Biodentine Reduces Tumor Necrosis Factor Alpha–induced TRPA1 Expression in Odontoblastlike Cells

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Abstract

Introduction: The transient receptor potential (TRP) ion channels have emerged as important cellular sensors in both neuronal and non-neuronal cells, with TRPA1 playing a central role in nociception and neurogenic inflammation. The functionality of TRP channels has been shown to be modulated by inflammatory cytokines. The aim of this study was to investigate the effect of inflammation on odontoblast TRPA1 expression and to determine the effect of Biodentine (Septodent, Paris, France) on inflammatory-induced TRPA1 expression. **Methods:** Immunohistochemistry was used to study TRPA1 expression in pulp tissue from healthy and carious human teeth. Pulp cells were differentiated to odontoblastlike cells in the presence of 2 mmol/L beta-glycerophosphate, and these cells were used in quantitative polymerase chain reaction, Western blotting, calcium imaging, and patch clamp studies. **Results:** Immunofluorescent staining revealed TRPA1 expression in odontoblast cell bodies and odontoblast processes, which was more intense in carious versus healthy teeth. TRPA1 gene expression was induced in cultured odontoblastlike cells by tumor necrosis factor alpha, and this expression was significantly reduced in the presence of Biodentine. The functionality of the TRPA1 channel was shown by calcium microfluorimetry and patch clamp recording, and our results showed a significant reduction in tumor necrosis factor alpha–induced TRPA1 responses after Biodentine treatment. **Conclusions:** In conclusion, this study showed TRPA1 to be modulated by caries-induced inflammation and that Biodentine reduced TRPA1 expression and functional responses. *(J Endod 2016;42:589–595)*

Key Words

Biodentine, dental pulp, human, inflammation, pulp capping, transient receptor potential channel

Members of the transient receptor potential (TRP) family of ion channels have emerged as important cellular sensors in both neuronal and non-neuronal cells. The TRP channel family comprises more than 30 calcium-permeable cation proteins that are activated by thermal, chemical, and mechanical stimuli (1). The functionality of TRP channels has been shown to be modulated by inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) (2), providing a potential mechanism for inflammatory hyperalgesia. Among the TRP channel family, TRPA1 plays a central role in nociception and neurogenic inflammation. It is a polymodal channel activated by chemical, mechanical, and thermal stimuli (3–5) and is the principal target of many endogenous reactive molecules produced at sites of inflammation and tissue injury (6, 7). A role for TRPA1 in mechanical and thermal hyperalgesia has been documented in a variety of inflammatory pain models (5, 8, 9).

In the dental pulp, TRPA1 is expressed by dental afferents (10, 11), and its expression is up-regulated in trigeminal neurons by nerve growth factor (NGF) (12) and after tooth injury (13). TRPA1 is also expressed in human odontoblasts (10, 14), and its activation has been shown to release signaling molecules, such as adenosine triphosphate (ATP), that mediate sensory transduction in teeth via an odontoblast neuronal signaling axis (15), supporting an emerging role for odontoblasts as sensory cells (16). Lying on the periphery of the dental pulp, odontoblasts also play important defense functions by regulating caries-induced pulp inflammation and cytokine production including TNF-α (17). During restorative procedures, odontoblast function can also be influenced by the application of restorative dental materials; however, the effect of these factors on odontoblast sensory receptors is not known.

Biodentine is a calcium silicate cement with promising regenerative potential and is proposed for use in both direct and indirect pulp capping (18–20). Pulp capping agents, in addition to their regenerative properties, are also ideally suited to have additional sedative and pain relief properties to enhance their clinical efficacy. Investigations of the interactions of Biodentine with dentin have revealed that this material penetrates into the dentin, forming taglike microstructures within the tubules. These structures obliterate the dentin tubules providing a hermetic seal and may be involved in reducing the postoperative hypersensitivity mechanically (21). In addition, promising clinical observations after the application of Biodentine as a restorative material have shown pain relief effects and the absence of postoperative sensitivity in cases of symptomatic pulpitis (22). However, the potential cellular and molecular mechanism by which Biodentine induces such pain relief effects is not known. We hypothesized that Biodentine modulates the expression and functionality of TRPA1 in

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TABLE 1. TaqMan Primer Details

Gene	Reference no.	Chromosome no.	Amplicon length
TRPA1	HS00175798	8	124
B2M	HS00984230	15	81
GUSB	HS00939627	7	96

odontoblasts to reduce pain and postoperative sensitivity. Therefore, the aim of this study was to investigate the effect of inflammation on odontoblast TRPA1 expression and to determine the effect of Biodentine on inflammatory-induced TRPA1 expression.

Materials and Methods

Cell Culture and Treatments

Dental pulp cells were derived by explant culture as previously described (23). Immature permanent third molar teeth were obtained in accordance with French ethics legislation, and cells were grown in minimal essential medium with L-glutamine supplemented with 10% fetal bovine serum, 100 UI/mL penicillin, and 100 μg/mL streptomycin. To differentiate pulp cells to odontoblast-like cells, the medium was supplemented with 2 mmol/L beta-glycerophosphate, as previously described (14). The odontoblast phenotype of the cells was confirmed by the expression of the odontoblast marker dentin sialophosphoprotein, as previously shown (14). For experiments involving TNF-α and Biodentine treatments, odontoblastlike cells (3×10^5) were grown in 6-well plates to 50%–70% confluence before treatment with cytokine in the presence or absence of Biodentine extracts. These were obtained after incubation of set Biodentine (0.05 mm^2/mL) in tissue culture medium for 24 hours, as previously described (24). Cells were then maintained in an incubator at 37°C and 5% CO_2 throughout.

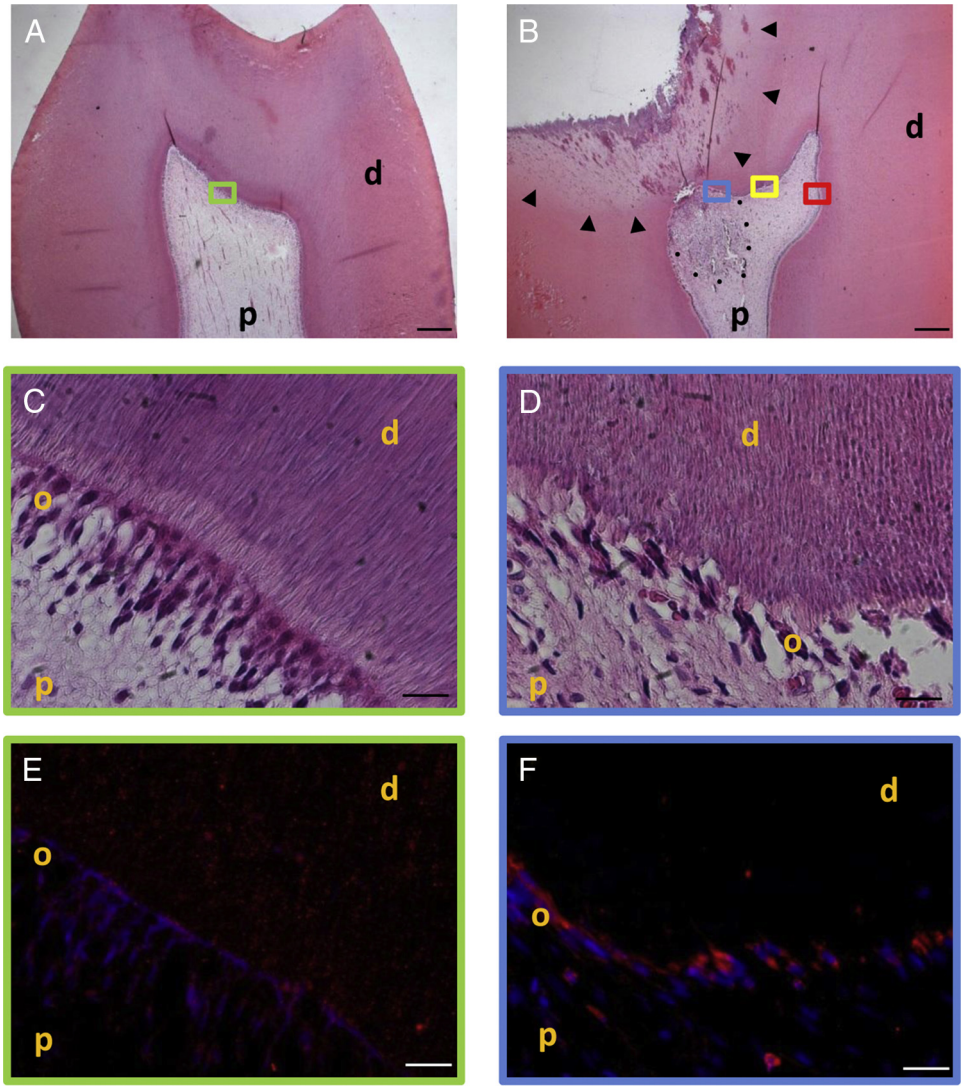

Figure 1. Detection of TRPA1 in human intact and carious teeth sections. (*A* and *C*) In intact teeth, odontoblasts (o) appear perfectly aligned at the pulp periphery. Bacteria infiltrating the dentin (d) are visible in carious tissue sections (*arrowheads*). (*B* and *D*) At the carious site, dentin is irregular and has an altered morphology, whereas inflammatory cell infiltration (*dotted line*) is visible in the underlying pulp (p). (*J*) This seems to be mainly caused by cariogenic bacteria (*red arrows*) as seen in Brown and Brenn staining. (*E*) Immunofluorescence reveals the expression of TRPA1 (red fluorescence) in odontoblast cell bodies as well as in the odontoblastic processes of intact teeth. This expression is intense in the odontoblasts of carious teeth. Immunofluorescence labeling appears intense (*F*) under the carious lesion and (*G* and *H*) elsewhere in the pulp at sites distant from the carious lesion. (*I*) No immunostaining was seen in the negative control. *A–D*: hematoxylin-eosin staining; *J*: Brown and Brenn staining; *I*: isotype control. *E–I*: immunofluorescence of TRPA1 (*red*) and DAPI counterstain of nuclei (*blue*). Scale bars: *A*, *B*, and *J* = 500 μm; *C–I* = 20 μm.

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