# Copper–zinc superoxide dismutase activity in healthy and inflamed human dental pulp

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## Abstract

**Varvara G, Traini T, Esposito P, Caputi S, Perinetti G.** Copper–zinc superoxide dismutase activity in healthy and inflamed human dental pulp. *International Endodontic Journal*, **38**, 195–199, 2005.

**Aim** To examine copper–zinc superoxide dismutase (Cu, Zn-SOD) activity in clinically healthy and symptomatic human dental pulps.

**Methodology** Twenty-five systemically healthy patients, 14 females and 11 males (age: 13.1–34.6 years; mean: 21.7 ± 6.3), were the source of the pulp tissue. The condition of the pulps was assessed using clinical and radiographic evaluation. The pulp tissue was collected by longitudinally grooving and splitting the teeth (if extracted) or during endodontic treatment, and were age- and sex-matched between the healthy and the irreversible symptomatic pulpitis tissue groups. Cu, Zn-SOD activity was determined through spectrophotometric methods and a Mann–Whitney test assessed the significance of differences between the groups.

**Results** The enzyme activities were 144.8 ± 42.2 and 68.1 ± 25.0 U $mg^{-1}$ in the healthy and irreversible symptomatic pulp tissue, respectively. The difference between the groups was statistically significant ($P < 0.001$).

**Conclusions** These results demonstrate a potential role for Cu, Zn-SOD during dental pulp inflammation in humans.

**Keywords:** dental pulp, inflammation, oxygen scavenging, superoxide dismutase.

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## Introduction

The vast majority of eukaryotic organisms require atmospheric oxygen in order to survive. Oxygen is the terminal electron acceptor in the respiratory chain of mitochondria during the production of ATP. However, mitochondrial respiration also results in the production of reactive oxygen intermediates (ROIs), which arise primarily within the matrix of the mitochondria (Chance *et al.* 1979, Boveris 1984). Eukaryotes have evolved specific defence mechanisms against these ROIs, including superoxide dismutase (SOD), glutathione peroxidase, catalase and a host of other enzymes, the function of which is to reduce the cumulative load of ROIs both within the cell and in the intercellular space (Halliwell 1999).

SODs are metalloenzymes that catalyse the dismutation of the superoxide ion into oxygen and hydrogen peroxide. Three distinct isoforms of SOD have been described (Andreadis *et al.* 2003): copper–zinc SOD (Cu, Zn-SOD), located in the cytosol; manganese SOD (Mn-SOD), located into the mitochondria; and an extracellular SOD (EC-SOD). However, most studies in the dental field have evaluated Cu, Zn-SOD activity selectively by means of cyanide ($CN^-$) in the reaction mixture (Marklund 1984). Moreover, in dental pulp, Cu, Zn-SOD has been shown to be present in fibroblasts and macrophages, as well as in the extracellular matrix (Davis *et al.* 1991).

The presence of Cu, Zn-SOD in animal dental pulp was initially described by Grossi *et al.* (1991). Subsequently, Davis *et al.* (1991) studied Cu, Zn-SOD activity

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in healthy human pulp and in irreversibly inflamed pulp tissue. However, their population study had a wide age range and included only seven subjects per group. Tulunoglu *et al.* (1988) evaluated the total SOD, rather than Cu, Zn-SOD, activity in human pulps diagnosed as being healthy, or as having reversible or symptomatic irreversible pulpitis, which were obtained from patients between 10 and 15 years of age. In the first of these studies, Cu, Zn-SOD activity in the pulp tissue was inversely correlated with subject age, and inflamed tissues had greater activity than healthy controls (Davis *et al.* 1991). In contrast, Tulunoglu *et al.* (1988) found no differences in total SOD activities between healthy and symptomatic irreversible pulpitis tissue, and a significantly lower activity was recorded in reversible compared with healthy and irreversible inflamed pulp tissue. However, this study neither reported a possible explanation of their results, nor evaluated possible correlations between enzymatic activity and patient age.

Considering these diverse results, further clarification is needed to determine whether significant changes in Cu, Zn-SOD activity occur in systemically healthy patients during pulp inflammation, and whether there is a correlation between Cu, Zn-SOD activity and subject age. The present investigation was, therefore, aimed at answering these questions.

## Materials and methods

### Subjects and pulp tissue samples

Informed consent was obtained from the patients and the parents of patients under 18 years of age, and the protocol was reviewed and approved by the Ethical Committee of the G. D'Annunzio University Medical Faculty.

Twenty-five patients, 14 females and 11 males (age: 13.1–34.6 years; mean: 21.7 ± 6.3 years), of healthy systemic and periodontal status were donors of the pulp tissue samples. The patients included had to comply with the following criteria: (i) good general health according to medical history, blood pressure, pulse rate and clinical judgement; and (ii) no use of antibiotics or anti-inflammatory drugs within 3 months prior to the start of the study. They were then stratified into three matched groups, from which healthy pulp and irreversible pulpitis tissues were obtained.

The control group contained patients presenting at the Unit of Orthodontics of the Department of Oral Sciences at the 'G. D'Annunzio' University who were scheduled for extraction of their first premolar teeth because of dental crowding. The teeth of this group were unrestored, asymptomatic and without pain on percussion, and had no radiographic evidence of caries or periapical radiolucency. Immediately after extraction, the teeth were longitudinally grooved on the buccal and lingual/palatal surfaces under copious water irrigation with a diamond disc (so as not to penetrate the canal), and then split in half with cutting pliers. Within this group, six premolars were mandibular, and six were maxillary.

The irreversible pulpitis group constituted the remaining patients, who arrived as emergencies at the Unit of Endodontics. The diagnostic criteria for this group were: (i) history of spontaneous pain; (ii) clinical and radiographic diagnosis of occlusal caries reaching the pulp chamber of the affected tooth; (iii) radiographically normal periapex or widened periodontal ligament with unbroken lamina dura; and (iv) prolonged episodes of pain caused by thermal and electrical tests. Previously restored teeth were excluded from the study. Within this experimental group, five teeth were mandibular molars, four were maxillary molars, three were mandibular premolars and one was a maxillary premolar.

For these teeth with irreversible pulpitis, the pulp chambers were carefully exposed using a high-speed drill, and the pulp tissue collected with a spoon excavator and endodontic reamers. When pulp specimens were removed from the experimental teeth, they were immediately placed in plastic vials and washed in ice-cold, heparinized, sterile saline to remove blood. Samples were then stored at −80 °C until analysed.

### Cu, Zn-SOD activity determination

The pulp samples were weighed immediately prior to biochemical analysis, and then homogenized in 1 mL of 10 mmol $L^{-1}$ potassium phosphate buffer, pH 7.0, with 0.1% sodium cholate. The homogenate was centrifuged at 100 000 ***g*** for 60 min at 4 °C, and the supernatant was recovered, diluted to a volume of 2 mL with phosphate buffer, and used for the enzyme activity determinations. One millilitre of supernatant was collected and immediately assayed for protein concentration using the Lowry method (Bio-Rad Protein Assay Kit; Bio-Rad, Hercules, CA, USA). SOD activity was determined as described by Sun & Zigman (1978). Briefly, the assay mixture contained 50 mmol $L^{-1}$ sodium carbonate buffer, pH 10.0, 0.1 mmol $L^{-1}$ epinephrine (Sigma, St Louis, MO, USA) and the tissue

 

fraction (containing up to 50 μg of protein), in a final volume of 2.5 mL. The Cu, Zn-SOD activity was determined by its ability to inhibit the autoxidation of epinephrine in an aqueous alkaline solution. Cu, Zn-SOD activity was assayed spectrophotometrically at 480 nm and at 25 °C. KCN (1.25 mmol $L^{-1}$) was used to separate the Cu, Zn-SOD activity from that of the other SODs. Percentage inhibition values were converted into activities using purified bovine Cu, Zn-SOD as standard. Results of Cu, Zn-SOD activity are expressed as U $mg^{-1}$ of pulp tissue.

### Statistical analysis

The Statistical Package for Social Sciences programme (SPSS® Inc., Chicago, IL, USA) was used for data analysis. Each data set was tested for the normality of the data by means of the Shapiro–Wilk's test and by Q–Q normality plots. Equality of variance was also tested by means of the Levene test and Q–Q normality plots of the residuals. Through this analysis, nonparametric methods were used in hypothesis testing. A chi-squared test and a Mann–Whitney test were used to assess the equality of groups by sex and age, respectively. The Mann–Whitney test also assessed the significance of the differences in Cu, Zn-SOD activities between the experimental groups. Moreover, within each experimental group, the strengths of the straight-line relationships between age and enzyme activity were tested by means of a Spearman correlation coefficient $\rho$. A $P$-value $<0.05$ was used for rejection of the null hypothesis.

## Results

The experimental groups were matched for both patient sex and age ($P > 0.1$; chi-squared and Mann–Whitney tests, respectively). The full results are reported in Table 1. The Cu, Zn-SOD activity in the symptomatic pulpitis group was significantly lower than that of the control group ($P < 0.001$, Mann–Whitney test). Finally, the Spearman correlation coefficient $\rho$ demonstrated a nonsignificant straight-line relationship between age and Cu, Zn-SOD activity in each group.

## Discussion

This study examined the Cu, Zn-SOD activity of human inflamed dental pulp tissues compared with healthy control pulps. The results demonstrate that a significant decrease in Cu, Zn-SOD activity is seen in irreversible pulpitis tissue in comparison with healthy controls. Finally, this enzyme activity did not correlate significantly with patient age in either group.

Over the last few decades, quantitative methods for assessing the condition of dental pulps have been investigated (Seltzer *et al.* 1963), and several studies have now evaluated the potential roles of numerous cell mediators and enzymes released into pulp tissue under different conditions (Tulunoglu *et al.* 1988, Davis *et al.* 1991, Bowles & Burns 1992, Barkhordar *et al.* 1999, Spoto *et al.* 2001). Recently, some studies have focused on the protective ROI-scavenging system (Roos *et al.* 1980, Baumgardner *et al.* 1999). This system consists of enzymes, such as Cu, Zn-SOD, catalase, glutathione peroxidase and reductase (Freeman & Crapo 1982), that have direct or indirect protective roles against oxidizing agents released by host cells during bacterial inflammation (Roos *et al.* 1980). Neutrophils are able to produce antioxidant enzymes (Roos *et al.* 1980), whereas macrophages have been shown to produce large amounts of multiple oxidizing agents in response to specific stimuli, such as bacterial toxins (Babior 1984, Forman & Torres 2001). These oxidizing agents are represented by the ROI radicals and $H_2O_2$ (Freeman & Crapo 1982). $O_2^-$ is one of the ROI radicals, and SOD converts it to oxygen and $H_2O_2$. The $H_2O_2$ is subsequently detoxified by the enzyme catalase and by the glutathione-dependent $H_2O_2$-detoxifying system, both of which reduce $H_2O_2$ to water (Weisiger & Fridovich 1973).

The presence of Cu, Zn-SOD in animal dental pulp was initially described by Grossi *et al.* (1991); subse-

**Table 1** Patient ages, Cu, Zn-SOD activities and their correlations in the experimental groups

| | | Age (years) | | Cu, Zn-SOD activity (U $mg^{-1}$) | | Correlations | |
|---|---|---|---|---|---|---|---|
| Group | *n* | Mean ± SD | Range | Mean ± SD | Range | $\rho$ | *P* |
| Healthy | 12 | 19.5 ± 4.5 | 13.1–28.2 | 144.8 ± 42.2 | 90–198 | − 0.01 | 0.99 |
| Irreversible pulpitis | 13 | 23.7 ± 7.1 | 14.3–34.6 | 68.1 ± 25.0* | 34–100 | 0.23 | 0.44 |

$\rho$, Spearman correlation coefficient.
*Significantly different from the healthy control group at $P < 0.001$.

quently, the presence of this enzyme in dental pulp tissue was also shown in humans (Tulunoglu *et al.* 1988, Davis *et al.* 1991). Thus, the detectable amount of Cu, Zn-SOD in healthy human dental pulp tissue found in the present study confirms these previous observations. Moreover, this activity was seen to decrease in irreversible pulpitis tissue. These results are in agreement with a previous catalase activity study (Esposito *et al.* 2003), which showed a similar behaviour in healthy pulp tissue and in reversible and irreversible pulpitis tissue.

During inflammation from bacterial infection, macrophage activity is present in the pulp tissue, as well as in other tissues (Forman & Torres 2001). Furthermore, such cells are able to release $H_2O_2$ into the extracellular environment (Babior 1984, Forman & Torres 2001). $H_2O_2$ is a powerful oxidizing agent and has been shown to be toxic to endogenous cells (Babior 1984, Forman & Torres 2001). This Cu, Zn-SOD activity increase seen by Davis *et al.* (1991) would thus demonstrate that an active defence system against $H_2O_2$ is present during inflammatory processes in dental pulp tissue (Roos *et al.* 1980, Esposito *et al.* 2003), as in other body tissues (Babior 1984, Forman & Torres 2001). However, the Cu, Zn-SOD activity was decreased in the symptomatic pulpitis tissues investigated in the present study. The lowering of this activity in irreversible pulpitis tissue could thus be attributed to the progression of inflammation that could result in a depletion or destruction of this enzyme, although the tooth pulp remains vital. Indeed, Spoto *et al.* (2001) demonstrated a significantly lower aspartate aminotransferase (AST) activity in irreversible pulpitis tissue, as compared with healthy pulp tissue, and these authors explained their results through an extensive destruction of AST due to inflammation. A similar result was found for catalase activity, where it is of interest that the reversible pulpitis tissue, which shows the ability to recover, had the highest catalase activity, as compared with the irreversible pulpitis tissue (Esposito *et al.* 2003). Hence, the difference in enzyme activities between the two tissues reported in the present investigation is supported by these studies. It is thus conceivable that the activity of the enzymes such as the Cu, Zn-SOD, and also catalase, is dependent upon the degree of progression of the pulpitis. In the present investigation, only pulpitis tissues with spontaneous pain, the last phase before pulp necrosis, were chosen. However, there is an apparent inconsistence of these data as compared with those of the Tulunoglu *et al.* (1988) (see Introduction). Conceivably, the different analytical procedures used may explain the different results; indeed, whilst in the present study Cu, Zn-SOD activity was determined, Tulunoglu *et al.* (1988) studied total SOD activity. This contrast would thus show that there may be different roles for each SOD isoform in dental pulp tissue during the inflammation process, a concept that remains to be fully elucidate in further studies.

A significant association between Cu, Zn-SOD activity and patient age was not found in either group in the present study. However, this appears not to be due to a limit in the statistical power, as the $\rho$ correlation coefficients are very low in both groups; therefore, an explanation for these results may derive from the age range of the patients included in the present study, which was of 13.1–34.6 years (overall value), which was more restricted than that considered in other studies (Davis *et al.* 1991). Hence, the lack of correlation between Cu, Zn-SOD activity changes in the dental pulp of both healthy and inflamed pulp tissues and patient age found here should be further investigated.

## Conclusion

This study was designed to investigate changes in Cu, Zn-SOD activity between clinically healthy and symptomatic human dental pulps in order to clarify the potential role of Cu, Zn-SOD during inflammation of pulp tissue in humans. The demonstration of a significantly lower activity in the symptomatic pulpitis tissue indicates a decreasing role for this enzyme during these final stages of pulp inflammation.

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