Odontoblast layer structure alteration as a response to carious lesions

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ABSTRACT

Background: Dental caries is a bacterial disease affecting the hard tissue of the teeth as well as the pulp. The human dental pulp consists of odontoblast which are organized as a densely packed cell layer. Odontoblasts is located at the periphery of the pulp; therefore, they are the first cells encountered by cariogenic bacteria and their products that are represented in the carious lesion.

Purpose: This study aimed to elucidate the effect of cariogenic bacteria to odontoblasts of human teeth.

Methods: Five intact third molars and 15 third molars with occlusal caries at various stages of decay were extracted because of orthodontic or therapeutic reasons. The tooth specimens were fixed, decalcified with 10% EDTA solution (pH 7.4), and embedded in paraffin. Serial sections of 5 μm thickness were cut and stained with haematoxylin eosin and Gram’s, in addition to nestin immunohistochemistry. The specimens were then examined under light microscopy.

Results: In normal teeth, odontoblast layer were aligned along the pulp chamber showing normal morphology of the cells. Slight disorganization of odontoblast layer was seen in the cases of carious lesions confined to enamel. In the cases of carious lesions confined to dentin, odontoblast layer was not observed in the areas subjacent to the lesions, only single cells showing flattened cell morphology were found. Odontoblasts beneath the lesion suffered severe damage and diminished nestin immunoreaction were observed in all cases of carious lesions with pulp exposure.

Conclusion: Cariogenic bacteria invasion may damage the odontoblasts by affecting the morphology and vitality of the cells. The severity of the damage of the odontoblasts may increase as the bacterial invasion progresses toward the pulp.

Key words: Dental pulp, odontoblast, carious lesion

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ABSTRAK

Latar belakang: Karies merupakan penyakit yang disebabkan oleh bakteri, yang dapat memengaruhi jaringan keras gigi maupun pulpa. Pada pulpa gigi manusia terdapat sel odontoblas yang tersusun atas lapisan sel. Odontoblas terletak pada tepi kamar pulpa, sehingga sel ini merupakan sel yang pertama kali bertemu dengan bakteri kariogenik dan produk-produknya yang terdapat dalam lesi karies.

Tujuan: Penelitian ini bertujuan untuk mengetahui pengaruh invasi bakteri kariogenik terhadap sel odontoblas gigi manusia.

Metode: Lima buah gigi molar ketiga utuh dan 15 gigi molar ketiga yang mengalami karies pada permukaan atas lapisan dentin. Gigi-gigi tersebut kemudian dipotong dengan ketebalan 5 μm dan ditempatkan dalam parafin. Spesimen gigi tersebut kemudian dianalisis menggunakan mikroskop cahaya.


Kesimpulan: Invasi bakteri kariogenik dapat menyebabkan kerusakan pada sel odontoblas dengan menyebabkan perubahan morfologi dan memengaruhi vitalitas sel. Kerusakan ini akan terus berlanjut dengan semakin dalam invasi bakteri ke arah pulpa.

Kata kunci: Pulpa gigi, odontoblast, lesi karies

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INTRODUCTION

Dental caries, the most common chronic infection in humans, is a classic biofilm disease that develops when changes in the oral environment enhance the growth of cariogenic bacteria. Microbiological assessment of caries-active sites and studies with experimental animals implicated that carious lesions may be caused by a range of bacteria, but the principal among the cariogenic flora are Gram-positive bacteria such as Streptococcus, Lactobacillus, and Actinomyces spp. Previous study showed that Gram-positive bacteria are frequently found in dentinal tubules of teeth with carious lesions and in teeth with irreversible pulpitis. The microflora changes its composition as the carious infection progresses to the pulp-dentin interface. It characterized by a decrease of the proportion of Gram-positive aerobic bacteria and an increase of Gram-negative anaerobic ones mainly Fusobacterium, Prevotella and Tannerella spp.

Cariogenic bacteria are highly efficient at converting carbohydrates to the organic acids that able to demineralize tooth enamel. This process may result in access for bacteria to the pulp tissue through the dentinal tubules and subsequently induce pulp inflammation. Odontoblasts are cells which is located at the periphery of the pulp chamber and organized as a densely packed cell layer. Due to their peripheral situation, odontoblasts are the first cells encountered by cariogenic bacteria that are represented in the carious dentin. Odontoblasts become exposed to the bacteria as the bacteria and their products progressively demineralize enamel and dentin and enter the disrupted tissues to gain access to the pulp.

Odontoblasts are responsible for both development and reparative formation of dentin. Odontoblasts localized beneath the damaged region can up-regulate their dentin secretory activity during mild tissue injury. Injury of greater intensity causes localized odontoblasts necrosis which are subsequently replaced by an odontoblast-like cell population.

Whilst the bacterial aetiology of caries is well established, there is limited understanding of the dynamic nature of the tissue changes within the dentin-pulp complex in response to lesions varying in their rate of progression. The purpose of the present study was to elucidate the effect of cariogenic bacteria to odontoblasts of human teeth.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the ethical committee of Medical Faculty of Universitas Gadjah Mada. Twenty volunteers ranging in age from 20 to 40 years who had been scheduled to undergo extraction for various therapeutic reasons, were enrolled in the study. Informed consent was obtained from all subjects after the proposed study was fully explained. Lesion depth assessments were performed clinically using explorer and confirmed using micro-computed tomography. Five intact third molars and 15 third molars with carious lesion involving enamel (n = 5), dentin (n = 5), and pulp (n = 5) were extracted. The teeth were fixed in 10% neutral buffered formalin solution, and demineralized using 10% ethylene diaminetetraacetic acid disodium salt (EDTA) solution (pH 7.4). The specimens were then embedded in paraffin and serially sectioned at 5 μm thickness. All sections coming through the cavity floor or pulp exposure site were stained with hematoxylin-eosin and Gram’s for identification of the bacteria, in addition to nestin immunohistochemistry, a specific marker for the odontoblast.

Nestin immunohistochemistry procedure were done by processed the sections with avidin-biotin-peroxidase complex (ABC) method, using polyclonal antibody to nestin (Chemikon International, Temecula, USA). Inhibition of endogenous peroxidase was done by treated the sections with 0.3% H2O2 in absolute methanol for 30 minutes. Any non-specific immunoreaction was inhibited by preincubation in 2.5% normal goat serum (Vector Laboratories Inc, CA, USA). After incubation with the primary antibodies, the sections were reacted consecutively with biotinylated anti-rabbit IgG and ABC (Vector Laboratories Inc, CA, USA). The sites of antigen-antibody reactions were visualized using 3-3’-diaminobenzidine tetrachloride in Tris buffer and 0.002% H2O2 and counterstained with 0.05% methylene blue. Immunohistochemical controls were performed by omitting the primary antibody, the biotinylated anti-rabbit IgG, or the ABC complex. These immunostained sections showed no specific immunoreaction. Sections of all 15 teeth were examined under light microscopy and evaluated for the quality of the odontoblast layer below the lesion.

RESULTS

In normal teeth, no bacteria was observed in all specimens. Odontoblast layer were seen aligned along the pulp chamber, showing normal cell morphology. Their cells appeared palisade tall columnar cells and a nucleus located in a basal position, adjacent to the predentin. Cell bodies and processes of the odontoblasts revealed an intense nestin positive-immunoreaction indicating that odontoblasts were vital. Cell-free zone located immediately subjacent to the odontoblast layer was observed (Figure 1).

In the cases of carious lesions confined to enamel, based on the clinical assessment and micro-computed tomography observation, the lesion already affected the enamel; however, no bacteria was stained in all samples. Although slightly disorganized, odontoblast layer was maintained its continuity and observed aligned along the pulp chamber in all specimens (Figure 2A). Some vacuolizations were observed in the odontoblastic layer subjacent to the lesion. The odontoblasts showed an intense...
nestin-positive immunoreaction indicating that odontoblasts were vital (Figure 2B).

Cell-free zone could not be identified in the area localized to the affected dentinal tubules in all specimens. A slight increased number of cells in the location of cell-free zone was observed. These cells had a morphology corresponding to that of fibroblasts and undifferentiated cells in the rest of the pulpal tissue. A few inflammatory cells were also found in this area.

In the cases of carious lesions confined to dentin, stained bacteria along the dentinal tubules was observed in all specimens. All specimens showed that odontoblast layer lost their continuity and could not be identified in the areas subjacent to the lesions, only some individual odontoblast cells were sparsely observed (Figure 3A). The cells had flattened cell morphology and showing nestin-positive immunoreaction. However, odontoblast layer was recognized in other areas of the roof of the pulp chamber showing an intense nestin-positive immunoreaction (Figure 3B). The odontoblast layer was aligned along approximately 30% (3 cases) and 50% (2 cases) of the roof of the pulp chamber. No predentin was observed in the area corresponding to the lesions.

Inflammatory cells infiltration was found in the area adjacent to the lesions. Polymorphonuclear (PMN) cells were infiltrated the odontoblast layer, occasionally they were observed close to the predentin. Nestin-positive filamentous structures were also shown surrounding the inflammatory cells infiltration.

In the cases of carious lesions with pulp exposure, all samples showed stained bacteria penetrated the pulp chamber. Odontoblasts beneath the lesion suffered severe damage. Odontoblasts layer could not be identified in their pseudostratified appearance at roof of the pulp chamber in all samples. Some individual nestin-immunopositive odontoblast cells showing flattened cells morphology were sparsely observed. Nestin-immunopositive odontoblast layer was found at the area at a distance from the lesions sites.
Severe inflammation characterized by intense infiltrations of PMN and mononuclear cells was observed in the pulp chamber, particularly in the area corresponding to the lesions. Nestin expression was found up-regulated in the dental pulp cells surrounding the inflammatory cells infiltration.

**DISCUSSION**

Dental caries is an infectious and transmittable disease which is caused by bacteria. This study showed that invasion of cariogenic bacteria in dentinal tubules may damage the odontoblasts by affecting the morphology and vitality of the cells.

Odontoblasts, the most highly specialized post-mitotic cells of the pulp, is located at the periphery of the pulp. Due to their peripheral situation, they are the first cells encountered by the cariogenic bacteria. Odontoblasts become exposed to cariogenic oral bacteria as the bacteria progressively demineralize enamel and dentin and enter the disrupted tissues to gain access to the pulp.

In the cases of carious lesions confined to enamel, no bacteria were observed in all specimens. Enamel was dissolved due to the decalcification process. Therefore, it was proven that the bacteria had not invade the dentinal tubule yet. All specimens showed odontoblast layer aligned along the pulp showing nestin-positive immunoreaction, indicating that odontoblast cells were vital thus, they were capable of elaborating reactionary dentin. However, there were some extracellular vacuolization in the odontoblast layer subjacent to the lesion indicating early damage of the odontoblast cells due to the penetration of soluble substance of bacterial origin to the dentinal tubule. During bacterial multiplication, various product such as bacterial enzymes, metabolic products, and other extracellular substances are released. Components of the bacterial cell structure may also be liberated after lysis and disintegration of the bacterial cells. Previous study has been demonstrated that a bacterial endotoxin was able to diffuse through dentinal tubules to the pulp chamber.

In the cases of carious lesions confined to dentin, all specimens showed that bacteria already penetrate the dentinal tubules. All samples showed disorganization of the odontoblast layer at the roof of the pulp chamber. The odontoblast layer lost its continuity particularly at the area subjacent to the lesions, however, some individual odontoblast cells were identified showing nestin-positive immunoreaction. Nestin was expressed in the processes of odontoblasts surrounding the carious lesion suggesting a role for nestin in the elaboration of the reactionary dentin.

Cariogenic bacteria and their products have ability to demineralize enamel and dentin, and penetrate the dentinal tubule. As bacteria invade enamel and enter the dentin, changes commence in the pulp. Carious lesion provide constraints on the free diffusion of ions and small molecules, therefore, the rate of ingress of microbial metabolic products might bear a linear relationship to disease progression. The diffusion kinetics of both these microbial products and the degradation products arising from their action will influence disease progression.

No odontoblast layer was observed in the cases of carious lesion with pulp exposure, only few single cells were observed revealed nestin positive-immunoreaction. Previous studies showed that nestin could be used for monitoring the degeneration and regeneration processes of damaged odontoblasts under pathological conditions in animal experimental models using mice and rats. Nestin contributes to the signaling cascade, resulting in odontoblast-like cell differentiation. Continuous bacterial invasion of dentinal tubules overcomes the pulp-dentin complex resulting in the infection of the pulp and may cause the death of odontoblasts. In addition, nestin expression was found up-regulated in the dental pulp cells surrounding the inflammatory cells infiltration in this case. Expression of
this molecule at the injured pulp may help to coordinate cell fate decisions as well as proliferative, migratory, and differentiation activities.\textsuperscript{15}

Various degree of inflammation were observed in all caries cases. It seemed that the severity of the inflammation was advanced as the caries progressed toward the pulp. Previous study showed that odontoblasts exposed to bacteria and their by-products expressed interleukin 8 mRNA and protein. Interleukin 8 is a potent chemotactic factor for neutrophil, which is the predominant inflammatory effector cell observed in those cases. Neutrophilic degranulation liberates lysosomal enzymes that digest host as well as bacterial cells.\textsuperscript{16}

No predentin was observed beneath the lesions in the cases of carious lesion confined to the dentin and with pulp exposure. Odontoblasts are responsible for both the developmental and reparative formation of dentin. They produce the components of the organic matrix of predentin and dentin, including proteoglycans, collagens, and noncollagenous proteins as well.\textsuperscript{17} Therefore, disorganization or death of the odontoblasts may affect their function in producing predentin.

In conclusion, cariogenic bacteria invasion may damage the odontoblasts by affecting the morphology and vitality of the cells. The severity of the damage of the odontoblasts may increase as the bacterial invasion progresses toward the pulp.

REFERENCES