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Porphyromonas gingivalis vesicles reduce MDA-LDL levels and aortic wall thickness in high fat diet induced atherosclerosis rats

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KEYWORDS
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Abstract *Background:* Recently, atherosclerosis-associated disease has been reported simultaneously increased. Whereas, to date, no atherosclerosis vaccine is available. Since the epitope mimicry between malondialdehyde low-density lipoprotein (MDA-LDL) and arginine

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specific epitope gingipain (Rgp) on the *Porphyromonas gingivalis* vesicles has been reported, it raises an opportunity to employ the potency of *P. gingivalis* as an atherosclerosis vaccine.
Objective: To evaluate the potency of *P. gingivalis* vesicles to prevent atherosclerosis, by assessing MDA-LDL level, visceral fat, body weight, and aortic wall thickness, in rats model.
Methods: Five groups of rats (n = 10 per group), three treatment groups, one positive and negative control group were assigned and adapted with high fat diet for 8 weeks. The treatment groups were injected with *P. gingivalis* vesicles with and without adjuvant with four booster doses. The level of MDA-LDL serum, visceral fat, body weight, and aortic wall thickness were measured in the end of the course.
Results: Our present study found that decreased in MDA-LDL levels ($p = 0.037$) and aortic wall thickness ($p = 0.016$) were observed in rats treated with vesicles and adjuvants, but not with vesicles or adjuvants only, compared to negative control. Moreover, MDA-LDL levels in rats immunized with vesicles and adjuvants were significantly lower than healthy rats. However, body weight ($p = 0.329$ and visceral fat ($p = 0.789$) were not significantly different in all treatment groups compared to control.
Conclusions: Immunization with *P. gingivalis* vesicles and adjuvants significantly reduces MDA-LDL level and aortic wall thickness in rats model.

Introduction

Atherosclerosis, narrowing of the arteries caused by plaque deposits, contributes to a high number of morbidity and mortality of cardiovascular and cerebrovascular diseases globally.[1,2] In 2015, there were 17.7 million deaths associated with cardiovascular diseases and representing 31% of all global deaths.[3] Although atherosclerosis management has been established,[4] the mortality rates due to atherosclerosis-associated diseases have increased in the last two decades.[5] This because the management of atherosclerosis is a complex, involving various aspects.[6] Therefore, management with a prevention approach such as vaccination is likely to bring better outcome. Several atherosclerosis vaccines have been studied and each of them has a different target site antigen, such as malondialdehyde low-density lipoprotein (MDA-LDL),[7–12] native LDL or copper oxidized-LDL,[13–15] and p210.[16–19] Of these antigens, MDA-LDL is the most widely studied. MDA-LDL is the most important oxidized LDL (ox-LDL) and considered more atherogenic than LDL.[20] A study found that the induction of antibodies against MDA-LDL was associated with reduced atherosclerotic lesion formation and lower serum cholesterol level.[10]

Recently, the correlation between infectious pathogens and the diseases has been proposed to elicit the potency of vaccination, for example: *Streptococcus pneumoniae*[21] and *Salmonella typhimurium*[22] were shown to have an effect on the decreased risk of atherosclerosis; influenza vaccination were found to be associated with reduced risk of stroke,[23] and pneumococcal polysaccharide vaccine had been disclosed beneficial for reduction of acute coronary syndrome risk.[24] Periodontitis is an inflammation of the periodontal and supporting structures of the teeth, associated with polymicrobial infections including *Porphyromonas gingivalis*, one of the major contributors of this disease. Scientific evidence revealed that periodontopathogens such as *P. gingivalis* during periodontitis have a pivotal role in inducing in the development of atherosclerosis.[25] This correlation is probably due to the structure mimicry between MDA-LDL and arginine specific epitope gingipain (Rgp) on the vesicles of *P. gingivalis*, structures with high immunogenicity.[26] Theoretically, two high immunogenic properties having similar fragment size have the potency to generate cross-immunity.[27] Based on this, this study sought to evaluate the potency of *P. gingivalis* vesicles to be an atherosclerosis vaccine in rats model assessed by MDA-LDL, visceral fat, body weight, and aortic wall thickness. The results of this study provide a primary data the potency of *P. gingivalis* vesicles as atherosclerosis vaccine candidate.

Methods

Animal and *P. gingivalis* outer membrane vesicles

Male albino wistar rats at eight weeks of age were purchased from Physiology Laboratorium, Brawijaya University. Ten of them were randomly assigned into each study group. All animal protocols in this study were approved by the Ethical Committee of Brawijaya University, Malang, Indonesia and were carried out in strict accordance with the Indonesian law and guidelines on the use of experimental animals. The study was conducted in animal facilities at Biomedical Laboratory, Brawijaya University.

P. gingivalis strain (ATCC® 33277™), kindly provided by Supriyono Hasan from Microbiology Laboratory, Faculty of Dentistry, Airlangga University, was used. The outer membrane vesicles of *P. gingivalis* vesicles were isolated according to previous study.[28] Briefly, after separating from the culture media, the bacterial cells were mixed with 40% ammonium sulfate for two hours and centrifuged at 20,000 g for 40 min. The pellet was suspended with Tris buffer (50 mM, pH 9.5) containing 0.5 mM dithiothreitol (DTT) (ThermoFisher, MA, USA) and was dialyzed overnight.