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ORIGINAL PAPER

Lactobacillus Acidophillus Stimulates The Activation of Nfκ-B and DMP-1 Odontoblast like Cells in The Dentinogenesis Process

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ABSTRACT

Lactobacillus acidophilus obtained from dentin caries and produces organic acids resulting in decalcification of dentin. On the process of dentinogenesis is active but will decrease in secondary dentinogenesis. Invasion of the bacteria causing the onset of the immune response by forming odontoblast dentin reparative, indicated by an increased expression of DMP-1, but the mechanism of the formation of dentin is still unclear. The purpose of this study is to show the odontoblast cell like exposure to Lactobacillus acidophilus expression against NFκβ and DMP 1. Pulp tissue is taken from the third molar of teeth that have been extracted then exposed to Lactobacillus acidophilus and expression of NFκβ and DMP 1 measured using imunocytochemistry techniques. Results show an increased NFκβ expression and decrease DMP 1 expression in the odontoblast like cell in exposure Lactobacillus acidophilus. In conclusion, Lactobacillus acidophilus increases the expression of NFκβ and inhibits the expression of DMP 1 on odontoblast like cell.

INTRODUCTION

Caries is a dynamic process which involves microorganism and host responses in the form of inflammation and immunity. Research that uses pulpitis sample has shown that bacteria antigen or bacteria metabolic products can spread through dentine tubulus which then generates the immune response from dental pulp. Immune complex and products from immunity response are nonspecific (Innated immunity), such as an extracellular proteolytic enzyme that is begun with phagocytosis (Bergenholtz, 2000). Dental caries is a disease on the hard tissue of the teeth, bacteria that started the occurrence of caries is streptococcus mutants while when already reached the dentine the most found bacteria is Lactobacillus acidophilus (Nadkarni et al., 2010; Martin et al., 2002).

Lactobacillus acidophilus is a positive gram bacteria that has Lipoteichoic acid (LTA) which contains peptidoglycan layer on cytoplasm membrane as the main component of its cell membrane. LTA causes secretion from proinflammatory mediator and will stimulate the activation of odontoblast, induce the inflammation reaction through Toll like Receptor 2 (TLR2) by producing some chemokines (Durand, 2006). LTA bound with TLR2, activated NF-kB, causes the release of NF-kB into the nucleus that will activate proinflammatory mediator producing cytokine IL1, IL6, IL8 dan TNF-α (Hahn dan Liewehr, 2007; Bratawidjaya & Rengganis, 2009).
Odontoblast is a postmitotic cell and is responsible for the formation of dentine matrix calcification. If injury is formed then the interphase part of dentine-pulp which contains mesenchymal stem cell will undergo cytodifferentiate into the odontoblast cell (Couve & Osorio 2013).Dentine Matrix Protein 1 (DMP1) is the noncollagenous extracellular matrix protein involved in mineralization process. This research examines the expression of DMP1 and NFκβ odontoblast-like cells exposed to Lactobacillus acidophilus in the process of the development of dentin reparative.

MATERIALS AND METHOD

Before this research is done, every procedure is proposed to Faculty of Dental Medicine Universitas Airlangga Ethic Commission.

Inactive Lactobacillus acidophilus

Before Lactobacillus acidophilus bacteria is being exposed to odontoblast culture, first the making of inactive Lactobacillus acidophilus by heating was done. Inactive Lactobacillus acidophilus was done by heating in 121˚C for 5 minutes. The dosage of bacteria exposure was done with the ratio of cell: 1: 25 and was incubated for 24 hours on 37˚CTo induce odontoblast like cells (Widjiastuti et al., 2014).

Odontoblast like cells Culture

Pulp cell culture is derived from impacted and extracted lower jaw third molar. The surface of the teeth was segmented with chlorhexidine gel 0, 3%, cleaned with 70% (v/v) alcohol. The roof of the pulp was prepared using fissure drill in the occlusal and bifurcation area so that the pulp cavity is opened. Pulp tissue, extirpated, isolated, and cultivated using digestion method. Pulp fibroblast cell was differentiated into odontoblast like cells. The differentiation was done using supplementation with 10 nM dexamethasone, 50 µg/ml ascorbic-acid and 10 mM glycerophosphate or with the addition of BMP-2 (100-200 ng/ml)
on proliferation medium (DMEM + 10% FBS + penicillin/streptomycin). After differentiation, then odontoblast-like phenotype characterization was done. Dentine matrix formation in differentiation process to be odontoblast will secrete specific matrix, such as dentine matrix protein 1 (DMP-1). Recognition of DMP1 was done using immunohystochemical technique, with anti DMP1 (SantaCruz), accordant to the guidance procedures using the manual from Immunostaining Kit assay (Biocare).

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RESULTS AND DISCUSSION

The expression of NF-κβ can be seen through immunocystochemical test. The test result is shown in figure 1.

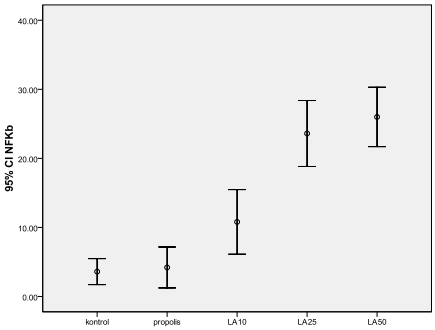

Fig 1. Average of cells expressing NF-κβ in odontoblast culture induced with inactive *Lactobacillus acidophilus*

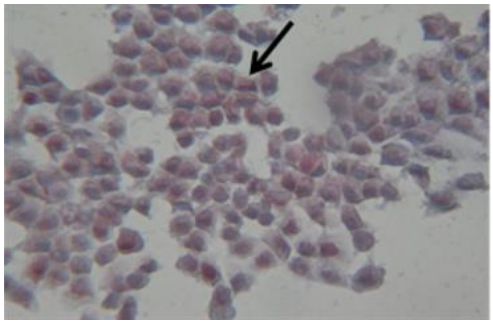

Fig 2. Odontoblast culture (AEC staining, 400x magnification), the black arrow shows cell that expresses NFkB is distributed in the cytoplasm (red color)

The expression of *DMP1* can be seen through immunocytochemical test. The test result data is shown in figure 3 and 4

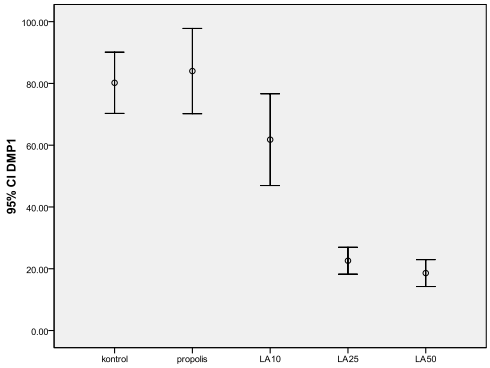

Fig 3. The average of cells expressing DMP1 in odontoblast culture induced with inactive *Lactobacillus acidophilus*

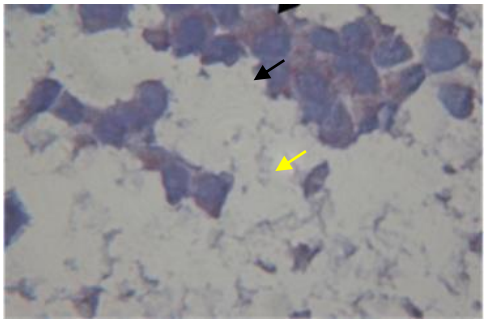

Fig 4. Odontoblast culture (AEC staining, 400x magnification), the black arrow shows cell that expresses DMP1 is distributed in the cytoplasm (red colour) and yellow arrow shows a broken cell

The data were examined using ANOVA with p=0.05 degree of significance and continued with Tukey HSD test

In vitro Odontoblasts are the main cells that form the peripheral layers of induced pulp tissue to express both cytokines and chemokines with a typical cellular morphology (Veerayutthwilai *et al*., 2007). In this experimentation to make the culture of odontoblasts as well as fibroblasts of pulp tissue obtained from the lower 3 molar tooth of the extracted impaction from patients aged 14-29 years (Alliot *et al*., 2001).

Based on this election is that during the process of dental growth requires only the minimum number of odontoblast progenitor cells undergoing mitosis before differentiating to form odontoblasts. In the process of mitosis produces 2 cells daugther: located adjacent to the basement membrane function to receive the signal so that induces the deference to odontoblast contribute to the layer of Hohl, while the other daughter cells are progenitor cells that have function as cells that will replace odontoblast if damaged in the healing process at Formation of reparative dentine and dentin bridge (Fitzgerald, 1979).

This study used odontoblast like cells cultures exposed to inactive Lactobacillus acidophilus bacteria to induce the expression of proinflammatory cytokines, and those examined were expression of DMP1 and NF-κβ. DMP1 is one of the proteins that function in regulating the formation of odontoblast like cells and is demonstrated by the increase and distribution of positive cells expressing DMP1 (David *et al.*, 2004).

The study was conducted to find out the distribution of cells expressing NF-κβ and DMP1 due to exposure of inactivated Lactobacillus acidophilus through immunocytochemical examination. In the in vitro study using pulp cell cultures, the identification of DMP1 showed that mesenchymal distinction of cells into odontoblasts would begin the process in which organic subtances are converted in organic substances. DMP1 induces cytodifferentiation of cells in the dental pulp tissue into odontoblast like cells in normal and pathological teeth. In the dentinogenesis process, it shows that DMP1 acts as a morphogen with the ability to reform dentine material to form dentin reparative (Almushayt *et al*., 2006). DMP1 has a dual function, both as a target transcription factor of the cell nucleus and as an extracellular matrix protein initiating process in which organic substances are converted into organic substances (Narayanan *et al*., 2003; Narayanan *et al*., 2006).

NF-κβ enters the cell nucleus and initiates the transcription of various target genes. Activation of NF-κβ proteins can secrete various cytokines including proinflammatory cytokines causing cell damage (Lee *et al*., 2004) which cause damage from odontoblast like cells. In the preliminary study (Widjiastuti *et al*, 2014) using the dose of exposure to Lactobacillus acidophilus inactives was 1:25, because exposure to the dose was slightly damaged cells, characterized by a decrease in the number of positive cells expressing DPM 1 smaller than the dose of Lactobacillus acidophilus inactivity 1:50. At that dose causes activated NF-κβ which is characterized by an increase in the number of cells expressing NFκβ. This indicates that at dose of Lactobacillus acidophilus inactive of 1:25 is the effective dose, the smallest dose that can activate NF-κβ but small cell damage so that in

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this research used dose of Lactobacillus acidophilus inactive 1:25 to induce odontoblast. This suggests that by induction of inactivated Lactobacillus acidophilus in odontoblast cell cells proved to increase NF-κβ activation and decrease the expression of DMP-1

CONCLUSION

Lactobacillus acidophilus increase the expression of NFκβ and inhibits the expression of DMP 1 on odontoblast like cell culture

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