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Research Article

The Effect of *Porphyromonas gingivalis* Lipopolysaccharide-Induced Periodontitis in Rats Fed a High-Cholesterol Diet on Macrophage Number

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KEYWORDS

high-cholesterol diet;
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periodontitis;
P. gingivalis

ABSTRACT

Introduction: Periodontitis is a chronic inflammatory condition of the tooth-supporting tissue. *P. gingivalis*, which produces virulence factors, including lipopolysaccharide (LPS), is the main pathogenic driver of periodontitis. However, the interaction between the innate immune system and periodontal pathogens in hyperlipidemia remains unclear. **Objective:** The aim of this study was to investigate the effect of a high-cholesterol diet (HCD) on macrophage activity in *P. gingivalis* LPS-induced periodontitis. **Methods:** Twenty-eight male Sprague Dawley rats were divided into four groups (n=7 rats each group): LPS-HCD, saline-HCD, LPS-basal diet (LPS-BD), and saline-BD. HCD group had been being feeding by high cholesterol diet (1% cholesterol (w/w) and 0.5% cholic acid (w/w)) for 30 days before were injected with 0.2 ml of *P. gingivalis* ATCC 3277 LPS (LPS-HCD group) and saline (saline-HCD group). The other two groups had been being feeding by normal basal diet for 30 days before were injected with 0.2 ml of *P. gingivalis* ATCC 3277 LPS (LPS-BD group) and saline (saline-BD group). Rats were sacrificed and lower jaws were harvested and embedded in paraffin. Paraffin section of lower right and left incisor were deparaffinized, rehydrated, and stained with hematoxylin & eosin (H&E). The total number of macrophages was counted using a light microscope at a magnification of 400× from 10 fields of view. **Results:** The number of macrophages in the LPS-HCD group was the highest compare to LPS- BD, saline-HCD, and saline-BD groups. In addition, LPS-BD group had higher number of macrophage than saline-BD group which had the lowest number of macrophages. **Conclusion:** HCD and *P. gingivalis* LPS-induced periodontitis can contribute to increasing of macrophage activity in periodontitis. Thus, HCD itself can enhance the process of inflammation in periodontitis.

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INTRODUCTION

Periodontitis is an inflammatory condition of tooth-supporting structures that leads to gradual weakening of these structures and can cause tooth loss. *Porphyromonas gingivalis* (*P. gingivalis*), a gram-negative bacterium, is the major etiological agent of periodontitis. *P. gingivalis*, which produces virulence factors, including lipopolysaccharide (LPS), is the main pathogenic driver of periodontitis. This bacterium produces virulence factors that cause damage to hard and soft tissue associated with the host immune system response.[1] LPS stimulates macrophages to secrete proinflammatory cytokines, which enhance the adhesion of monocytes to endothelial cells by upregulating endothelial cell expression of adhesion molecules.[2] Macrophages play crucial roles in the innate immune system and contribute to microbial phagocytosis and the production of cytokines, which activate inflammatory mediators.[3]

Periodontitis initiated by *P. gingivalis* play a critical role in possible mechanisms linking periodontal disease with various systemic diseases, including insulin resistance, diabetes, atherosclerosis, and nonalcoholic fatty liver disease. In previous research, oral administration of *P. gingivalis* in mice altered the production of interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) in the liver and spleen. In the same study, pathogen-associated microbial patterns (PAMPS) entered the systemic circulation and activated host defense cells to induce the secretion of cytokines, chemokines, and other types of immune factors in endothelial cells and hepatocytes.[4]

The prevalence of both periodontal disease and hyperlipidemia are increasing worldwide.[5] Consumption of a diet high in saturated fats increases total serum cholesterol and triglycerides and can lead to obesity-related metabolic disorders.[6] Recent data revealed an association between periodontal disease and hyper-lipidemia.[7]

A previous study reported that hyperlipidemia can induce systemic chronic inflammation.[8] The use of lipid-lowering agents in hyperlipidemia has been found to be beneficial against periodontal attachment loss and periodontal treatment in subject with hyperlipidemia & chronic periodontitis can improve serum lipid levels and decrease level of pro-inflammatory cytokine.[9,10] However, the underlying mechanism by which hyper-lipidemia influences periodontal disease progression, especially the interaction between the innate immune system and periodontal pathogens, remains to be established.[11]

MATERIALS AND METHODS

Animals

Three-month-old male Sprague Dawley rats (N = 28) were used in this study. The rats were divided into four groups (n = 7 rats in each group): LPS-HCD, LPS-basal diet (LPS-BD), saline-HCD, and saline-BD. HCD group had been being feeding by high cholesterol diet (1% cholesterol (w/w) and 0.5% cholic acid (w/w)) for 30 days before were injected with 0.2 ml of *P. gingivalis* ATCC 3277 LPS (LPS-HCD group) and saline (saline-HCD group). The other two groups had been being feeding by normal basal diet for 30 days before were injected with 0.2 ml of *P. gingivalis* ATCC 3277 LPS (LPS-BD group) and saline (saline-BD group). On day 10 after injection, periodontitis was confirmed by gingival recession bellow the cementoenamel junction.

P. gingivalis LPS ATCC 33277 was obtained from the Microbiology Laboratory, Faculty of Dentistry, Universitas Airlangga, Surabaya, Indonesia. *P. gingivalis* LPS (0.2 ml) was injected in the interdental gingival sulcus of the lower right and left incisors using a 30 G tuberculin syringe. Prior to the injection, the rats received an injection of intramuscular ketamine hydrochloride (ketamine HCl). On day 10, periodontitis was confirmed by gingival recession bellow the cementoenamel junction.[12,13] The protocol of this study was approved by the ethics committee of the Faculty of Dentistry, Universitas Gadjah Mada (Number: 00111/KKEP/FKG-UGM/EC/ 2019).

Histological analysis

All of the animals were sacrificed by cervical dislocation. The thumb and index finger were placed on side of the neck at the base of the skull. Before harvesting the organ, perfusion of the organ was performed from the left ventricle using natrium chloride 0.9% (NaCl solution) to prevent blood contamination in samples. The lower jaws were harvested and fixed in 4% paraformaldehyde (PFA) 24 hours. Paraffin was used for the tissue embedding process. Paraffin sections (4 µm) were deparaffinized and rehydrated using serial xylene and alcohol. The specimens were then stained with hematoxylin & eosin (H&E). The total number of macrophages in 10 fields of view was counted using a light microscope at a magnification of 400×.

Statistical Analysis

Data were analyzed with Shapiro Wilk test to determine the normality and Leavene's Test to determine

the homogeneity. The data were continue analyzed by One-Way Analysis of Variance (One-Way ANOVA) and Post-Hoc Least Significant Difference (LSD test).

RESULTS

The number of macrophages were counted by 2 observers. Infiltration of inflammation cells was observed in the periodontal tissue in LPS-HCD, LPS-BD, saline-HCD, and saline-BD groups. The majority of the macrophages contained a round or oval nucleus, with one or more small nucleoli and often coarse azurophilic granules as shown in Fig. 1. The results of a One-Way Analysis of Variance (One-Way ANOVA) pointed to a significant difference in the number of macrophages between the groups (p=0.000). As shown in Fig. 2, the highest number of macrophages was found in the LPS-HCD group ($\bar{x} \pm SD = 3.0 \pm 0.81$), with a statistically significant difference (Post-Hoc LSD test) between LPS-HCD group and LPS-BD, saline-HCD, and saline-BD groups (p=0.001, p=0.000, and p=0.000, respectively). The lowest number of macrophages was found in the saline-BD group ($\bar{x} \pm SD = 0.29 \pm 0.48$). The number of macrophages in the LPS-BD group was higher than that in the saline-BD group. There was no significant difference between the number of macrophages in the LPS-BD and saline-HCD groups.

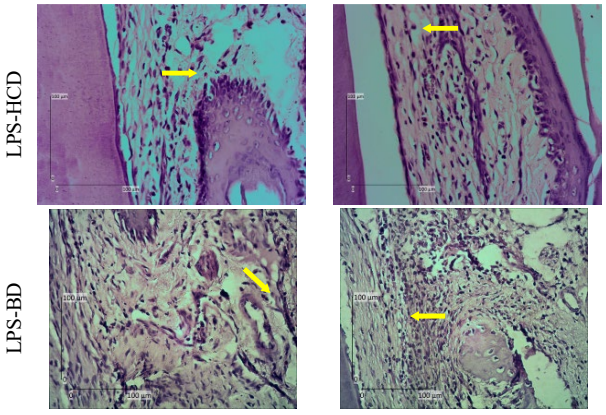

Figure 1. Representative images of macrophages (yellow arrows) in the LPS-HCD and LPS-BD groups (400×; H&E).

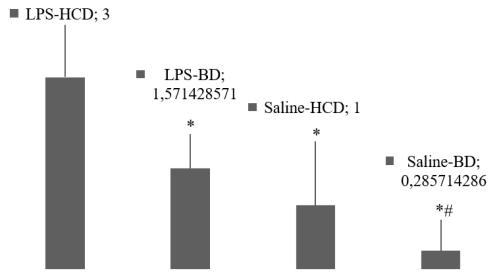

Figure 2. Mean number of macrophages.
*p <0.05 versus LPS-HCD; #p <0.05 versus LPS-BD.

DISCUSSION

The main function of macrophages is phagocytosis of foreign bodies. Macrophages can be activated by a variety of stimuli during the immune response, such as cytokines secreted by helper T cells, interferon-γ (IFN-γ), increase the activity of macrophages. Macrophages initiate an immune response due to their toll-like and scavenger receptors. Macrophages express major histocompatibility complex class II molecules and activate T lymphocytes to release cytokines, which activate B lymphocytes, leading to antibody production.[3]

In the present study, the number of macrophages in the LPS-BD group was higher than that in the saline-BD group. The induction of LPS increases the number of macrophages. Previous study showed that induction of LPS can activate toll-like receptors (TLRs) recognize PAMPS and activate acute inflammation through secretion of inflammatory cytokines. To ensure efficient pathogen removal, specific TLRs recognize specific PAMPS. TLR4 is a specialized TLR that recognizes LPS and binding of TRL4 to LPS triggers Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFκB) or Mitogen-Activated Protein Kinase (MAPK) signaling pathways, leading to the production of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, IL-12, and type-1 interferon, resulting in pathogen destruction.[14] Proinflammatory cytokines activates macrophage to phagocyte microorganism and initiate immune response by express major histocompatibility complex (MHC) class II molecules and present antigens to lymphocytes. This recognition activates T lymphocytes release cytokine and B lymphocytes secrete antibodies.[3]

The highest number of macrophages was found in the LPS-HCD group, with a significant difference in the number compared to that in the LPS-BD, saline-HCD, and saline-BD groups. In a previous study, the authors reported that a HCD impairs lipid metabolism and enhances total cholesterol and triglyceride levels in serum.[6] In the same study the periodontal pocket is deeper in patients with hyperlipidemia indicating that that a HCD influences the progression of periodontitis. Another study reported that a HCD increases oxidative stress in various organs. Both a HCD and periodontopathic bacteria contribute to the level of oxidative stress, which can affect periodontal disease progression. Tissue damage induced by oxidative stress can cause cell damage either directly or indirectly and lead to cell death and in periodontitis, fibroblasts are injured by this mechanism. Indicators of tissue damage induced by oxidative stress include 8-hydroxydeoxy-guanosine, IL-1β, and TNF-α. [15]

Hyperlipidemia increases susceptibility to periodontal diseases, and inflammation in periodontal tissue can contribute to hyperlipidemia. During periodontal inflammation, proinflammatory cytokines induced by reactive oxygen species and bacterial products are released into the bloodstream and exacerbate inflammation in various organs.[16] LPS induction increases the secretion of proinflammatory IL-1β, IL-6, and TNF-α, leading to systemic low-grade inflammation.[6] Upregulation of the expression of TLRs activates macrophages, which increase the production of proinflammatory cytokines. Previous research showed bidirectional relationship between periodontitis and hyperlipidemia, for instance bacterial invasion of the cardiovascular system can initiate atherosclerotic plaques. Cytokines induced in response to periodontal inflammation results in a shift in the hypothalamic-pituitary-adrenal axis, increasing the concentration of adrenocorticotropic hormones cortisol, adrenaline, noradrenaline, and glucagon, thereby altering lipid metabolism. A high level of cytokines can enhance the levels of free fatty acids, low-density lipoprotein (LDL), and triglycerides via hepatic lipogenesis, not only in the presence of periodontal disease but also hyperlipidemia and atherosclerosis.[5]

In the present study, there was no significant difference between the number of macrophages in the LPS-BD and saline-HCD groups. Although periodontitis was not induced by LPS in saline-HCD group, a high-fat diet can lead to a shift in gut microbiota populations and activation of the TLR pathway, leading to enhance endotoxin (such as LPS) permeability so LPS can enter to the circulation. LPS then activates macrophages and the production of proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β.[6] The findings reported in the literature and those of the present study indicate that high cholesterol diet on its own can lead to an increase in the number of macrophages.

CONCLUSION

In this study, the highest number of macrophage was found in the LPS-HCD group. HCD and *P. gingivalis* LPS-induced periodontitis contributed to increasing of macrophage activity in periodontitis. Thus, HCD itself can enhance the process of inflammation in periodontitis.

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Conflict of Interest

The authors certify that there is no actual or potential conflict of interest regarding this study. .

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