Changes in Proteinase-Activated Receptor 2 Expression in the Human Tooth Pulp in Relation to Caries and Pain

Claire R. Morgan, BSc (Hons), PhD
Postdoctoral Research Associate
Department of Oral and Maxillofacial Medicine and Surgery
School of Clinical Dentistry
University of Sheffield
Sheffield, United Kingdom

Helen D. Rodd, BDS, FDS (Paed), PhD
Professor of Paediatric Dentistry
Department of Oral Health and Development
School of Clinical Dentistry
University of Sheffield
Sheffield, United Kingdom

Nick Clayton, BSc
Senior Scientist
Neurology and GI Centre of Excellence for Drug Discovery
GlaxoSmithKline
Harlow, Essex, United Kingdom

Fiona M. Boissonade, BDS, PhD
Professor of Oral Neuroscience
Department of Oral and Maxillofacial Medicine and Surgery
School of Clinical Dentistry
University of Sheffield
Sheffield, United Kingdom

Correspondence to:
Dr Claire Morgan
Department of Oral and Maxillofacial Medicine and Surgery
School of Clinical Dentistry
University of Sheffield
Claremont Crescent, Sheffield, S10 2TA
United Kingdom
Fax: 0114 271 7863
Email: c.morgan@shef.ac.uk

Aims: *To investigate the presence of proteinase-activated receptor 2 (PAR2) in the human tooth pulp and to determine whether there are any changes in receptor expression with caries and pain.* ***Methods:*** *Forty-four mandibular first permanent molars were collected from children (n = 36, mean age 9.96 years ± 2.11) requiring dental extractions under general anesthesia. Teeth were categorized as either intact (n = 22) or carious (n = 22). Carious teeth were further subdivided into asymptomatic (n = 10) and painful (n = 12). The coronal pulp was removed and processed for indirect immunofluorescence by using antibodies raised against PAR2 and double labeled with either a neuronal marker (protein gene product 9.5) or both a smooth muscle cell (αSMA) and endothelial (UEIL) marker, in order to examine PAR2 presence in both neuronal and vascular tissue. In addition, hemotoxylin and eosin staining was performed to identify pulpal fibroblasts.* ***Results:*** *PAR2 expression was found to be present in pulpal nerve fibers, vascular tissue, and pulpal fibroblasts. PAR2 neuronal expression was not affected by the presence of caries (*$P > .05$*) but was significantly less in carious painful teeth than in carious asymptomatic teeth (*$P < .05$*). No changes in vascular PAR2 expression were found (*$P > .05$*); however, the number of PAR2-labeled fibroblast-like cells per mm² was significantly greater in carious teeth (*$P < .05$*).* ***Conclusion:*** *These findings indicate that PAR2 receptors and changes in their level of expression may have relevance and clinical importance in nociception.* J OROFAC PAIN 2009;23:265–274

Key words: caries, human tooth pulp, immunohistochemistry, pain, proteinase-activated receptor 2

Proteinase-activated receptors (PAR) belong to a family of G-protein coupled receptors that are irreversibly activated by proteases released under inflammatory conditions. To date, four PAR2 receptors have been identified, and much research has been carried out into the role of PAR2 in inflammation and nociception.

PAR2 is expressed on peripheral nerve fibers[1,2] and appears to have a role in neurogenic inflammation, with the activation of PAR2 on sensory nerves resulting in release of substance P (SP) and calcitonin gene-related peptide (CGRP). Mast cells, the majority of which contain the proteases trypsin and mast cell tryptase,[3] known PAR2 agonists, are routinely found associated with SP- and CGRP-expressing nerve fibers in normal and inflamed tissues.[4] Furthermore, tryptase stimulates the release of these neuropeptides from peripheral nerve endings.[1] PAR2 also appears to be involved in nociception; administration of sub-inflammatory

doses of PAR2-agonists results in long-lasting thermal and mechanical hyperalgesia accompanied by elevated spinal Fos expression.[2]

PAR2 and the transient receptor potential vanilloid receptor TRPV1 (a noxious heat sensor) are co-localized in sensory neurons of the dorsal root ganglia (DRG)[5,6] and bladder C-fibers.[7] Evidence suggests that PAR2 and TRPV1 interact in a novel pathway to induce nociception.[5,6] Recently, PAR2 has been found on trigeminal TRPV1-expressing nociceptors.[8] Investigations in our own laboratory have demonstrated TRPV1 expression in the human tooth pulp.[9] Furthermore, a study investigating neuropeptide release in the human tooth pulp found PAR2 receptor activation to be involved, demonstrating a possible further link between changes in PAR2 expression in the pulp and development of inflammation and pain.[10]

Dental caries is one of the most common diseases in man; fortunately, the vast majority of cases of pulpitic pain can be treated, either by restoration or removal of the affected tooth. However, there is evidence to suggest it can lead to the development of other types of chronic orofacial pain.[11] The tooth pulp is generally regarded as a useful model of nociceptive input. Stimulation of the tooth pulp rarely evokes any sensation other than pain; however, there is some evidence that nonpainful sensation may be elicited under certain conditions such as very low electrical stimulation.[12–15,16] Stimulation of the tooth pulp in humans usually causes pain irrespective of the stimulus applied, and there is little evidence for pulpal primary afferents (including Aβ fibers) with selective sensitivity for other sensations.[17,18] Thus, the use of the human tooth pulp allows the study of a group of nociceptive afferent nerve fibers and permits investigation into peripheral changes as a result of local inflammation. It also provides an excellent source of human tissue in which changes in the periphery can be correlated with the patient's clinical pain history. Furthermore, comparisons can be made between intact samples, inflamed asymptomatic samples, and inflamed pulps that are painful. In this way, changes that are due to the presence of inflammation can be separated from changes related to the development of pain.

The present study was designed to address the hypothesis that changes in PAR2 expression in the human tooth pulp may be associated with the development of inflammation and/or pain. Using the human tooth pulp as the experimental model, our aim was to investigate the presence of PAR2 in the human tooth pulp and to determine whether there are any changes in receptor expression with caries and pain.

Materials and Methods

Experimental material consisted of 44 mandibular first permanent molars obtained from children (n = 36) with a mean age of 9.96 years (SD 2.11, range = 6.83 to 14.08) requiring dental extractions under general anesthesia. Three groups of teeth were used in this study; intact (n = 22), carious asymptomatic (n = 10), and carious painful (n = 12) first permanent molars. First permanent molars of poor prognosis are ideally extracted between the ages of 9 to 11 years and, depending on the orthodontic status, intact first permanent molars may also be removed as compensating extractions (this is the removal of the opposing tooth to prevent its overeruption),[19] as they were in this case. Carious asymptomatic teeth were removed if they were of poor prognosis and beyond restoration. Ethical approval was sought from and granted by the South Sheffield Research Ethics Committee and informed consent was obtained from the parent or guardian of each patient to sanction the use of the extracted teeth for the purpose of the stated research. Prior to tooth extraction, a self-reported pain history was sought from each patient. Samples were considered symptomatic if the patients themselves reported spontaneous toothache in the previous two days and if parents or guardians were able to confirm this pain history by their having noticed that the child had suffered disturbed sleeping, or interference with normal school or social activities that was attributable to pulpitic pain over the past 48 hours. It was established that pain was attributed to irreversible pulpitic pain as only teeth associated with spontaneous, prolonged pain were included. Teeth painful on eating were not included, as this usually indicates a periapical infection. In this way, carious teeth were subdivided into carious asymptomatic and carious painful groups. Only carious teeth with occlusal caries were used in this study and all teeth with additional pathologies were also excluded. In addition, teeth were assessed radiographically and macroscopically to ensure that there was mature apical development, and any teeth showing open apices were omitted from the study.

Immediately following a simple forceps extraction, a groove was cut on the buccal side of the tooth and the teeth were split longitudinally using an osteotome and surgical mallet to reveal the pulp. The halves of the teeth were immediately placed into Zamboni's fixative (4% paraformaldehyde and 0.2% picric acid in 0.1M phosphate buffer, pH 7.4) at 4°C for 24 hours. The coronal pulp was then carefully dissected from the tooth pulp chamber and placed in phosphate-buffered saline (PBS)

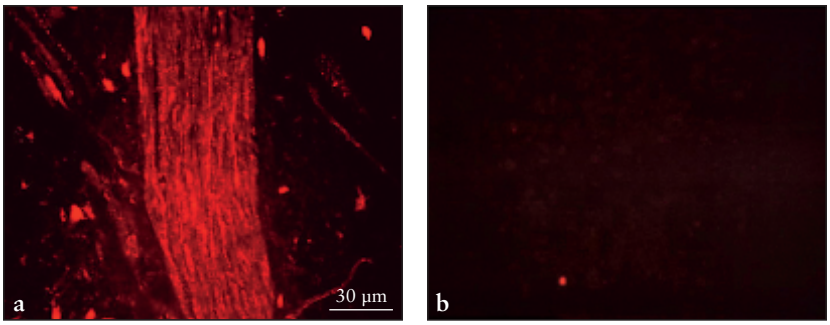

Fig 1 Photomicrograph showing pulp sections with positive labeling for *(a)* neuronal PAR2 in the MCP and *(b)* a PAR2 control section showing no positive labeling. Scale bar = 30 µm.

(0.2M, pH 7.4). At this point, the teeth were visually assessed under a dissection microscope at ×20 magnification. The extent of occlusal caries was determined using the following criteria: teeth categorized as intact showed no color changes within the dentin but possible staining of the enamel, whereas teeth categorized as grossly carious showed marked yellow/brown color changes extending beyond half the dentinal thickness.

Tissue Processing

Following the immersion of the coronal pulps in PBS for 24 hours, the pulps were placed in 0.1M PBS containing a 30% sucrose solution for cryoprotection (5 hours at 4°C). The pulp tissue was then embedded in Tissue-Tek OCT compound (Bayer Diagnostics) and 60 10-µm-thick longitudinal sections were cut from each pulp and thaw-mounted, in 20 sets, with three sections on each poly-D-lysine coated slide (Sigma Aldrich) so that sections 1, 21, and 41 (each 200 µm apart) were mounted on the first slide and so on. Slides were left to air dry at room temperature for 60 minutes before being placed in storage (-80°C) until ready for use.

All tissues were processed in parallel for indirect immunofluorescence. Slides were removed from storage as required and left for 60 minutes at room temperature. The slides were then washed in PBS containing 0.2% Triton X-100 (PBST) for 2 × 10 minutes. To minimize nonspecific staining, sections were first incubated in PBST containing 10% normal donkey serum (NDS) (Jackson Immunoresearch) for 30 minutes at room temperature. Sections were then incubated with a mixture of a polyclonal antibody to human PAR2 raised in rabbit (1:2000, Santa Cruz) and either the general neuronal marker, protein gene product 9.5 (PGP 9.5), a monoclonal antibody raised in mouse (1:1000, Ultraclone), or both a monoclonal antibody raised in mouse against human alpha smooth muscle actin (αSMA) (1:50, Novocastra) and the biotinylated Ulex europaeus agglutinin Iectin (UEIL) (1:100, Vector Labs), which were used to fully label the pulp vasculature. The antisera and UEIL were diluted in PBST containing 5% NDS and slides were left to incubate in this solution for 24 hours at 4°C in a humid atmosphere.

Slides were washed again in PBST for 2 × 10 minutes before incubation at room temperature for 90 minutes with either a mixture of donkey anti-rabbit IgG conjugated to indocarbocyanine (Cy3, 1:400, Jackson Immunoresearch) and donkey anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC, 1:40, Jackson Immunoresearch) to visualize PAR2 and PGP 9.5 expression, respectively, or a mixture of donkey anti-rabbit Cy3 (1:400), donkey anti-mouse FITC (1:40), and a FITC conjugated streptavidin (1:25). This second protocol was utilized to visualize PAR2 expression and the fully labelled pulp vasculature. The secondary fluorescent antisera and streptavidin were diluted in PBST containing 2% NDS. Finally, slides were washed in PBST for 2 × 10 minutes, carefully dried, and mounted in Vectashield (Vector).

Immunohistochemical controls were performed on all primary antisera. The specificity control for UEIL was carried out by inhibiting the lectin binding reaction by pre-incubating with 0.2M α-L-fucose (Vector Labs) dissolved in PBST for 60 minutes at room temperature.[20] Controls for PAR2, PGP 9.5, and αSMA were performed by omitting the primary antisera. No positive labeling was observed in any of the controls (Fig 1).

Analysis of Labeling

A Zeiss axioplan fluorescent microscope was used to examine the pulp sections. All analyses were performed blind to the status of each pulp sample. Primarily, descriptive analysis of the immunolabeling of each sample was undertaken followed by

quantitative analysis. Previous studies have shown that there are no differences in levels of immunolabeling between the three sections, each 200 μm apart, of each slide[21] and so the first section was taken for analysis. Quantitative analysis was undertaken on three different areas of the pulp; the mesiobuccal pulp horn (PH), the mid-subodontoblastic plexus (SOP), and the mid-coronal pulp (MCP). Each field was viewed using the ×20 objective and representing 0.22 mm^2 of tissue. Quantitative analysis was performed by the same operator and always using the same methodological order; PH, SOP, and MCP. After analysis of the SOP, the field of view was moved down into the MCP by 0.22 mm and the first large nerve trunk observed was analyzed. This ensured that all operator bias was removed. Computer-assisted image analysis software (Image-Pro Plus v3.0, Media Cybernectics) was used to obtain a digital image from the microscopic image. The method used to analyze expression of immunolabeling has previously been described in the human tooth pulp.[9] Essentially, the percentage area staining (PAS) of neuronal (PGP 9.5) or vascular labeling (αSMA and UEIL) was measured and the "common to both" function in the image analysis software was used to measure the PAS of PAR2 found within either the pulp nerve or vascular tissue. From this, the proportion of nerve tissue and vascular tissue containing PAR2 could be calculated. In addition to this analysis, the total number of αSMA/UEIL-labelled blood vessels and PAR2-labelled blood vessels were counted in order to calculate the percentage of blood vessels that expressed the PAR2 receptor. Furthermore, the numbers of PAR2-immunoreactive (-IR) fibroblasts were noted within the three different areas of the pulp.

Hematoxylin and Eosin Staining

Following analysis of immunolabeling, five slides were randomly selected to further investigate the presence of fibroblasts in the tooth pulp. The slides were placed in a glass trough containing PBS and left for 1 hour or until the coverslip could easily be removed. All five slides were stained using the hematoxylin and eosin protocol for fixed tissue sections with hematoxylin used to stain nuclei and eosin used to stain cytoplasm. Sections were viewed using a Nikon light microscope and staining was compared to associated PAR2-labeled images previously captured using image analysis software.

Statistical Analysis

A one-way analysis of variance (ANOVA), followed by post-hoc multiple comparison of means (Tukey Kramer) was employed to test for any statistical differences in neuronal and vascular PAR2 expression according to tooth status (intact, carious asymptomatic, or carious painful). All statistical analysis was performed on normalized data. Further ANOVAs were used to investigate whether the number of PAR2-expressing blood vessels or fibroblasts were altered in each of the three groups. Significance levels were set at $P < .05$. Pearson's correlation coefficients were used to examine whether the age of the patient was a determining factor on levels of neuronal and vascular PAR2 and numbers of PAR2-expressing fibroblasts.

Results

In total, 44 mandibular first permanent molars were utilized in this investigation. These consisted of 22 intact, 10 carious asymptomatic, and 12 carious painful samples. The mean age of the children from whom teeth were collected was 9.96 ± 2.11 years. No relationship was found between age and PAR2 levels in neuronal tissue ($P > .05$, $r = -0.13$) or vascular tissue ($P > .05$, $r = -0.06$) or numbers of fibroblasts expressing the PAR2 receptor ($P > .05$, $r = 0.08$) (data not shown). Preliminary examination of PAR2 by the use of indirect immunofluorescence revealed the presence of this receptor in nerve fibers and the pulp microvasculature. Furthermore, PAR2 expression was consistently found in pulpal fibroblast-like cells. Odontoblasts, when observed still present around the periphery of the pulp, were found not to be immunoreactive for PAR2 regardless of pulp status.

Analysis of Neuronal PAR2 Expression

Qualitative analysis revealed that PAR2-IR nerve fibers were present in all three areas of the pulp with expression found in the majority of samples. In the PH and SOP, PAR2 expression was found in the peripheral nerve endings, with a small number extending into the surrounding odontoblast layer. Unfortunately, many of these fibers were obscured by the vast numbers of labeled fibroblast-like cells that were also present within these areas. Throughout the pulp, neuronal PAR2 expression showed little change with caries; however, expression in the larger nerve trunks of carious painful samples appeared to be minimal. Figure 2 shows

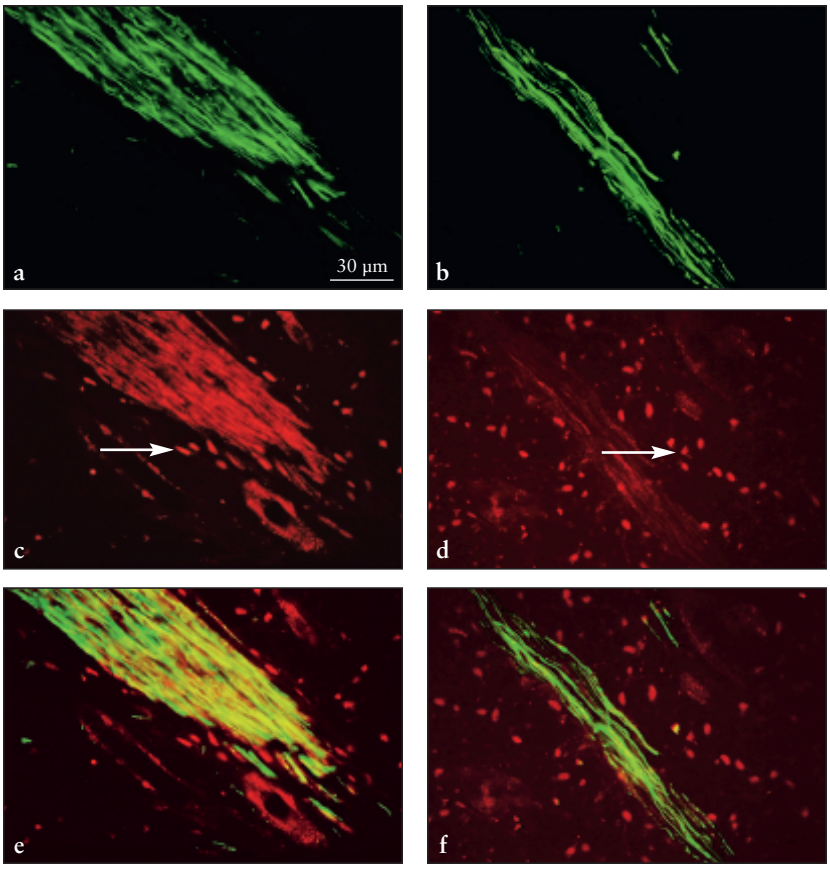

Fig 2 Photomicrographs demonstrating differences in neuronal PAR2 expression between carious asymptomatic and carious painful teeth. Photomicrographs *(a)* and *(b)* show labeling of pulpal nerve fibers with the general neuronal marker, PGP 9.5. Photomicrographs *(c)* and *(d)* show corresponding nerve fibers labeled for PAR2. A nerve trunk labeled for PAR2 in a carious asymptomatic pulp *(c)* shows an abundance of PAR2-IR fibers compared to *(d)*, a carious painful pulp, in which the nerve trunk contains far fewer positively labeled nerve fibers. *(e)* and *(f)* show co-localization of PGP 9.5 and PAR2-IR nerve fibers. In addition, photomicrographs *(c)* and *(d)* show cells *(arrows)* presumed to be pulpal fibroblasts, labeled for PAR2. Scale bar = 30 µm.

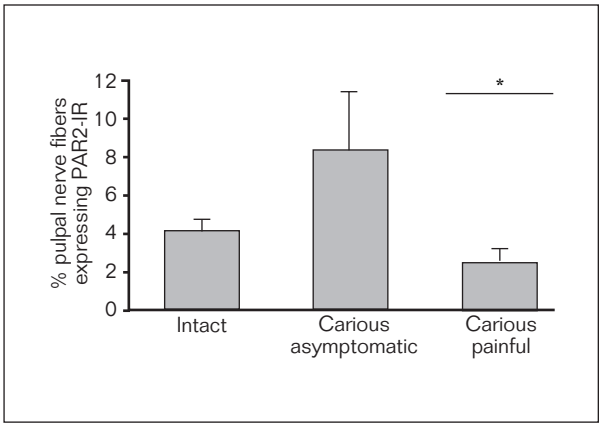

Fig 3 Bar chart showing mean (± SEM) percentage area of PGP 9.5-labeled tissue also expressing PAR2 in the MCP. No changes in PAR2 expression were found to occur with the presence of caries; however, neuronal PAR2 expression was significantly decreased in carious painful teeth compared to the carious asymptomatic samples (*$P < .05$, ANOVA).

PGP 9.5 and PAR2 double labeled nerve trunks from a carious asymptomatic sample and a carious painful sample. The co-localized photomicrographs (Figs 2e and 2f) show that only a few fibers were found positively labeled for PAR2 in carious painful samples compared to the carious asymptomatic samples where the majority of fibers in the nerve trunk expressed PAR2-IR.

Quantitative analysis of neuronal PAR2 expression was only undertaken in the MCP, as many of the smaller fibers in the PH and SOP were obscured by the presence of the fibroblast-like cells. The quantitative analysis of neuronal PAR2 confirmed that expression was not notably altered with caries, but levels were significantly lower in carious painful samples when compared to the carious asymptomatic group ($P < .05$, ANOVA). No significant differences were observed between intact and carious painful teeth (Fig 3).

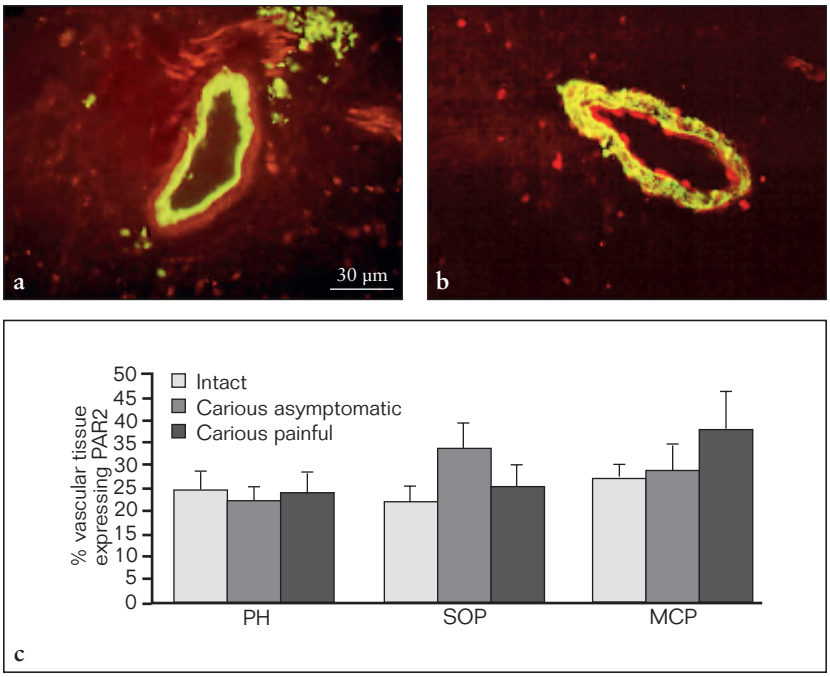

Fig 4 *(a)* Double exposure photomicrograph showing PAR2 expression in inner endothelial layer of blood vessel *(yellow immunofluorescence)* performed by double labeling of PAR2 *(red)* with the endothelial marker UEIL *(green)*. *(b)* Double exposure photomicrograph of arteriole for smooth muscle cells *(green)* and PAR2 *(red)*. The yellow fluorescence indicates PAR2 is also present in the outer smooth muscle layer of blood vessels. Scale bar = 30 µm. *(c)* Bar chart showing mean (± SEM) percentage area of UEIL/αSMA-labeled vascular tissue also positive for PAR2 in each of the three areas of the pulp. No differences were found in vascular PAR2 expression in the teeth with caries or pain ($P > .05$, ANOVA).

Additional Observations

PAR2 expression was also consistently found in the microvasculature of the pulp, including both the endothelial and smooth muscle layer of the blood vessels (Figs 4a and 4b). Capillaries in the PH and SOP and arterioles and venules in the MCP were all found to express PAR2-IR, although interestingly, PAR2-IR was less prominent in venules and there was minimal expression in what appeared to be lymphatic vessels. All vessels were identified using previously described criteria.[22–24] No significant differences were found in vascular PAR2 expression with either caries or the onset of pain ($P > .05$) (Fig 4c). The numbers of blood vessels expressing PAR2 also did not change with either caries or pain (data not shown).

In addition to PAR2 expression in pulpal nerve fibers and vascular tissue, PAR2 was also found to be expressed in fibroblast-like cells. Hematoxylin and eosin staining carried out after the original archive staining run confirmed this to be the case by allowing further identification of these elongated spindle-shaped cells (data not shown). Descriptive analysis appeared to show the numbers of PAR2-IR-fibroblast-like cells increased in the pulp with caries progression (Figs 5a and 5b). In the MCP, quantitative analysis suggested an increasing number of fibroblast-like cells expressed PAR2 in teeth with caries and with the presence of pain, however, only the difference between numbers of fibroblast-like cells in the intact pulp and carious painful samples was significant ($P < .05$) (Fig 5c). No increase in the numbers of PAR2-expressing fibroblast-like cells was found in either the PH or SOP with caries or, in any region of the pulp, with pain ($P > .05$).

Discussion

This investigation has shown PAR2 to be expressed in a variety of tissues in the human tooth pulp including endothelial cells, smooth muscle cells, and pulpal nerve fibers. This is consistent with previous studies that have found PAR2 to be present on various tissues in other systems, such as epithelial and endothelial tissues, human platelets,[25] smooth muscle cells,[26] and peripheral nerve fibers.[1,2] Furthermore, the present study also found PAR2 expression in what were presumed to be pulpal fibroblasts. This again is consistent with another study in which pulpal fibroblast-like cells were found to express PAR2 and the neuropeptides SP and CGRP.[10]

PAR2 Neuronal Expression

As this is the first study to describe PAR2 expression in pulpal nerve fibers, there is a paucity of data with which to corroborate our findings. It is already known that PAR2 is expressed on C fibers of the DRG[1] and shows co-expression with both TRPV1 on cultured nociceptors of the trigeminal

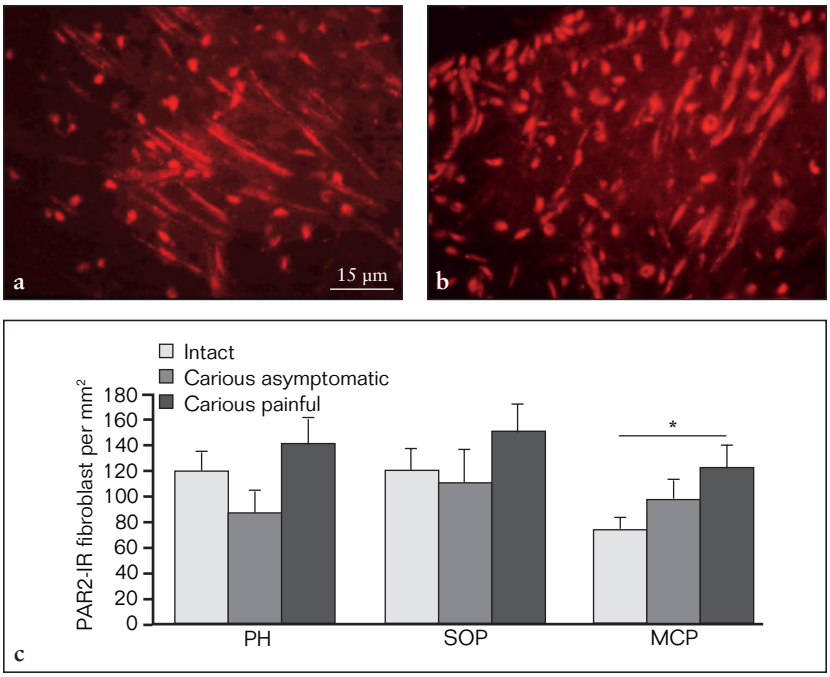

Fig 5 *(a)* PAR2-expressing fibroblast-like cells in the MCP from an intact tooth. *(b)* The numbers of PAR2-expressing fibroblasts in the MCP appear to be increased in carious teeth. Scale bar = 15 µm. *(c)* Bar chart showing the numbers (± SEM) of fibroblasts expressing PAR2 per mm^2 throughout the pulp. Numbers of PAR2-IR fibroblasts were significantly increased in the MCP between the intact and the carious painful samples. ($P < .05$ ANOVA).

ganglion[8] and transient receptor potential ankyrin 1 (TRPA1) on rat DRG neurones.[27] With TRPV1 and TRPA1 both being present in the trigeminal system, it appears likely that PAR2 may also be expressed on small-diameter neurons in the dental pulp. However, in the human tooth pulp, it was not possible to determine the afferent fiber types expressing PAR2 through the measurement of conduction velocities or examination of cell body size. Future studies could examine this further in an animal model.

The present investigation revealed that the proportion of pulpal nerve fibers expressing PAR2 was significantly less in painful teeth compared with carious asymptomatic samples. This decrease in neuronal PAR2 expression may indicate an increase in receptor activation at the cell surface with the onset of pain. PAR2 receptors are irreversibly activated following cleavage of its N-terminus. This unmasks a tethered ligand which binds to the main body of the receptor to initiate a cellular response. Termination of this signal only occurs as the receptor uncouples from the G-protein and PAR2 is internalized, via endocytosis, into early endosomes and lysosomes where it is degraded. Receptors are then replaced by de novo synthesis and mobilization of intact PAR2 from stores in the Golgi apparatus back to the cell membrane (for review, see reference 28). Bohm and colleagues[29] showed that after single exposure to trypsin, PAR2 receptors are replaced at the cell membrane, even after disruption of Golgi stores with brefeldin A, an inhibitor of intracellular protein transport. However, after repeated exposure to trypsin, resensitization of PAR2 is attenuated by brefeldin A. Cyclohexamide, an inhibitor of protein synthesis, also attenuates PAR2 resensitization after continued exposure to trypsin. These data indicate that continuous synthesis of PAR2 is needed to replenish receptors at the cell membrane. Therefore, the decrease in neuronal PAR2 observed in the present study could be a result of PAR2 receptors being continually activated and rapidly internalized in painful teeth, so much so that stores in the Golgi apparatus are more quickly depleted than in normal or carious asymptomatic teeth. However, we are unable to verify this hypothesis and so further work is needed. Interestingly, a decrease in PAR2 expression was also found in urothelial cells of the inflamed mouse bladder,[30] thus lending further support to this hypothesis. A recent study investigating protease activity in visceral pain presented biopsy samples from irritable bowel patients that showed increased trypsin and tryptase concentrations and also increased levels of proteolytic activity.[31] This proteolytic activity was responsible for activation of sensory neurons and generation of hyperalgesia. This is consistent with our studies where the decrease in neuronal PAR2 expression suggests an increase in PAR2 activation and internalization.

PAR2 Vascular Expression

PAR2 expression was observed in the microvasculature of the pulp, including both endothelial and smooth muscle tissue which is consistent with many other studies.[30,32,33] Vascular PAR2 mRNA has been shown to be upregulated in inflammatory conditions[34,35]; however, in this study, vascular PAR2 protein expression was not found to be altered with either caries or pain. It is possible that the detected increase in vascular PAR2 mRNA is compensating for the internalization of PAR2 receptors in vascular tissues and this could account for the stable protein levels observed at the cell surface. However, again, as this is the first study to investigate vascular PAR2 expression in the human tooth pulp, there is no data with which to compare these findings. The findings are also of interest as we have previously shown TRPV1 to be expressed in both endothelial and smooth muscle cells of the pulp microvasculature, with an increase in pulps from patients with a recent history of pain.[9] Again, the interactions between these two receptors may lead to TRPV1 activation on the vascular tissue, resulting in increased vasodilation of the pulpal blood vessels. As the pulp lies in a low compliance environment, any increase in blood flow could lead to pain.[36]

PAR2 Fibroblast Expression

PAR2 expression was found on pulpal fibroblast-like cells throughout the pulp and this expression was confirmed by the use of hematoxylin and eosin labelling. The hematoxylin-labeled nucleic structures clearly revealed the elongated structure of the fibroblast cell and also showed co-localization with PAR2-labeled structures. Previous studies have also shown PAR2 to be expressed on human fibroblasts[37,38] and intracellular Ca^{2+} signaling has shown PAR2 to be functional in human bronchial fibroblasts.[39] In pathological states, PAR2 expression on fibroblasts has been shown to be increased in the immediate surroundings of the injury site.[40]

Fibroblasts are the most common cell type in the dental pulp. When the pulp is compromise, increased mitotic activity among fibroblasts present in the cell-rich zone of the pulp can be observed.[41] Fitzgerald et al also showed that fibroblasts migrate to the pulp-dentin border and differentiate into secondary odontoblasts following damage to the primary odontoblasts. This migration and differentiation into other cell types may help explain why the numbers of PAR2-IR fibroblast-like cells were only increased in the MCP of teeth with caries.

Mast cell tryptase is a potent stimulant of fibroblast proliferation via activation of PAR2 receptors present on fibroblasts.[37,42,43] However, at present, the presence of mast cells in the tooth pulp is still somewhat controversial. A number of studies have failed to find evidence of mast cells in the normal healthy pulp,[44–49] yet Farnoush[50] and Walsh et al[51] both identified mast cells in tissues of the oral cavity, including the normal tooth pulp. Some of these same studies have shown that mast cells are present in the inflamed pulp.[47–49] It is often argued that, in the pulp, mast cells are more difficult to detect as removal of the tissue elicits mast cell degranulation due to tissue injury and neuropeptide release. It is possible that in the intact pulp, mast cells are often not present or are present but at very low levels, but with the course of inflammation these levels are increased—a process that may be similar to increased leukocyte expression in the carious tooth.[52] Interestingly, mast cells are found in increased numbers at sites of excessive tissue remodeling,[53] this again lending support to the notion that mast cells and fibroblasts interact to induce fibroblast proliferation. Furthermore, it appears that the factors that can induce fibroblast proliferation may also have the capacity to increase fibroblast PAR2 expression.[43] These data indicate that PAR2 expression on fibroblasts may be of importance in pulpal repair as proliferation of fibroblasts is key for reparative dentinogenesis.

It is possible that tryptases originating from sources other than mast cells are released in the tooth pulp to activate PAR2 receptors. It is well known that periodontitis can lead to irreversible pulpitis[54] with *Porphyromonas gingivalis* (*P gingivalis*) being an important factor. Cell surface-associated and secretory trypsin-like cysteine proteinases produced by P gingivalis, such as RgpB, have been shown to have proinflammatory effects[55] and it was recently demonstrated that RgpB is able to induce SP and CGRP release in pulpal cells through a PAR2-dependent pathway.[10] Although the teeth investigated in the present study had caries confined to occlusal surfaces, with any teeth showing additional pathologies (including associated periodontitis) omitted from the study, it may be possible that other bacterial proteinases are present in the grossly carious teeth and are involved in a similar pathway leading to activation of the PAR2 receptor.

Clinical Significance

This investigation has shown that PAR2 is present in the human dental pulp and is expressed by a variety of different tissues where it is likely to have a variety of different effects. Neuronal PAR2 appears to be of importance in the nociceptive pathway. As previously mentioned, activation of PAR2 on sensory neurones leads to the local release of neuropeptides.[1] Persistent activation of PAR2 on peripheral nerves can lead to central sensitization and the release of neuropeptides from central projections in the dorsal horn of the spinal cord where they participate in pain transmission.[2] In addition, PAR2 agonist-induced hyperalgesia is abolished in animal models deficient in PAR2.[2] The present study has demonstrated a decrease in neuronal PAR2 expression in painful teeth; this indicates a likely rapid activation of this pathway and thus, an important role in nociception. As mentioned above, this receptor is internalized following activation, thus reduced levels of the PAR2 protein may indicate high levels of receptor activation. This indicates a link between ongoing intense activation of this receptor and the presence of pain. Therefore, a PAR2 antagonist to block this effect might be a useful analgesic tool in a variety of painful conditions. However, as PAR2 is also expressed by vascular tissues and fibroblasts, receptor expression on these tissues may be important in maintaining homeostasis and wound healing, respectively. Thus, in these situations the loss of a functional PAR2 receptor may lead to adverse effects on tissue repair.

In conclusion, these findings indicate that PAR2 may be a potential target for the treatment of dental and other types of inflammatory pain. The recent studies documenting the co-localization of PAR2 and TRPV1 and their interactions[5,6,8] are interesting as TRPV1 is found in the human tooth pulp and its expression is associated with inflammatory pain.[9] Therefore, it is tempting to speculate that disruption of this pathway, with a PAR2 antagonist, may be important in the treatment of pulpitic pain. However, any treatments targeting PAR2 must take into account its role in tissue repair and wound healing.

Acknowledgments

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