Catalase activity in healthy and inflamed pulp tissues of permanent teeth in young people

KMÇ Topçu, DÖ Kırıcı, MS Evcil

Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum, Ministry of Health, State Hospital of Dentistry, İzmir, Turkey

Abstract

**Aim:** To evaluate catalase (CAT, EC 1.11.1.6) activity in healthy and inflamed dental pulp of young patient’s teeth and to investigate if an active defense system oxidizing agents is present as a response to bacterial invasion.

**Materials and Methods:** Twenty young patients between 15 and 25 ages, who were diagnosed to be healthy, were the source of the pulp tissue. The situation of the dental pulps was evaluated using clinical and radiographic assessments. The patients were divided two groups from healthy, and inflamed pulp tissues were obtained; each participant provided one pulp tissue specimens. The specimens were collected during endodontic treatment or by longitudinally grooving and splitting the teeth (if extracted). Catalase activity was determined through spectrophotometric methods and an independent sample t-test assessed the significance of differences between the groups.

**Results:** There was statistically a difference between healthy pulp tissue and inflamed pulp tissue ($P < 0.005$, independent sample t-test). The catalase activity of healthy group was significantly lower than inflamed pulp groups.

**Conclusion:** The present study has shown that a significant increase in catalase activity is determined in inflamed dental pulps, which is due to pulpitis in comparison to healthy dental pulp.

**Key words:** Catalase, enzyme, pulp tissues, teeth

Date of Acceptance: 02-Nov-2015

**Introduction**

Many cell mediators and enzymes have been demonstrated to have a possible role in the development and spreading of pulp inflammation. Recent studies have studied the role of bradykinin, substance P, neurokinin A, interleukin-1β, prostaglandin E2, F2α, 6-keto prostaglandin F1α, a-thrombin, superoxide dismutase, nitric oxide, and interleukin-6.

Differences between healthy and inflamed dental pulps have been evaluated in several studies.[7,11,12] During tissue inflammation, oxidizing agents are released from host cells and these agents are able to break down bacterial components as well as normal components of the surrounding cells and matrices.[13,14] Conversely, host cells release many enzymes that can degrade these oxidizing agents to avoid excessive tissue destruction.[15]

One of them is catalase, which is an enzyme directly involved in active oxygen scavenging. Catalase breaks down $\text{H}_2\text{O}_2$ to yield oxygen and water. Moreover, catalase reduces $\text{H}_2\text{O}_2$ via oxidation of lower-molecular-weight alcohols. For these reasons, catalase is considered to be a defensive enzyme against the deleterious effects of hydrogen peroxide.
Bowles and Burns\textsuperscript{16} were the first to show the presence of catalase in healthy human dental pulp tissue. Limited investigations have evaluated catalase activity in healthy and inflamed human dental pulp tissue. The aim of this study was to investigate catalase activity in healthy and inflamed human dental pulps obtained from young and systemically human subjects.

**Material and Methods**

**Pulp tissue samples**

Each participant or their parents (if < 18 years old) signed a confirmation form acknowledging their voluntary and nonprejudicial participation in the study, and the protocol was approved by the Ethical Committee of the Atatürk University Dentistry Faculty, Erzurum, Turkey.

Twenty patients, 12 females and 8 males (age range: 15–25 years) with healthy systemic and periodontal states were the source of the pulp tissue specimens. Each participant or their parents (if < 18 years old) signed a confirmation form acknowledging their voluntary and nonprejudicial participation in the study, and the protocol was approved by the Ethical Committee of the Atatürk University Dentistry Faculty, Erzurum, Turkey. The patients included had to comply with the following criteria: (1) Good general health according to medical history, blood pressure, pulse rate, and clinical judgment and (2) no use of antibiotics or anti-inflammatory drugs within 3 months prior to the start of the study. They were then stratified into two matched groups, from which healthy pulp and inflamed pulps tissues were obtained. The healthy pulp group contained patients who presented to the Department of Orthodontics of the Faculty of Dentistry at the Atatürk University. They were scheduled for extraction of their first premolar teeth because of orthodontic treatment. The teeth of this group had no radiographic evidence of caries or periapical radiolucency, were unrestored, asymptomatic, and without pain on percussion. After extraction, the teeth were longitudinally grooved with a fissure bur and then split in half with cutting pliers to extirpate of pulp specimens.

The inflamed pulp samples were obtained from patients that had teeth with spontaneous pain and prolonged event of pain caused by thermal and electrical tests, with clinically and radiographically occlusal caries reaching the pulp chamber of the affected tooth to avoid tissue damage, care was taken in extirpating dental pulp samples from both the extracted and subsequently endodontically treated teeth. When the pulp specimens were removed from the experimental teeth, they were immediately placed in plastic vials and washed in ice-cold, heparinized, and sterile saline to remove blood. Then, samples were stored at 80°C until analyzed.

**Preparation of the crude enzyme extract**

Prior to biochemical analysis, the specimens were weighed and homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 7.0). This homogenate was centrifuged at 10,000 g for 15 min at 4°C, and the supernatant was recovered, and used for the enzymatic activity determinations.

**Determination of catalase activity and quantitative protein**

Catalase activity was determined spectrophotometrically by a two-step procedure.\textsuperscript{17,18} All determinations were performed in a single session. Quantitative protein determination was determined according to Bradford’s (1976) dye binding method with a ultraviolet-visible spectrophotometer at 595 nm.\textsuperscript{19} Catalase activity was converted to U mg\textsuperscript{-1}.

**Statistical analysis**

The Statistical Package for Social Sciences program (IBM SPSS Inc., Chicago, IL USA) was used to perform the data analysis. A Chi-squared test and a Mann–Whitney test were used to assess the equality of groups by sex and age, separately. An independent sample t-test assessed the significance of the differences in catalase activities between the experimental groups.

**Results and Discussion**

Differences among the groups were statistically significant at $P < 0.005$. Catalase activity was $2.1274 ± 1.7003$ U mg\textsuperscript{-1} (range $5.593–0.258$ U mg\textsuperscript{-1}) in the healthy pulp group, $8.3996 ± 3.6472$ U mg\textsuperscript{-1} (range $12.742–2.575$ U mg\textsuperscript{-1}) in the inflamed group. A statistically significant difference in catalase activity between the inflamed pulp and the healthy pulp groups ($P = 0.000$) is shown in Table 1.

This study examined the catalase activity of human inflamed dental pulp tissues compared with healthy dental pulps. The results demonstrate that a significant increase in catalase activity is seen in inflamed pulpitis tissue in comparison with healthy controls.

Clinical signs and symptoms in teeth with inflamed pulp are correlated poorly with the histological findings.\textsuperscript{20} Thus, quantitative methods for assessing the condition of dental pulps have been investigated. Many studies have evaluated the potential roles of enzymes and cell mediators

**Table 1: Catalase activities in the experimental groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase activity (U/mg)</th>
<th>(n)</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflamed pulp</td>
<td>8.3996 ± 3.6472*</td>
<td>10</td>
<td>12.742–2.575</td>
<td></td>
</tr>
<tr>
<td>Healthy pulp</td>
<td>2.1274 ± 1.7003</td>
<td>10</td>
<td>5.593–0.258</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from the healthy control group at \(P<0.005\). SD=Standard deviation.
released into pulp tissue.\textsuperscript{[7,15,21]} The inflammatory processes that occur in the pulp are extremely complex and consist of many elements. During inflammation from bacterial infection, macrophage activity is present in the pulp tissue, as well as in other tissues.\textsuperscript{[14]} Furthermore, such cells are able to release H\textsubscript{2}O\textsubscript{2} into the extracellular environment.\textsuperscript{[13,14]} H\textsubscript{2}O\textsubscript{2} is a powerful oxidizing agent and has been shown to be toxic to endogenous cells. In addition, host cells are able to produce specific enzymes (copper-zinc superoxide dismutase [Cu, Zn-SOD], catalase, glutathione peroxidase, and reductase) that have been demonstrated to have protective roles against these oxidizing agents.\textsuperscript{[15,22]} The presence of these enzymes in dental pulp tissue has been also shown in several studies.\textsuperscript{[16,21,23]}

Bowles and Burns\textsuperscript{[16]} were the first to show the presence of catalase in human healthy dental pulp. This study included only healthy tissue. Esposito et al.,\textsuperscript{[24]} found an increase in catalase activity in inflamed pulp tissue, compared with healthy tissue. They attributed these results to the protective roles against oxidizing agents of catalase enzyme. This result was harmonious with the results of the present study.

Davis et al.\textsuperscript{[23]} have described the correlation of Cu, Zn-SOD activities with the subject’s ages; therefore, in the present study only young and systemically healthy patients were included. Previously restored and caries teeth were also not included to avoid iatrogenic pulp tissue alterations, bacteria within caries lesion may induce pulp reaction and catalase activity. Further studies are needed to evaluate effect of caries lesion on enzymes activity in dental pulp.

Conclusions

Our study demonstrates a natural biological defense system against H\textsubscript{2}O\textsubscript{2} and a potential role for catalase during inflammation in human dental pulp.

Acknowledgment

This study was supported in part by an Ataturk University Research Fund (project no: 2012/69). The authors deny any conflicts of interest related to this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References