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The expression of HMGB1 in Dentin Pulp Complex Induced by Resin Monomer HEMA

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ABSTRACT

High mobility group box protein 1 (HMGB1) is a nonhistone nuclear protein that can stimulate innate immunity and drive the pathogenesis of various inflammatory diseases and can be triggered by infections, tissue damage and metabolic imbalance. HMGB1 can exit cells during stimulation of the inflammasome during a process called pyroptosis that induced by non microbial particles such as asbestos and chemical agents such as resin monomer 2-hydroxyethtyl dimethacrylate (HEMA). This condition will drive innate immune response towards invading pathogens and cellular damage in dentin pulp complex. However, it is still unclear how resin monomer HEMA could trigger the expression of HMGB1 to activate innate immun response in the pulp. The objective of this research is to identify the molecular mechanism which activate HMGB1 in dentin pulp complex induced by resin monomer HEMA. We used nine healthy whistar rat teeth on in vivo experiments. Tooth cavity was applied with HEMA (Sigma Aldrich) in concentration of 0,016µg/ml, then covered with glass ionomer cements (Fuji IX LC, GC Japan) as cement. Teeth were extracted after 24, 48 and 72 hours. The teeth were decalsificated using EDTA for 8 weeks and the paraffin block were made after the teeth were cut 5 milimicron by microtome. Immunohistochemistry staining was applied and using antibody anti HMGB1 to investigate regulation these antibody on odontoblast pulp cells in dentin pulp complex. The sample were analyzed statistically by Anova and Tukey HSD. HEMA upregulated the expressions of HMGB1 in odontoblast cells of dentin pulp complex after 12,24 and 48 hours. HMGB1 induced innate immunity response in dentin pulp complex

INTRODUCTION

According to the survey in the United States in 2007, there are 290 million cases of dental filling treatments each year but 200 million of them failed. Filling using resin-based materials such as composites has become one of the clinical choices of clinicians in the field of dentistry due to the chemical and physical properties of both materials, although in several studies said that the toxicity of the material still remains high. One of the most widely used resin material is 2 hydroxyethyl methacrylate (HEMA). The reasons for using HEMA beside having good attachment strength and not easy to degrade so as to generate longevity of restorations (Annusavice 2003), be able to penetrate into the dentine and bind to collagen fibrils to form peptide bonds and produce strong attachment. Some studies demonstrated HEMA monomers will be able to release residual monomers that potentially cause negative effects on the teeth and oral environment. The use of resin can damage the pulp cells and disrupt the immune system. Several studies have reported the negative effects caused by the resin monomers. Schweikl (2014) mentioned that HEMA triggers apoptosis by involving reactive oxygen species (ROS). The pulp inflammation reaction is reported after filling cavity of resin-based composites and adhesives or when micro perforation occupied in the pulp (Bouillaguet, 2004).High mobility group 1 (HMGB1) is a complex protein that have been implicated in the pathogenesis of several inflammatory diseases, including pulp inflammation. When released by necrotic cells in the extracellular , HMGB1 serves as a molecular signaling damage, which has function as an alarming or related pattern of molecular damage (DAMP). DAMPs are self-molecules with the ability to activate inflammation via pattern recognition receptors (Sangiuliano,2014). HMGB1 is a typical DAMP and mediates the sterile inflammatory response in response injury to multiple tissues (Tsung, 2014). Several studies demonstrated that HMGB1 mediates severe damage-associated inflammatory responses. Extracellular HMGB1 upregulated inflammatory responses by directly activate on pattern recognition receptors, including NOD-like receptors (Yang, 2013). NLRs have widespread specificity in response to injury either caused by microbes or by non microbial agents such as resin monomer (Abbas, 2012). Previous studies shown NLRP3, member of NLRs, involved in ischemic injury by promoting the excite of HMGB1 (Chi *et al*, 2015). However, mechanism that induced the expression of HMGB1 in dentin pulp complex caused by resin monomer HEMA is still unclear. We hypothesized that expression of HMGB1 may be involved into the oxidative damage at the pulp.

MATERIAL AND METHODS

The research is a true experimental laboratory experimental. Sample unit is an adult Sprague Dawley rat, male, 24 weeks of age, weight between 300-350 grams, healthy condition and no abnormalities in the tooth and general condition were purchased from the Research Center at Laboratory of Biochemistry Medical School of Airlangga University. This studies using HEMA monomer resin concentrations of 0.016 µg / ml as an inducer in odontoblasts of dentin pulp complex. All mice were randomly distributed into 4 groups, ie 7 mice for the group. The mice anesthetized with a combination of ketamine HCl and diazepam (100mg: 10mg at a dose of 0.2cc per kg of intra muscular body weight). Class 1 cavity preparation was performed on the occlusal surface of the mandibular left first molars using a low speed round diamond bur with a diameter

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of 0.84mm. The depth of the preparation is about 1-1.5 mm. HEMA solution is applied to the cavity using fine microbrush. The cavity is filled by a glass ionomer cement after application of HEMA. At the time points (24, 48, 72 hours), mice were terminated, the alveolar and molar teeth were immersed in 10% formalin buffer solution for 24 hours. After 24 hours, the formalin buffer is replaced with ethylene-diamine tetra acetic acid (EDTA) of 10% and replaced daily for 60 days at room temperature (decalcified process). the paraffin block were made after the teeth were cut 5 milimicron by microtome. Immunohistochemistry staining was applied and using primary antibody anti HMGB1. The images were collected and magnify to 400x, then were analyzed with a light microscope. Statistical analysis was used to compare the differences expression of HMGB1 at difference point time (24, 48, 72 hours). For statistical analysis, the data were presented as mean ± SD. The significance level used is 5% which is then followed by the Multivariate Analysis of Variance (Manova) statistical test. If the variance of the research data was not different (homogeneous, p> 0.05) then the analysis could be continued using a multivariate Tukey HSD test.

RESULT

DAMPs triggered the release of HMGB1 in response to oxidative damage caused by the monomer resin HEMA . In our study, we tought to examine the pattern of HMGB1 in the development of dentinal injury caused by elevating free radical from residual monomer that release from HEMA. In dentin pulp injury models, the damage occurs after the using of HEMA solution at different point time. Immunohistochemistry staining showed there was increased trend as early as 24 h after induction of resin monomer. Additionally, damage of odontoblast pulp cells rapidly initiated the release of HMGB1 at 24 h after induction and peaked at 72 hours (Fig.4). Stimulation of monomer HEMA increased the severity of pulp injury. These results indicated the main role of HMGB1 in mediating oxidative damage in dentin especialy in odontoblast pulp cells which to be the first part contacted with external injuries.

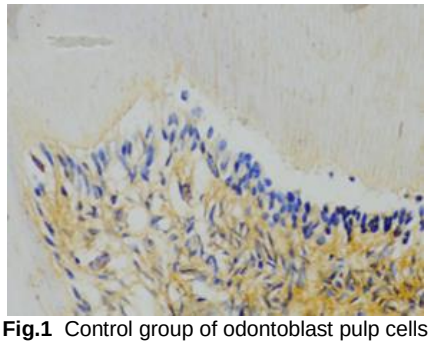

Fig.1 Control group of odontoblast pulp cells

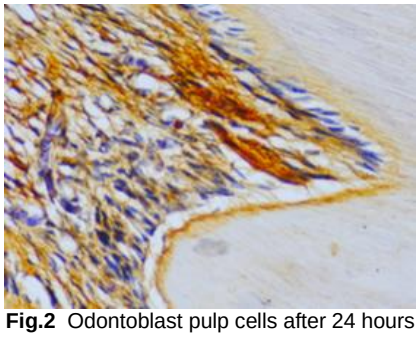

Fig.2 Odontoblast pulp cells after 24 hours

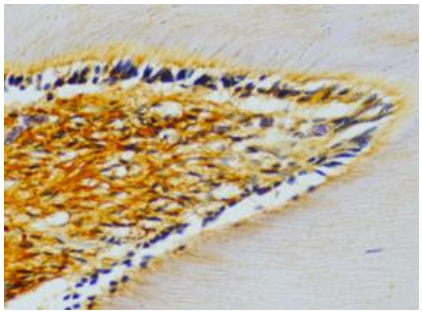

Fig.3 Odontoblast pulp cells after 48 hours application

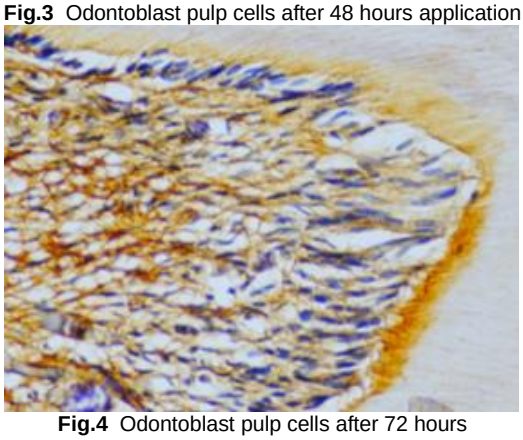

Fig.4 Odontoblast pulp cells after 72 hours

Table 1 The mean and standard cross-sectional expression of HMGB1 in the control group and HEMA administration in the three treatment time groups (24.48 and 72 hours)

HMGB1	N	ẋ	SD
Kontrol 1	7	2,4286	0,5345
HEMA 24	7	5,7143	1,2535
Kontrol 2	7	3,2857	1,3801
HEMA 48	7	6,2857	1,2535
Kontrol 3	7	3,5714	0,5345
HEMA 72	7	6,8571	1,5735

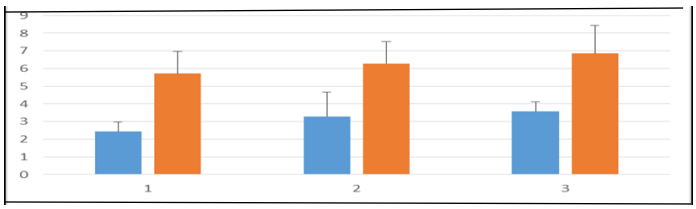

Fig.5 The mean graph and standard cross-sectional expression of HMGB1 in the control group and HEMA administration at the three points time groups (24.48 and 72 hours)

DISCUSSION

Sensitivity and inflammation in pulp cells commonly find after filling cavity of the tooth. If not treated promptly it will continue to be a process of chronic inflammation and towards the process of cell death. We previously demonstrated (data not shown) that NLRP3 inflammasome involves in the inflammatory injury of dentinal pulp complex especially at odontoblast pulp cells which are the first defense mechanism of the tooth. The object of this study is to explore the expression of HMGB1 odontoblast pulp cells after induced by resin HEMA. Chen (2011) showed that NLRP3 had responsibility for the invasion of pathogens that enter the body's tissue cells by recognizing and binding to the essential molecules of PAMP and DAMP located in the cytoplasm. Danger signals captured in this process are derived from resin HEMA. Yu et all (2015) demonstrated that oxidative stress is common likely mechanism of HMGB1's release and activity in inflammation and cell death. Means that expression of HMGB1 proved the activation of an immune innate

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response to the invasion of pathogen injury and cell damage. In pathological conditions, where excessive free radical production occurs due to chemicals exposure, so that can not be muted by cellular antioxidants will occur oxidative stress (Sathyasaikumar et al., 2007). Oxidative damage due to HEMA exposure induced increased ROS. We demonstrated in previous studies that application of resin HEMA at dentin pulp complex triggered the increased of free radical such as hydroxyl radical (OH) which lead to upregulated ROS and generate oxidative stress. Oxidative stress is harmful to the body because it can damage the macromolecules of DNA, fat and protein in the cell causing disruption of cellular sinyaling resulting in cell death (Cotran, 1999). If there is an excessive accumulation of ROS resulting in oxidative stress, antioxidants can not develop a defense mechanism against oxidative damage, it will occur cell death. Our study showed that HMGB1 increased in odontoblast pulp cells as early as 24 h after application of HEMA (Figure 2) and peaked at 72 hour (Figure.4). This research suggests that HMGB1 release from the odontoblast pulp cells and signaling through the pattern recognition receptor. NLRP3 might be a key mechanism of the immune response after pulp injury that has profound consequences on clinical results.

CONCLUSION :

Oxidative stress is a central regulator of HMGB1's release and activity in inflammation that could lead to cell death.These results indicated that HMGB1 contributed to the inflammation of the pulp through oxidative damage caused by resin HEMA and induced immunity at dentin pulp complex.

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