

Research Report

Crude toxin of *Aggregatibacter actinomycetemcomitans* serotype-B increase PARP-1 expression in gingival epithelium

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ABSTRACT

Background: *Aggregatibacter actinomycetemcomitans* (A. *actinomycetemcomitans*) serotype-B has long been associated with aggressive periodontitis. Gingival epithelial cell is exquisitely sensitive to the toxin so that may lead to disruption of the epithelial protective barrier, facilitating invasion and perturbation of the underlying connective tissue. Currently suggested that Aa serotype-B produce protein toxin that caused DNA strand breaks. PARP-1 is an abundant nuclear protein functioning as a DNA nick-sensor enzyme. PARP-1 was one of the first identified substrates of caspases, the main executioners of apoptosis. Therefore, a role for PARP-1 in the regulation of apoptosis has been suggested. **Purpose:** The purpose of this study was to prove PARP-1 expression in gingival epithelium caused by toxin exposure of A. *actinomycetemcomitans* serotype-B. **Methods:** This is an experimental study involving twenty adult mice strain Swiss Webster (balb C) divided randomly into two groups: control group (Group A) and toxin group (Group B). Both group were acclimated for one week before treatment. Group A was applied topically with sterile distilled water every 12 hours. Group B was applied topically by 100µg/ml of crude toxin A. *actinomycetemcomitans* serotype B at the buccal area of mandibular anterior teeth using Hamilton syringe. The mice were sacrificed at 24 hours after toxin application, and then the tissue sections of gingival epithelium were stained with immunohistochemistry to reveal the PARP-1 expression. The data were analyzed with t-test. **Results:** The PARP-1 expression exhibited an increase with the toxin group (mean= 48.9; SD= 2.01) compared with the control group (mean= 25.21; SD= 1.72). DNA fragmentation appeared from the agarose gel examination, marked as DNA laddering, indicate the cell apoptosis. **Conclusion:** In conclusion the crude toxin exposure of A. *actinomycetemcomitans* serotype-B leads to DNA fragmentation and increase PARP-1 expression.

Key words: Crude toxin of *Aggregatibacter actinomycetemcomitans* serotype-B, PARP-1, DNA fragmentation

ABSTRAK

Latar belakang: *Aggregatibacter actinomycetemcomitans* (A. *actinomycetemcomitans*) serotype-B merupakan etiologi utama periodontitis agresif. Sel epitel gingiva sangat sensitif terhadap toksin sehingga dapat mengganggu epitel sebagai pertahanan awal gingiva, membantu invasi toksin dan mengganggu jaringan ikat dibawahnya. Saat ini diketahui bahwa toksin bakteri Aa serotype-B menyebabkan putusannya rantai DNA. PARP-1 merupakan protein dalam intisel yang berfungsi sebagai DNA nick-sensor enzyme. PARP-1 merupakan penanda awal apoptosis, sehingga peran PARP-1 dalam pengaturan apoptosis perlu diteliti **Tujuan:** Tujuan dari penelitian ini adalah untuk meneliti ekspresi PARP-1 pada epitel gingiva yang dipapar toksin bakteri A. *actinomycetemcomitans* serotype-B. **Metode:** Penelitian eksperimen pada 20 mencit strain Swiss Webster (balb C) dibagi secara random dalam 2 kelompok, kelompok kontrol (Group A) dan kelompok perlakuan (Group B). Kedua kelompok diaklimasi sebelumnya selama 1 minggu. Kelompok diaplikasi secara topikal dengan air destilasi steril setiap 12 jam. Kelompok B diaplikasi 100 µg/ml toksin A. *actinomycetemcomitans* serotype-B secara topikal dengan menggunakan Hamilton syringe. Mencit dimatikan 24 jam setelah aplikasi toksin kemudian potongan epitel gingiva dilakukan pemeriksaan secara imunohistokimia untuk melihat ekspresi PARP-1. Data dianalisis dengan uji-t **Hasil:** Ekspresi PARP-1 menunjukkan peningkatan pada kelompok perlakuan (Mean = 48,9; SD = 2,01) bila dibanding kelompok kontrol (Mean = 25,21; SD = 1,72). Tampak adanya gambaran DNA fragmentasi pada pemeriksaan gel elektroforesis yang menunjukkan adanya apoptosis. **Kesimpulan:** Dapat disimpulkan bahwa paparan toksin A. *actinomycetemcomitans* serotype-B menyebabkan DNA fragmentasi, dan meningkatkan ekspresi PARP-1.

Kata kunci: Toksin *Aggregatibacter actinomycetemcomitans* serotype-B, PARP-1, fragmentasi DNA

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INTRODUCTION

Aggressive periodontitis is a form of periodontal disease, which has a sign of aggressive destruction of periodontal ligament and alveolar bone. The loss of attachment is three times higher than chronic periodontitis causing premature tooth loss. The prevalence of aggressive periodontitis can reach a significant number of 10 to 15 percent.¹ The trigger of aggressive periodontitis is the predominant specific bacterium of *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) serotype-B.² The bacterium and its end-products can interact with gingival epithelium during the chronic infection, furthermore will penetrate into fibroblast, periodontal ligament and alveolar bone. *A. actinomycetemcomitans* inhibits the proliferation and supporting the apoptosis of gingival cell, fibroblast, osteoblast, macrophage, lymphocyte B and lymphocyte T.^{3,4}

Apoptosis plays an important role in the homeostasis of cells and tissue in response to damage. PARP-1-mediated poly (ADP-ribosylation) of nuclear proteins is required for apoptosis. During apoptosis, caspase-7 and caspase-3 cleave PARP-1 into two fragments: p89 and p24.⁵ PARP-1 also plays a central role in a caspase-independent apoptosis pathway mediated by apoptosis-inducing factor (AIF). The induction of apoptosis at the host cell triggered by specific pathogenic bacterium is a new phenomenon in the pathogenesis of periodontal diseases.⁶ The incidence of apoptosis at the underlying gingival pocket epithelium can be continuously occur and can caused further destruction by decreased fibroblasts, diminished lymphocytes functions and declined osteoblast. If it occurs, tooth will lose its attachment to periodontium and will cause premature tooth detachment and loss.⁷ The gingival cell acts as the port d'entrée of *A. actinomycetemcomitans* into the periodontium, thus taking part in the development of inflammation and the progressivity of periodontal disease. This research is aimed to examine of PARP-1 expression in gingival epithelium that exposed to *A. actinomycetemcomitans* serotype-B toxin.

MATERIALS AND METHODS

This experimental research was using male rat (*Mus musculus* strain Swiss Webster balb c). The methodology was using post test only group design. The analysis unit was gingival epithelial cell of the bucco-anterior rat mandible, physically fit, aged at 2.5 months with body weight of 25–35 gram obtained from Pharma Veterinary Centre Surabaya. First treatment (control group): ten rats were given sterile distilled water topically

at the bucco-anterior mandibular gingiva every twelve hours with aid from disposable oral sponge swab (Rynell Inc, USA) immersed into sterile distilled water until thoroughly wet, then applied with double lateral strokes. Second treatment (treatment group): ten rats were applied topically with *A. actinomycetemcomitans* serotype-B toxin at 100µg/ml at the bucco-anterior mandibular gingiva using Hamilton syringe (Reno, Nevada USA). After twenty-four hours, the rats were eliminated and the mandibular gingiva were incised as biopsy specimen.

The immunohistochemistry examination with the method of Streptavidin –Biotin-Complex were used to get the expression of PARP-1. The counting of gingival epithelial cells which expressed PARP-1 was done under light microscope with 400 times magnification. Cells which proved to be positive gave brownish color between bluish/greenish epithelial cells. Every reserves were examined at 4 different places clockwise 3, 6, 9, and 12. Each field of vision are examined and counted at two places according to 6 and 12 needle using counting room and counter. This research used the t-test statistical analysis with 95% sensitivity ($p < 0.05$). To strengthen the image of early apoptosis using PARP-1 expression, the DNA fragmentation test was performed with electrophoresis gel. DNA was purified from epithelial gingiva cultured cells using a DNA extraction kit (Stratagene #200600; La Jolla, CA). The purified DNA was electrophoresed using a 2% agarose gel with ethidium bromide staining.

RESULTS

The results of immunohistochemical examination shows that the number of cells expressing PARP-1 is shown in table 1. On the induction of Aa serotype-B crude toxin shows an increasing number of cells expressing PARP-1 with an average rating of 48.9, whereas in the control group obtained the results of 25.21.

Normality test is done using Kolmogorov-Smirnov test. Normal distribution group continued by parametric test (t-test) to know the difference in the group with 5% significant rate. Table 1 shows that significant difference in number of cells which express PARP-1 in gingival

Table 1. Mean, standard deviation, the significance of number of cells which express PARP-1 in gingival epithelial cells that exposed to *A. actinomycetemcomitans* serotype-B bacterial toxin

Group	N	Mean	SD	Significance
Control	10	25.21	2.01	0.01
Toxin	10	48.9	1.72	0.01

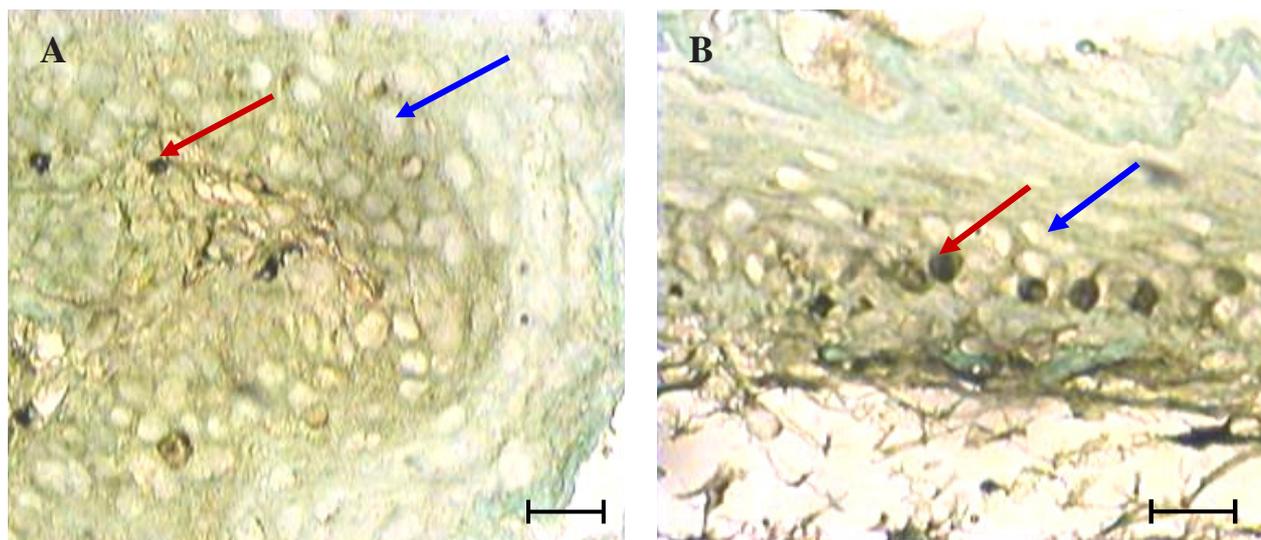


Figure 1. Overview of PARP-1 expression in rat gingival epithelial cells using monoclonal antibody against PARP-1 with a magnification of 400 \times . A) control, B) the provision of *A. actinomycetemcomitans* serotype-B bacterial toxin. Red arrow shows the positive results of brown spots on the nucleus of gingival epithelial cells, whereas blue arrows indicate a negative result of no brown spots on gingival epithelial cell nucleus.

epithelial cell that exposed Aa serotype b toxin. In figure 1 can shown that the results of immunohistochemical examination, the gingival epithelium biopsy of mice to detect the expression of PARP-1 with a magnification of 400 \times . At figure 2, there are many DNA laddering which indicates the DNA has been fragmented at the intoxicated cells. Apoptosis indicated by the fragmentation at 180–200

basepairs and its multiples. On the control group, there was no DNA fragmentation.

DISCUSSION

Periodontitis, one of the most common human infectious diseases, is an acute or chronic infectious condition that can result in the inflammatory destruction of periodontal tissues such as periodontal ligaments and alveolar bone. Among more than 300 species of bacteria in the oral cavity, *Porphyromonas gingivalis* and *A. actinomycetemcomitans* (Aa) are the major periodontopathic bacteria, and several virulence factors related to the pathogenesis of periodontitis. Therefore, identification of certain virulence factors of Aa serotype-B bacteria would aid in the development of preventive strategies against periodontal diseases, especially aggressive periodontitis.

Apoptosis plays an important role in the homeostasis of cells and tissue in response to damage. The process of apoptosis/programmed death of cells shows changes in cell morphology include chromatin condensation, cell membrane shrinkage, the outbreak of nucleus and apoptotic bodies was a sign of DNA fragmentation in nucleosom, releasing PARP-1. Based on these results, it seems that the exposure of Aa serotype-B crude toxins can increase the number of PARP-1 in the cells of the gingival epithelium. This is caused by the Aa serotype-B bacterial toxin, both endotoxin and exotoxin can cause apoptosis via different paths. Endotoxin (lipopolysacharida) through LPS binding protein binds the CD14 in membrane cells will activate IP3 receptors that activate calcium release from the endoplasmic reticulum. LPS also activates TNF alpha which will trigger apoptosis in an external way. LPS induces apoptosis signal in fibroblast through tumor necrosis factor receptor 1 (TNFR1), thus

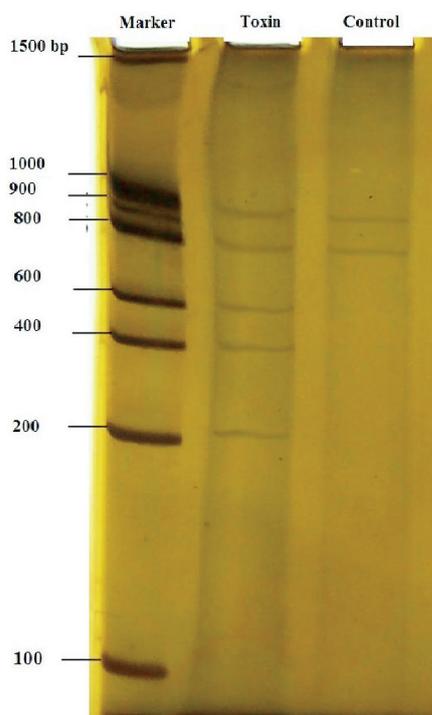


Figure 2. DNA fragmentation from electrophoresis gel examination. By using marker (100 bp DNA ladder Promega) shows DNA laddering at 200 bp and its multiples at the first lane.

increasing the activity of caspase 3 and caspase 8. Cytotoxic toxin (CDT) from *actinomycescomitans* bacteria inhibit epithelial cell growth, CDT bind to GM3 ganglioside specific receptor in nucleus using the enzyme DNA-like a nuclease that will lead to DNA fragmentation thus inhibiting cell cycle G0/G1 and G2/M and inhibit cell to enter the mitotic phase. The breakdown of DNA internucleosome chain is an early sign of apoptosis. The breakdown of DNA chain will activate PARP-1 enzyme, which is an enzyme that activate DNA repair in the nucleus. But if there is excessive activation, PARP-1 will result in the loss of NAD⁺, causing apoptosis.^{8,9}

PARP-1 is an enzyme in the nucleus which always present in eukariota. It consists of proteins with a molecular weight of 116 kDa. PARP-1 is the most dominant member of the poly ADP-ribose polymerases (PARPs) protein in the nucleus, representing more than 85%. PARP-1 plays a role in DNA repair that suffers injury. In every 1000 bp of DNA, there is 1 PARP molecule. If the DNA is damaged, there is an increase of 500 times of PARP to bind to the broken DNA chain. PARP-1 produces 50–200 Poly ADP-ribose (PAR) to be given to the histones protein, DNA-polymerases, topoisomerase, DNA ligase, and transcription factors. PARP-1 catalyzes the breakdown of nicotinamide adenine dinucleotide (NAD⁺) into Nicotinamide and ADP ribose, subsequently polymerized forming a branch of poly ADP-ribose nucleic acids. In basal state, PARP-1 activity is very low, but if there are damages to DNA, PARP-1 will hyperactively break NAD⁺ to create poly ADP-ribose branch, in turn, will generate the histone proteins and enzymes that help the DNA repair. At low levels of DNA damage, PARP-1 has the function of repairing the DNA. But at medium or high level of DNA damage, PARP-1 may lead to cell's death through apoptosis or necrosis. PARP-1 requires NAD⁺ to be hydrolyzed and ADP-ribose units. PARP-1 that is too active will cause NAD⁺ depletion, which continues to deplete the ATP.¹⁰

In the present study, we demonstrated that crude toxin periodontopathic bacteria Aa serotype b increase PARP-1 expression which early marker of cell death. This cell death is due to apoptosis, since of the crude toxin induced DNA ladder formation. *A. actinomycescomitans* serotype-B have three cytotoxic factors such as leukotoxin, cytolethal distending toxin (CDT), and endotoxin LPS which induces both cell cycle arrest and apoptosis. Leukotoxin induced apoptosis in HL-60 cells, which was consistent with the present.¹¹ Furthermore, it has been reported that both cell cycle arrest and apoptosis were induced by partially purified *A. actinomycescomitans* serotype-B toxin in mouse hybridoma cell line HS-72 cells. It is likely that *A. actinomycescomitans* serotype-B produces several types of toxins which induce different cytotoxic effects against mammalian cells. Treatment with the extract from the *A. actinomycescomitans* serotype-B induced pore formation on the cell membrane, nucleosomal DNA ladder formation, and caspase-3 activation.¹²⁻¹⁴ The DNA breakage resulted in the rapid activation of PARP-1. At the

same time, ATP and NAD⁺ concentrations decreased and nicotinamide accumulated extracellularly. These findings collectively indicated the rapid activation and central role of PARP in the pathogenesis of periodontitis.¹⁵ This research showed that the *A. actinomycescomitans* serotype-B crude toxin cause DNA fragmentation and increased PARP-1 expression in gingival epithelial cells. The author recommends providing cytoprotection materials on gingival epithelium to promote epithelial cell survival against Aa serotype-B crude toxin. Inhibition of PARP-1 may represent a novel host response modulatory approach for the therapy of periodontitis. In conclusion, the crude toxin exposure of *A. actinomycescomitans* serotype-B leads to DNA fragmentation and increase PARP-1 expression.

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