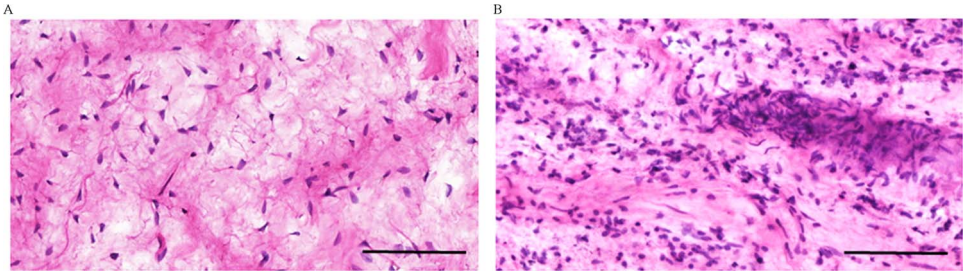

Fig. 1. Histological sections of hematoxylin and eosin-stained dental pulp of a non-symptomatic tooth (A) and inflamed tooth (B). The scale bar represents 100 µm.