

SCIENTIFIC ARTICLES

The Concentration of Prostaglandin E₂ in Human Periradicular Lesions

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Prostaglandins (PG) have been implicated in the genesis of periapical lesions. Periapical specimens from patients with clinical signs and symptoms of chronic and acute apical periodontitis were obtained and immediately frozen in liquid nitrogen. Periradicular tissues from unerupted third molars were frozen and used as controls. The concentration of PGE₂ was determined by radioimmunoassay. Low levels of PGE₂ were found in the control tissues as compared with those detected in chronic and acute lesions. Significantly higher levels of PGE₂ were found in acute lesions than those found in chronic lesions. The results show that acute lesions have higher concentrations of PGE₂ than chronic lesions and confirm the role of prostaglandins in the pathogenesis of human periapical lesions.

Tissue damage usually results in the release of an array of endogenous chemical mediators of inflammation. Among these are arachidonic acid metabolites such as leukotrienes, thromboxanes, and prostaglandins. Prostaglandins have been assayed from nearly every tissue in the human body and are among the most thoroughly studied of the chemical mediators of inflammation. Prostaglandin E₂ (PGE₂) has been shown to induce vascular dilation, vascular stasis, pain, and migration of leukocytes (1). It mediates these changes either directly or by potentiation of the effects of other mediators of inflammation such as histamine or the kinins (2).

It has been 20 yr since the effects of prostaglandins were first studied in bone resorption (3). Several investigators have determined the presence or absence of prostaglandins in the gingival tissues (4–6), inflamed and uninfamed dental pulps (7–9), and studied their roles in bone resorption and cyst formation (10–12).

Investigations into the production of prostaglandins in human periapical lesions have provided valuable information about the participation of these mediators of inflammation in the pathogenesis of these lesions (10–12). No reports, however,

could be found dealing with the levels of prostaglandins in acute apical lesions. The purpose of this investigation was to determine the levels of prostaglandin E₂ in human acute apical lesions.

MATERIALS AND METHODS

Sixteen human periradicular tissue samples were taken from 16 different individuals during endodontic surgery. The grouping of the "chronic" and "acute" tissues was accomplished by following the definitions of those clinical states as defined by Cohen (13) and Torabinejad and Walton (14). A lesion was considered acute when it was associated with a radiolucency and symptoms such as pain and swelling. Chronic lesions were associated with radiolucencies and no pain or swelling. An attempt was made to avoid inclusion of patients who were taking medications. However, due to the scarcity of patients with acute lesions who were not taking analgesics, two patients on medications were included in the acute category.

Group 1 consisted of periradicular tissues taken from teeth that had been diagnosed as having chronic apical periodontitis. Teeth were placed in this group based on the following criteria: a clinical and radiographic examination that determined the existence of periradicular pathosis involving destruction of cortical bone, no or slight sensitivity to percussion, and absence of periodontal pockets (greater than 3 mm). Surgeries in these cases were performed according to the criteria used by Cummings et al. (15).

Group 2 consisted of lesions from teeth that had been diagnosed as having acute apical lesions. Teeth were placed in this group based on the following criteria: a clinical and radiographic examination that determined the existence of periradicular pathosis involving destruction of cortical bone, and painful sensitivity to percussion and/or palpation and absence of periodontal disease. All lesions included in this category were associated with pain and/or swelling.

Uninflamed "normal" periradicular tissues were obtained from periapical regions of unerupted and incompletely formed third molars and used as controls (group 3). The teeth included in this group had to meet the following criteria: a verbal history confirming no history of pulpal pain, a clinical

and radiographic examination after extraction assuring that these teeth had no caries.

Patients in groups 1 and 2 were anesthetized by using Lidocaine with 1:100,000 epinephrine. In each case a full-thickness flap was reflected and a bony access was made to the periapical region by use of a high-speed bur with saline irrigation. The periradicular tissues were then carefully curetted, placed in plastic vials, and immediately frozen in liquid nitrogen and stored at -70°C . The uninflamed control periradicular tissues were obtained after extraction of unerupted wisdom teeth by removal of the connective tissue from the incompletely formed apices of these teeth. These samples were also frozen in liquid nitrogen and then stored at -70°C .

The periradicular tissues (for each group) were homogenated individually. The homogenate for radioimmunoassay was mixed in a Brinkman Polytron (Brinkman Instruments, Westbury, NY). Fifty milligrams of periradicular tissue per 2 ml of 0.05 M Tris buffer (pH 7.4) were homogenized at 4°C using the Polytron, activated 10 s out of each minute for 3 min. When less than 50 mg of tissue sample were available, an equivalent concentration of homogenate was accomplished by adjusting the tris-buffer quantity using the formula:

$$\frac{50 \text{ mg}}{2000 \mu\text{l}} = \frac{X \text{ mg tissue}}{Y \mu\text{l Tris buffer}}$$

A pilot study was run using one tissue sample from each group in order to determine correct dilutions of the tissue homogenate and duration of incubation in arachidonic acid.

Tissues that were to be incubated were added to arachidonic acid (25 mM pH adjusted to 7.4 in Tris buffer). The ratio was 500 μl of tissue homogenate with 500 μl of arachidonic acid. The mixture was vortexed for 5 s and allowed to incubate in a 37°C water bath for 10 min. The reaction was stopped by addition of 1000 μl of ethyl alcohol (95%). The samples were then centrifuged at $4500 \times g$ at 8°C for 30 min. The supernatant was decanted and used to prepare dilutions for the radioimmunoassay test. The optimum dilution, as determined by the pilot study, was 1:10 supernatant:0.05 M Tris buffer (pH 7.4).

The radioimmunoassay procedure to determine the amount of PGE_2 synthesized was accomplished according to the procedures described in the commercial kit purchased from New England Nuclear Co. (E. I. du Pont de Nemours & Co., Inc., NEN Products, Billerica, MA).

The amount of radioactivity was measured by using a gamma counter for a period of 1 min. The data were analyzed by entering the raw counts for the standard curve and the test

samples on an IBM computer and analyzing it with the IBM-PC Ria Data Reduction software (M. L. Jaffe and Assoc., Silver Spring, MD).

To evaluate any significant statistical difference among the three groups, the Kruskal-Wallis one-way analysis of variance was used.

RESULTS

Sixteen periradicular tissues were assayed for prostaglandin E_2 in the final test. Six lesions comprised group 1, the chronically-inflamed tissues, and six lesions comprised group 2, the acute tissues. Four of them comprised group 3, the "uninflamed" control tissues. The concentration of Prostaglandin E_2 for each sample is shown in Table 1 and represents the calculated value of prostaglandin E_2 in picograms (pg) per mg of wet sample tissue weight.

The mean value recorded for endogenous levels of prostaglandin E_2 in the uninflamed tissue was 6.288 pg per mg wet tissue wt; for the chronic tissues the mean was 37.840 pg per mg wet tissue wt; and in the acute tissues the mean was 105.872 pg per mg wet tissue wt. The Kruskal-Wallis test showed that there was a significant difference in concentration of PGE_2 between the uninflamed tissues and the chronic lesions ($p < 0.01$). There was also significant difference in the levels of PGE_2 production between the chronic and the acute lesions ($p < 0.050$) (Table 2).

The mean calculation of prostaglandin E_2 for the tissues incubated in arachidonic acid in the uninflamed tissue was 12.992 pg per mg wet tissue wt; for the chronic lesions the mean was 49.472 pg per mg wet tissue wt; and in the acute tissues, the mean was 117.456 pg per mg wet tissue wt. The Kruskal-Wallis test showed no significant difference between the concentration of PGE_2 in uninflamed tissues when compared with the chronic lesions. However, there was a significant difference between the levels of PGE_2 produced in the chronic lesions and in the acute lesions at $p < 0.03$ (Table 3).

DISCUSSION

Attempts have been made to correlate the clinical signs and symptoms of pulpal pathosis with their histological findings (16, 17). As yet, no one has been able to relate a given histological characteristic to clinical symptoms with absolute repeatability. Due to the small amounts of tissue in each sample size, histopathological examination was not performed to determine the nature of the tissues evaluated in this study.

TABLE 1. Concentration of PGE_2 in periradicular tissues

Tissue Type	Sample (pg/mg wet tissue wt)						Mean
	1	2	3	4	5	6	
Uninflamed							
Endogenous	5.264	6.128	6.640	7.088			6.288 \pm 0.782
AA*	12.400	14.304	11.968	13.296			12.992 \pm 1.036
Chronic							
Endogenous	60.880	17.808	11.232	8.752	121.648	6.736	37.840 \pm 45.776
AA	63.664	57.360	16.064	11.216	135.824	12.736	49.472 \pm 48.268
Acute							
Endogenous	128.592	81.696	156.512	56.688	70.256†	7.392†	105.872 \pm 45.024
AA	138.128	98.368	161.600	71.744	84.720†	17.696†	117.456 \pm 40.124

* Reaction "pushed" with arachidonic acid.

† Not included in statistical analysis.

TABLE 2. Statistical summary (analysis of variance) for endogenous levels of PGE₂

Source	SS	df	MS	F
Between groups	81.9413	2	40.9707	6.9655
Within groups	64.7010	11	5.8819	
Total	146.6423	13		
Kruskal-Wallis (0.05)				
Chronic versus acute: significant				
Kruskal-Wallis (0.01)				
Uninflamed versus chronic: significant				
Uninflamed versus acute: significant				

TABLE 3. Statistical summary (analysis of variance) for PGE₂ in tissues pushed with arachidonic acid

Source	SS	df	MS	F
Between groups	88.5839	2	44.2920	7.5671
Within groups	64.3853	11	5.8532	
Total	152.9692	13		
Kruskal-Wallis (0.05)				
Uninflamed versus chronic: no significance				
Kruskal-Wallis (0.03)				
Chronic versus acute: significant				

Rather, the samples were divided on the basis of their radiographic appearance and clinical signs and symptoms. Periapical tissues from developing third molars were used as controls. The location of this tissue was similar to that of the test samples, and it was assumed that this tissue would be uninflamed.

It is important to note that in the selection of the acute lesions, we chose lesions that were associated with pain and swelling, regardless of a possible previous association with a chronic lesion. This may explain some of the variability in prostaglandin E₂ encountered in the samples of chronic and acute lesions. There are variations that occur between what is seen histologically and what is observed clinically (18).

Variations are also to be expected from one laboratory to another with regard to the process of tissue collection and radioimmunoassay procedures. The way the sample tissue is collected may even result in tissue damage that leads to release of prostaglandin (19). Other differences can result from the storage temperature of the tissue samples. The radioimmunoassay is quite technique sensitive as well. Data from one run, even in the same laboratory, may not be comparable to data from another run.

Researchers in the field of periodontics have contributed to the quantification of PGE₂ in the gingival tissues (4, 5). These investigators found an increase of 10- to 20-fold in PGE₂ levels in inflamed gingiva compared with uninflamed gingival tissue.

Cohen et al. (8) have quantified the levels of PGE₂ and PGF₂ in uninflamed, chronically inflamed, and acutely inflamed human dental pulp tissues. The values of PGE₂ in their inflamed tissue was 28 times the value in the uninflamed tissue. The results of the present study found an increase of about 17-fold when comparing the acute with the uninflamed tissues. The well-developed peripheral circulation at the periapex is quite similar to that of the gingival tissues, which may explain the similarity of concentration of PGE₂ in periapical lesions and inflamed gingiva.

In addition to determining the endogenous levels of prostaglandin E₂ in periradicular lesions, we also "pushed" the reaction toward production of PGE₂ by incubating the homogenates in arachidonic acid. This enabled us to measure the maximum values of PGE₂ that the tissue samples were capable of producing.

The data from this comparison are noteworthy. It appears that the uninflamed tissue has a capacity to produce more than twice the measured levels of PGE₂ produced endogenously. The chronic lesions can be pushed to produce more PGE₂ as well. The relative amount, though, is somewhat less than that of the uninflamed tissues. Chronic lesions were only able to be pushed to produce about 1.3 times the amount of the PGE₂ produced endogenously. The acute lesions were even less capable of producing excessive levels of PGE₂ in the presence of arachidonic acid. The acute lesions were only able to be pushed to produce 1.1 times the amount produced endogenously. This data may be a good illustration of the "burn-out" potential of the cyclooxygenase enzyme. It has been shown that this enzyme breaks down relatively quickly, becoming inactivated after approximately 400 substrate turnovers (20). In an acute inflammation, this enzyme is one of the limiting steps in the production of PGE₂. It wears out due to the excessive amounts of arachidonic acid substrate provided by the inflammatory process. Once the supply of cyclooxygenase has been exhausted, the tissue is unable to produce more prostaglandins, even if substrate is available.

Samples 5 and 6 in Table 1 in the acute category were excluded from the statistical evaluations. These two patients had taken nonsteroidal anti-inflammatory medications before the time of their surgeries, and thus the tissues might have been influenced by the medications. Patient 5 had taken "one or two Advil (200 mg of ibuprofen) tablets during the past 48 h." He presented with both pain and swelling. Patient 6 had taken 600 mg of ibuprofen every 6 h for a few days prior to periapical surgery. He presented with swelling but no pain. There is not enough data to perform statistical analysis on the two patients. However, the findings are noteworthy.

Low doses of ibuprofen in patient 5 had little effect in diminishing the amounts of prostaglandin E₂ in his periapical lesion. In contrast, patient 6, who was on high levels of ibuprofen, had PGE₂ levels that were almost identical to those seen in the uninflamed control tissues. Further clinical studies are needed to confirm the effects of nonsteroidal anti-inflammatory medications on the level of prostaglandin E₂ in acute periapical lesions.

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