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Porphyromonas gingivalis infection modifies oral microcirculation and aortic vascular function in the stroke-prone spontaneously hypertensive rat (SHRSP)

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ABSTRACT

The functional modulation of vascular endothelial cells associated with stroke and periodontal disease has not yet been clarified. The objective of this study is to analyze the vascular endothelial function of periodontitis and stroke animal models. We examined endothelial function and gingival blood flow in oral microcirculation *in vivo* and measured the isometric tension *in vitro* of the aorta in animal models for lifestyle-related diseases, such as periodontitis and stroke. Gingival reactive hyperemia (GRH) was measured using laser Doppler flowmetry. Wistar Kyoto rats (WKY) were used as control animals; *Porphyromonas gingivalis* (*P. gingivalis*) infected WKY (WKY + *Pg*) as the periodontitis model; stroke-prone spontaneously hypertensive rat (SHRSP) as the stroke model; and a final group consisting of *P. gingivalis* infected SHRSP (SHRSP + *Pg*). Furthermore, for each group, the relaxation of descending aortic ring preparations was measured using a force transducer. The GRH was estimated by maximum response (peak), time taken for the maximum response to fall to one half ($T_{1/2}$), and increased total amount of blood flow (mass). The relative change in $T_{1/2}$ and mass increased in SHRSP + *Pg* compared to WKY. However, mass significantly increased in WKY (758.59 ± 88.21 ml/min/100 g s to 1755.55 ± 226.10 ml/min/100 g s) and SHRSP (1214.87 ± 141.61 ml/min/100 g s to 2674.32 ± 675.48 ml/min/100 g s) after treatment with acetylcholine. In addition, $T_{1/2}$ and mass significantly increased in WKY + *Pg* (624.18 ± 96.36 ml/min/100 g s to 2629.90 ± 612.01 ml/min/100 g s) and SHRSP + *Pg* (1116.36 ± 206.24 ml/min/100 g s to 1952.76 ± 217.39 ml/min/100 g s) after treatment with nitroglycerin. Furthermore, the endothelium-dependent relaxation of ring preparations, evoked by acetylcholine, was attenuated in SHRSP compared with WKY, but not in SHRSP + *Pg*. This attenuation effect in SHRSP could be prevented by superoxide dismutase pretreatment. Our results suggest altered endothelial function may occur in gingival tissue in animal models experiencing both periodontitis and stroke. Therefore, these results indicate the disruption of vascular function in oral microcirculation may be caused by the interaction between the oxidative stress induced by periodontitis and nitric oxide in periodontitis, similar to the interactions present in stroke cases.

1. Introduction

The significant association between cardiovascular and periodontal diseases is receiving increasingly more attention. Epidemiological studies show periodontal disease may be a risk factor for systemic diseases, such as hypertension and diabetes

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[1–4]. Periodontal diseases can significantly affect systemic diseases, which can inversely be risk factors for periodontal diseases. The relationship between periodontal and systemic diseases is a basic concept in periodontal medicine [5], increasing the prevalence of this field [6,7]. Periodontal disease is characterized by inflammation and bone resorption; the study of the relationship between inflammation and bone resorption has resulted in a new field of study, providing context for a better understanding of the pathogenesis of periodontal disease [7]. Garrett et al. demonstrated that the formation of osteoclasts was stimulated in bone by the generation of reactive oxygen species (ROS) and that bone resorption occurred both *in vivo* and *in vitro* [8].

Oxidative stress arises when the generation of ROS exceeds the capacity for the cell to detoxify potentially injurious oxidants using endogenous antioxidant defense systems [9]. Conditions associated with oxidative stress induced by ROS include hypertension [10–12] and stroke [13,14].

We previously developed an electron spin resonance (ESR)-based technique to assess oxidative stress, including ROS, in biological systems [15–22]. We demonstrated increased generation of ROS in the brain of the stroke-prone spontaneously hypertensive rat (SHRSP), where ROS ultimately contributed to the mechanisms causing hypertension or stroke [16,19–22]. Additionally, vascular endothelial cell function has been previously estimated by flow-mediated dilation (FMD) and plethysmography, verified by reactive hyperemia of the forearm. We previously reported that measuring reactive hyperemia in oral microcirculation could be used to estimate the vascular endothelial function of general circulation, similar to using FMD or plethysmography of the forearm [23]. Periodontal disease-reduced gingival vascular reactivity could be accelerated by diabetes due to increased oxidative stress in the microcirculation of the oral and maxillofacial regions of the rodent model [15]. A recent study also suggested that periodontitis might be associated with endothelial dysfunction in individuals without cardiovascular risk factors, as well as in hypertensive patients [24].

Porphyromonas gingivalis (*P. gingivalis*) is an anaerobic gram-negative coccobacillus associated with periodontal disease progression, including bone and tissue destruction [25]. Gram-negative bacterial lipopolysaccharides (LPS) are known to induce tissue damage and injury via the generation of ROS [26]. Therefore, we hypothesized that oxidative stress induced by ROS may play a critical role in altering oral vascular function due to periodontal disease caused by *P. gingivalis*. However, the relationship between alteration of oral vascular function due to periodontal disease and a vascular disease model such as SHRSP, which is a model associated with increased oxidative stress, has not yet been examined. We have previously reported using animals orally challenged with *P. gingivalis* as a chronic inflammation model. Our previous results suggested that *P. gingivalis*-induced alveolar bone loss could occur in periodontitis and also “hypertension and stroke” animal models, such as SHRSP [27].

This study investigates the effects of *P. gingivalis* in the SHRSP rodent model, representing hypertension or stroke. By measuring reactive hyperemia in the oral microcirculation, we examined *in vivo* endothelial function and gingival blood flow in the oral microcirculation animal models of lifestyle-related diseases, such as periodontitis or stroke. Furthermore, we measured isometric contraction changes using ring preparations that we extracted from these model animals *in vitro*. We examined *P. gingivalis*-induced alteration of oral vascular function in both SHRSP and WKY, and found that vascular function changed in SHRSP infected with *P. gingivalis*.

2. Materials and methods

2.1. Animals

In this study, male Wistar Kyoto rats (WKY, 4–25 weeks old, weighing 65–450 g) were used as control animals and SHRSP rats (4–25 weeks old, weighing 65–350 g) were used as an animal model for stroke. For both groups, 3-week-old male animals were purchased from a commercial farm (Nihon SLC, Shizuoka, Japan). The procedures used in this study were in accordance with the guidelines of the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1985). Our protocols were approved by the Animal Care Committee of Kanagawa Dental University (Yokosuka, Japan).

Animals were housed in groups of five per cage in a room maintained under standardized light (12:12 h light–dark cycle) and temperature (22 ± 3 °C) conditions with free access to food pellets and tap water. Animals were divided into four groups: Group 1, *P. gingivalis*-non-infected WKY; Group 2, WKY infected with *P. gingivalis* (WKY + *Pg*); Group 3, *P. gingivalis*-non-infected SHRSP; and Group 4, *P. gingivalis* infected SHRSP (SHRSP + *Pg*). Each group comprised 5–8 rats.

2.2. Bacteria and culture conditions

The bacterial strain used was *P. gingivalis* American Type Culture Collection (ATCC) 33277. *P. gingivalis* was grown at 37 °C for 18 h in an anaerobic chamber (Anaerobox, Hirasawa, Tokyo, Japan) with an atmosphere of 85% N_2, 10% H_2, and 5% CO_2 in a brain heart infusion broth (Difco, Detroit, MI, USA) supplemented with 5 mg/ml yeast extract, 5 μg/ml hemin, and 0.2 μg/ml vitamin K_1.

2.3. P. gingivalis infection in rats

As shown in Fig. 1A, rats were given sulfamethoxazole (1 mg/ml) and trimethoprim (200 μg/ml) in their drinking water for three days *ad libitum* to reduce the original oral flora, followed by a 4-day antibiotic-free period before *P. gingivalis* infection. Each rat received 0.5 ml (1.5×10^9 cells per ml) *P. gingivalis* ATCC 33277 suspended in 5% carboxymethylcellulose (Sigma Chemical, St. Louis, MO, USA) by oral gavage three times per week at the ages of 4, 5, and 15 weeks [27,28].

2.4. Gingival blood flow

Animals were anesthetized with sodium pentobarbital (45 mg/kg, IP) and were subsequently given small maintenance doses as necessary. After determining body weight, each rat was laid on a wooden board (20 × 24 cm) in the supine position. All limbs were fixed at an angle of 45° to the body midline with adhesive tape and the upper and lower jaws were anchored in an open position with a thin rope via the incisors. Gingival blood flow (GBF) was measured at the palatal gingiva by a laser Doppler flowmeter (TBF-LN1, Unique Medical Co., Ltd., Tokyo, Japan) with a laser Doppler probe (diameter 2.0 mm). Heart rate was monitored to determine the effects on systemic hemodynamics of the administered agents or gingival reactive hyperemia (GRH). To evaluate vascular endothelial and smooth muscle functions, 100 mg/ml acetylcholine (ACh) or 5 mg/ml nitroglycerin (NTG) were topically absorbed from an area of gingival mucosa approximately 2 mm in diameter for 1 min before gingival compression for 1 min. Reactive hyperemia was elicited at the same place as ACh and NTG application by the release of occlusive gingival compression by the laser Doppler probe for 1 min. As shown in Fig. 1B, the reactive hyperemia in the gingival circulation was estimated by basal blood flow (basal), maximum