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EXPRESSION OF ENDOGENOUS ANTIOXIDANT IN DENTINE PULP COMPLEX AFTER HEMA RESIN APPLICATION

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ABSTRACT : Free radicals can react with polyunsaturated acid in cellular membrane in DNA and caused cellular damage. In order to prevent tissue damage, reactive oxide metabolite should be eliminated through and enzymatic antioxidant such as superoxide dismutase (SOD), catalase, thioredoxin and glutathione peroxidase. *Sprague Dawley* rats were divided into 4 groups. 1 control group and 3 experimental groups. The tooth cavity in the control group was filled with glass ionomer cement without HEMA liquid resin. Tooth cavities in experimental groups were treated with HEMA liquid resin and then filled with glass ionomer cement. The teeth were extracted after 24, 48, and 72 hours. Immunohistochemistry staining was applied to investigate the expression of SOD and catalase. The observation was done under a light microscope. Test data were analyzed using the ANOVA test and Tukey HSD. Significant differences between SOD and Catalase were observed in the control group and experimental groups. There was no significant difference between the experimental groups (24, 48 and 72 hours). There was increased production of Catalase and SOD in dentine pulp complex after being applied with resin HEMA.

Key words : Superoxide dismutase, catalase, immunohistochemistry, medicine, odontoblasts.

INTRODUCTION

Highly reactive molecules called free radicals can cause tissue damage by reacting with polyunsaturated fatty acids in cellular membranes, nucleotides in DNA and critical sulfhydryl bonds in proteins (Machlin and Bendich, 1987). In order to prevent tissue damage, the causative, which is a reactive oxidative metabolite, should be eliminated through an enzymatic antioxidant system such as superoxide dismutase (SOD), catalase, thioredoxin and glutathione peroxidase.

An antioxidant is a compound that gives out an electron. In other words, a compound that is able to muffle out the negative impact of oxidant. The exaggerated Reactive Oxygen Species (ROS) will be dissolved by primary endogenous antioxidant. The antioxidant system will keep the redox balance of the tissue, which will trigger pathogenic lesion cleansing and regulate the signaling of the important molecules to keep the balance of the physiology process (Sadi *et al*, 2014).

Resin monomer is a material in dentistry that is broadly used in composite resin, bonding and cement resin. Hydroxyethyl methacrylate (HEMA) is one of the material that is contained in dentin bonding. HEMA has a favorable characteristic for a restoration material such as having good bonding because it is hydrophilic and is not easily degraded, therefore, increasing the restoration survivability (Anusavice *et al*, 2003; Gerzina *et al*, 1996).

The usage of HEMA in dentistry needs intraoral polymerization that this material should have at least 30% unpolymerized monomer (Bakopoulou *et al*, 2009).

Some research mentioned that these monomers would release residual monomer that can potentially have a negative impact on the tooth and oral cavity (Goldberg *et al*, 2008; Hamid *et al*, 1998; Paranjpe *et al*, 2005). HEMA can lead to apoptosis by involving ROS in oxidative stress conditions (Schweikl *et al*, 2006).

The objective of this research was to analyze and prove should there be any increased expressions of SOD and catalase in dentine pulp complex as the result of the HEMA application.

MATERIALS AND METHODS

This was an experimental laboratory study with a randomized post-test only control design. This study was approved by the Health Research Ethical Clearance

Commission of Faculty of Dental Medicine, Universitas Airlangga (15 / KKEPK.FKG / IV / 2014). The sample is male Sprague Dawley rats, aged 24 weeks, weighed 300-350 grams, healthy, no abnormalities on general health nor teeth. Pure HEMA solution with 97% concentration was obtained from Sigma-Aldrich as much as 0.16 ml, dissolved in PBS 1000 ml. The final concentration of HEMA for the experiment was 0.016 µg/ml.

Sprague Dawley (SPD) rats were divided into 4 groups, 1 control group, and 3 experimental groups. The tooth cavity in the control group was filled with glass ionomer cement (Fuji IX LC) without HEMA liquid. Tooth cavities in experimental groups were treated with HEMA liquid then filled with glass ionomer cement.

The teeth were extracted after 24, 48, and 72 hours. The teeth were decalcified using EDTA for 8 weeks, and the paraffin blocks were made. After that, all the teeth were cut into 5 millimicron by a microtome. The sample was mounted on object glass with 5% gelatin and was deparaffinized.

The monoclonal antibody was SOD and catalase. Immunohistochemistry staining was applied to investigate the regulation of the antibody on the odontoblast cell in dentin pulp complex. The observation was done under a light microscope.

RESULTS

Expressions of SOD after HEMA application in treatment group 24, 48 and 72 hours and the control group are presented in Fig. 1. The mean cell that expressed SOD in experimental groups showed that there was an increase along with increased exposure time.

Data were analyzed with Analysis of Variance (ANOVA) and Tukey HSD. ANOVA test on groups shows a significant difference (p= 0.000). To know the differences between experimental groups, Tukey HSD test was done. Although there was an increase in SOD expression, there was no significant difference between experimental groups (24, 48 and 72 hours). Based on the result, it was known that the HEMA application effects SOD expression.

Expressions of catalase after being HEMA application in treatment group 24, 48 and 72 hours and the control group are presented in Fig. 3. The mean cell that expressed catalase in experimental groups showed that there as an increase along with increased exposure time.

ANOVA test on groups shows a significant difference (p= 0.000). To know the differences between experimental groups, Tukey HSD test was done. There was an increase in catalase expression as time goes by. However, there was no significant difference between experimental groups (24, 48 and 72 hours). Based on the result, it was known that the HEMA application affects catalase expression.

DISCUSSION

SOD and catalase are endogenous antioxidants acting as a defense mechanism from oxidative stress. These enzymes play an important role in the defense mechanism against the effect of toxic from oxygen metabolism. Free radicals, which are produced by SOD are formed as H_2O_2, which stays persistently inside the cell, and then by Haber Weiss reaction, it becomes hydroxyl radical that is overly reactive upon the cell and the tissue. The most frequently used dental restoration material contains acrylate and methacrylate monomer. Acrylate and methacrylate monomer are grouped in Esther material. Bouillaguet's study mentioned that methacrylate monomer is a cause of negative damage in the pulp dentinal complex. The causative agent of dental pulp necrosis is varied, but many studies confirmed that HEMA exposure is one of them (Gallorini *et al*, 2015).

The elevation of SOD and catalase indicates the

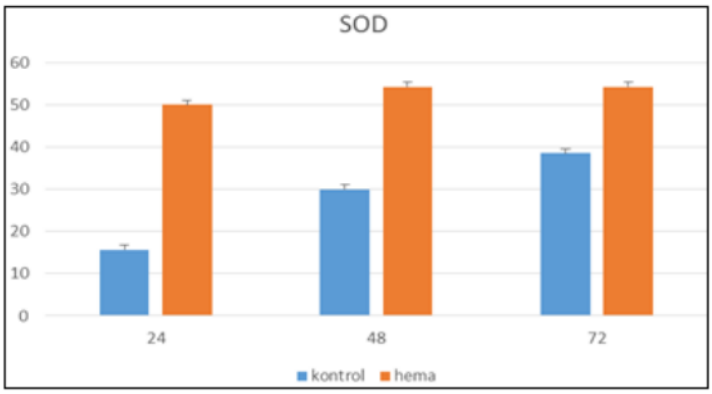

Fig. 1 : Mean graph of SOD expression in control and experimental groups.

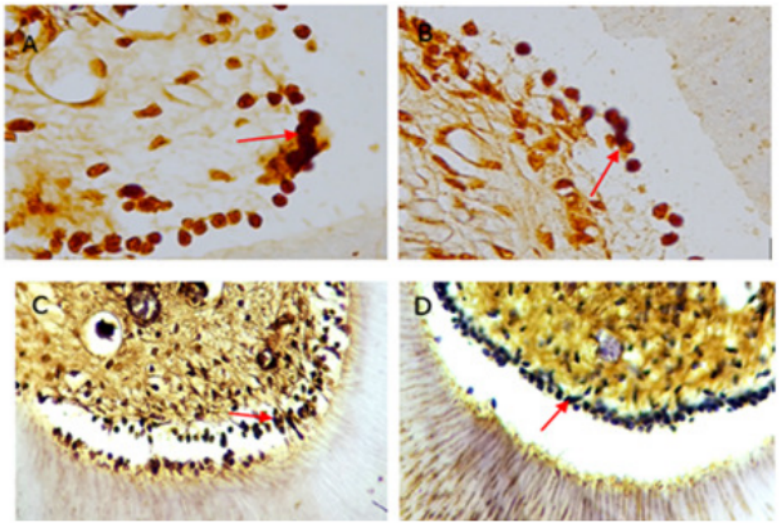

Fig. 2 : SOD expression in odontoblast cell with immunohistochemistry test. The red arrow shows cell that positively expressed SOD. Those positive cells are looked round, colored dark brown, and has brownish cytoplasm **A**. Control group. **B.** 24 hours experimental group. **C.** 48 hours experimental group. **D.** 72 hours experimental group.

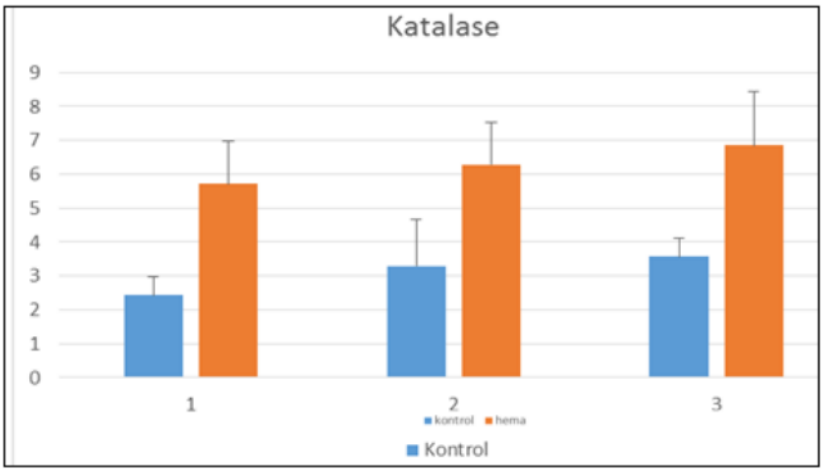

Fig. 3 : Mean graph of catalase expression in control and experimental groups.

activation of the defense mechanism of the body after the HEMA exposure. This process occurs through the regulation of reduction and oxidation reaction (redox) to decrease the accumulation of excessive ROS in the body (Krifka *et al*, 2010). ROS can exist in many forms, such as superoxide anions (O^{2-}), hydrogen peroxide (H_2O_2), and highly reactive hydroxyl radicals (HO). They are formed as a result of uncomplete oxygen molecules reduction (D'Autréaux & Benoît, 2007). Superoxide anions are the most reactive and unstable, among others (Krifka *et al*, 2010). H_2O_2 has important roles as a signaling molecule for its ability to react with Cys residues in a protein. The process can affect the regulation through a redox reaction that shows sensitivity toward protein and then affected by the high level of H_2O_2 as a result of oxidative stress conditions. Severe toxicity due to the existence of ROS is mainly originated from hydroxyl radical formation consequences, which are derived from via Fenton reaction (D'Autréaux and Benoît, 2007; Suryohusodo, 2000).

The elevation amount of hydroxyl radicals due to the HEMA exposure will enhance the endogenous antioxidant activity such as SOD and catalase to reduce the activity and component of antioxidants. The statistical analysis of this study shows an increasing number of cells that are expressing SOD and catalase in the experimental group after the HEMA application. The effect of HEMA application on pulp odontoblast cells in this study was

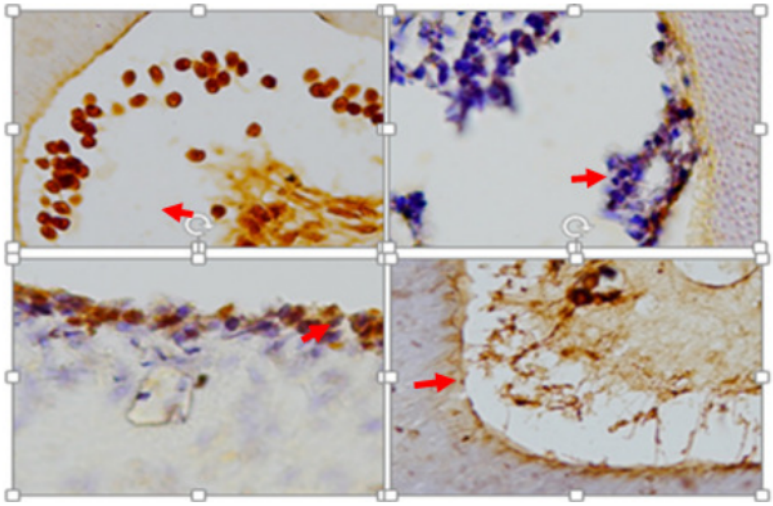

Fig. 4 : Catalase expression in odontoblast cell with immunohistochemistry test. The red arrow shows cell that positively expressed catalase. Those positive cells are looked round, colored dark brown, and has brownish cytoplasm. **A.** Control group. **B.** 24 hours experimental group. **C.** 48 hours experimental group. **D.** 72 hours experimental group.

evaluated through analysis by immunohistochemistry test.

In this study, the expression of SOD and catalase shows positive values in the three treatment groups based on time of 24, 48 and 72 hours. The increase in SOD was significantly expressed in each group as well as catalase ($p < 0.05$). Increased SOD and catalase expressions indicate the presence of endogenous antioxidant activity in compensating oxidative lesions from the HEMA resins application.

SOD expression was insignificantly increased between experimental groups because of the need to decompose the superoxide anion formed during the application period of the HEMA resin as well as the high H_2O_2 level that was formed for the compensatory mechanism. Superoxide anions are unstable; they make superoxide anions rapidly metabolized and become H_2O_2. Most H_2O_2 is metabolized and become H_2O by catalase, which is an enzymatic antioxidant (Marinho *et al*, 2014). H_2O_2 is more persistent in cells because, it is not too reactive, but if it is excessively produced, hydroxyl radicals (OH^-), which is reactive will be formed. Toxicity caused by H_2O_2 is likely a consequence of the formation of hydroxyl radicals from the Fenton reaction. In addition, hydroxyl radicals can be formed from the conversion of O^{2-} with H_2O_2 (Haber Weiss reaction) (D'Autréaux and Benoît, 2007). Hydroxyl radicals that are formed can cause changes in the DNA structure. If oxidative damage occurs to DNA continuously, the DNA mutations or cell death can occur (Schweikl *et al*, 2014).

ROS elevation and oxidative stress condition, as the result of the HEMA application, play an essential role in activating a process of inflammasome as the defense mechanism of odontoblast cell. The severity of the damage depends on the balance of ROS producing and muffling. If the amounts of free radicals are greater than anti-free radicals, the cell will be in an oxidative stress state. In this state, the cell will activate the intracellular enzyme and cause the disturbance of cell integrity. The elevation of antioxidant enzyme activity by the HEMA application to odontoblast cells was not adequate enough to compensate for the damage as the result of oxidative stress.

The impacts of enzyme activation are described as follows: cell membrane damage as the result of phospholipid degradation by phospholipase, cytoskeleton membrane damage by protease, DNA fragmentation by an endonuclease and at the end, cell apoptosis will occur (Sudiana, 2008; Suryohusodo, 2000). The elevating ROS as the result of the HEMA application will disturb the cell function, such as mineralization and differentiation, response upon innate immune, and lead to cell apoptosis (Krifka *et al*, 2010). According to Sudiana (2008), ROS in hydroxyl radical (OH^-) shape, can break down the DNA chain. This can cause changes in the nucleotide arrangement that leads to mutation and cell apoptosis. Apoptosis is programmed cell death without an inflammation process. Apoptosis occurs following the age as a homeostasis mechanism to keep the cell population and microenvironment. In the beginning, the apoptosis exists in physiologic condition (physiologic apoptosis), but in the next study, the apoptosis can be accelerated by an agent inside the cell in stress state (pathologic apoptosis) (Fink Susan *et al*, 2005).

CONCLUSION

There was increased production of Catalase and SOD in dentine pulp complex after HEMA resin application.

Conflict of interest

The authors declare no conflicts of interest.

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