Relationships between periodontal inflammation, lipid peroxide and oxidative damage of multiple organs in rats

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ABSTRACT

Gingival response to periodontal inflammation generates excessive lipid peroxide and such a condition may augment systemic health through increased circulating lipid peroxide. The purpose of the present study was to investigate whether the generation of lipid peroxide in periodontal inflammation could induce tissue injury in the liver, heart, kidney and brain using a rat model. Twelve Wistar rats (8 week-old male) were divided into 2 groups: the periodontal inflammation group, receiving topical application of lipopolysaccharide and proteases to the gingival sulcus for 4 weeks, and the control group using instead pyrogen-free water. After blood samples were collected, specimens from the brain, heart, liver and kidney were resected to determine the concentration of 8-hydroxydeoxyguanosine (an indicator of oxidative DNA damage). Gingival and serum levels for hexanoyl-lysine were measured to evaluate lipid peroxide. Administration of lipopolysaccharide and proteases induced periodontal inflammation, with increasing gingival and serum levels of hexanoyl-lysine. The level of 8-hydroxydeoxyguanosine increased 2.27, 2.01, 1.49 and 1.40 times in mitochondrial DNA from the liver, heart, kidney and brain of rats with periodontal inflammation, respectively. The results reveal that excessive production of lipid peroxide following periodontal inflammation is involved in oxidative DNA damage of the brain, heart, liver and kidney.

Periodontal inflammation is a chronic disease caused by oral bacterial infection (30). Evidences have implicated that periodontal inflammation may be a potential risk factor for systemic diseases, including diabetes mellitus (14), cardiovascular disease (4), preterm low birth weight (17), chronic kidney diseases (1) and hepatocellular carcinoma (25). The mechanisms by which periodontitis affects these systemic diseases are still unclear. However, the prerequisite is believed to be the host inflammatory response to long-term exposure to bacterial pathogens, such as lipopolysaccharide (LPS) and proteases (27).

In a periodontal lesion, polymorphonuclear leukocytes produce reactive oxygen species (ROS) as the initial host defense against bacterial pathogens (21). However, excessive ROS production impairs tissue oxidative/anti-oxidative balance that contributes to generate lipid peroxide (10). Clinical and animal studies have shown that periodontal inflammation induces over-production of lipid peroxide in the periodontal lesion (13, 29). In addition, studies have also reported that lipid peroxide produced by periodontal inflammation diffuses into the blood stream.
(8, 18, 24). While the toxicity of circulating lipid peroxide is low, it remains within the body for a long time, gradually affecting multiple organs. Since lipid peroxide is involved in progression of brain (3, 23), heart (5), kidney (31) and liver diseases (19), increased circulating lipid peroxide following periodontal inflammation may be detrimental to these organs. However, little information regarding this is available.

8-Hydroxydeoxyguanosine (8-OHdG) is formed when the guanine in DNA undergoes oxidative damage by ROS and lipid peroxide (12). 8-OHdG is generally accepted as a reliable indicator of tissue oxidative damage (26). The purpose of the present study was to examine whether the generation of lipid peroxide in periodontal inflammation could induce oxidative damage in the liver, heart, kidney and brain using a rat model. In order to evaluate lipid peroxide, the level of hexanoyl-lysine (HEL) was determined (22). In addition, the serum level of C-reactive protein (CRP) was measured because previous studies indicated that periodontal inflammation results in low-grade systemic inflammation (7, 9, 16).

MATERIALS AND METHODS

Animals. All experimental procedures were performed in compliance with guidelines approved by the Animal Research Control Committee of Okayama University Dental School. Male Wistar rats (n = 12, 8 weeks old) were maintained under controlled temperature (23–25°C) and light (12-hour light/12-hour dark cycle, lights on at 6 : 00 AM), with ad libitum access to powdered food and water.

Experimental design. Rats were randomly divided into two groups: the periodontal inflammation group received topical application of bacterial pathogens [25 μg/μL Escherichia coli (E. coli) LPS (Sigma Chemical Co., St. Louis, MO, USA) and 2.25 U/μL proteases from Streptomyces griseus (S. griseus) (Sigma Chemical Co.) suspended in pyrogen-free water], and the control group received topically applied pyrogen-free water instead (26). LPS (0.5 μL × 3 times) and proteases (0.5 μL × 3 times) or pyrogen-free water (0.5 μL × 6 times) were introduced into the gingival sulcus of both maxillary first molars daily for four weeks by micropipette, under inhalative anesthesia with an O₂-isoflurane mixture (26). The tip of the micropipette was placed close to the gingival sulcus and 0.5 μL of the LPS or protease solutions was dropped into the sulcus.

Analysis of blood samples. Blood samples were collected at four weeks directly from the heart of 24-hour-fasted animals. Blood was allowed to clot at room temperature, and serum was separated by centrifugation at 1,500 × g for 15 min. The level of serum HEL, a biomarker for early stage lipid peroxidation, was measured using an ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan) (8). The concentration of serum LPS was determined using a kinetic Limulus Amebocyte Lysate test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) (27). Serum levels of CRP were quantified by a highly sensitive ELISA (Life Diagnostics, Inc., West Chester, UK) (9).

Histological analysis. After the animals were sacrificed under general anesthesia, the maxillary molar regions (tooth and periodontal tissues), liver, heart, kidney and brain were resected from each rat and immediately fixed with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 1 day. The tooth and periodontal tissue were decalcified with 10% tetrascalium-ethylenediaminetetraacetic acid aqueous solution (pH 7.4) for 14 days at 4°C. Paraffin-embedded bucco-lingual sections (4 μm thick) were stained with hematoxylin and eosin.

In the periodontal tissue, the immunostaining of HEL was further performed using a commercial kit (Histofine Simple Stain MAX PO; Nichirei Co., Tokyo, Japan) (15). Tissue sections were immersed in 0.01 M citrate buffer (pH 6.0) and autoclaved for 15 min at 121°C to retrieve antigen. The monoclonal antibody against HEL (Japan Institute for the Control of Aging, Shizuoka, Japan) was diluted at 1/50 in phosphate buffered saline. The color was developed with 3,3’-diaminobentizine tetrahydrochloride, and sections were counterstained with Mayer’s hematoxylin.

A single examiner (T. T.), blinded to the treatment assignment, performed the following histometric analyses using a microscope (Olympus Co., Tokyo, Japan). The distances between the cemento-enamel junction and the alveolar bone crest (CEJ-ABC) and between the cemento-enamel junction and the most apical portion of the junctional epithelium (CEJ-JE) were measured with a microridge at a magnification of ×200 (27). The polymorphonuclear leukocytes per unit area (0.05 mm × 0.05 mm) of the connective tissue subjacent to the junctional epithelium were counted at a magnification of ×400 (27). The numbers of HEL-positive fibroblasts and total fibroblasts per unit area (0.01 mm × 0.01 mm) were determined in the connective tissue subjacent to the
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The control group showed little pathological changes (Fig. 1). However, neither tissue damage nor inflammatory changes were observed in sections from the heart, kidney and brain of either group. In addition, the levels of mitochondrial 8-OHdG in the gingiva, liver, heart, kidney and brain were 3.94, 2.27, 2.01, 1.49 and 1.40 times higher in the periodontal inflammation group than in the control group and the differences between the two groups were significant in all tissues ($P < 0.05$) (Fig. 3).

**DISCUSSION**

In the present study, chronic administration of LPS and proteases induced not only periodontal inflammation but also increased gingival level of HEL expression, resulting in an increase in serum level of HEL. In addition, increased levels of tissue 8-OHdG were found in the brain, heart, liver and kidney in the periodontal inflammation model. HEL and 8-OHdG are accepted as parameters of lipid peroxide and oxidative DNA damage, respectively (22). These results suggest that increased blood lipid peroxide following periodontal inflammation could induce oxidative DNA damage of the brain, heart, liver and kidney.

A clinical study demonstrated that the plasma level of thiobarbituric acid reactive substances released from endogenous lipoperoxides, reflecting the status of the lipid peroxidation process, was 116% higher in patients with periodontitis than that in healthy subjects (18). It was also reported that ligature-induced periodontitis resulted in an increase of 121% in serum malondialdehyde (end-products of lipid peroxidation) level in the rat model at 21 days (24). These observations are consistent with the present results showing that the periodontal inflammation samples exhibited an increase of 127% in serum HEL.

The periodontal inflammation model rats showed an increase of 127, 101, 49 and 40% in the level of mitochondrial 8-OHdG in the liver, heart, kidney and brain, respectively. The increase in tissue 8-OHdG level seemed to be greater in the liver than in the other tissues.

### RESULTS

No significant differences were observed between the control and periodontal inflammation groups with regard to food consumption, body weight or growth pattern during the experimental period.

The serum levels of HEL and CRP were higher in the periodontal inflammation group than in the control group at 4 weeks ($P < 0.05$) (Table 1). However, there were no significant differences in serum LPS concentration between the two groups.

In the periodontal inflammation group, the periodontal tissues showed apical migration of the junctional epithelium, extension of blood vessels and inflammatory cell infiltration; however, the control group showed no pathological changes (Fig. 1). The CEJ-ABC, CEJ-JE, and densities of polymorphonuclear leukocytes in the periodontal inflammation group were greater than those in the control group ($P < 0.05$) (Table 2). HEL was strongly detected in the cytoplasm of fibroblasts in the periodontal inflammation group compared to the control group (Fig. 2). The ratio of HEL-positive fibroblasts to total fibroblasts was also significantly higher in the periodontal inflammation group than in the control group ($P < 0.05$) (Table 2).

The liver tissue in the periodontal inflammation group showed mild hepatic steatosis, while that in the control group showed little pathological changes (Fig. 1). However, neither tissue damage nor inflammatory changes were observed in sections from the heart, kidney and brain of either group. In addition, the levels of mitochondrial 8-OHdG in the gingiva, liver, heart, kidney and brain were 3.94, 2.27, 2.01, 1.49 and 1.40 times higher in the periodontal inflammation group than in the control group and the differences between the two groups were significant in all tissues ($P < 0.05$) (Fig. 3).

### Statistical analysis

Comparisons between the groups of rats were made by Mann-Whitney $U$-test using a statistical software package (SPSS 17.0J for Windows, SPSS Japan, Tokyo, Japan).

### Measurement of tissue 8-OHdG

Mitochondrial DNA was isolated from rat gingiva, liver, heart, kidney and brain using a DNA extractor kit (Wako Pure Chemical Industries). Level of 8-OHdG in the isolated mitochondrial DNA was analyzed using an ELISA kit (Japan Institute for the Control of Aging) (26).

### Table 1 Serum parameters in rats (mean ± SD)

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<th>Control group (n = 6)</th>
<th>Periodontal inflammation group (n = 6)</th>
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<tr>
<td>HEL (pmol/mL)</td>
<td>0.64 ± 0.04</td>
<td>1.45 ± 0.22*</td>
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<tr>
<td>CRP (ng/mL)</td>
<td>0.79 ± 0.29</td>
<td>1.99 ± 0.59*</td>
</tr>
<tr>
<td>LPS (pg/mL)</td>
<td>210 ± 61</td>
<td>155 ± 58</td>
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* $P < 0.05$, compared to the control group, using Mann-Whitney $U$-test.
brain, heart and kidney. Furthermore, in the sections stained with hematoxylin and eosin, pathological changes induced by periodontal inflammation were observed only in the liver tissue. These results suggest that periodontal inflammation damaged the liver more than the brain, heart and kidney. Since the liver plays a central role in detoxification, it may have higher sensitivity to circulating lipid peroxide than the other organs. This concept is in agreement with the previous study showing that long-term hy-

Fig. 1 Pathological changes in rat periodontium and liver. The control group showed little pathological changes in both periodontium (A) and liver (C). The periodontal inflammation group exhibited apical migration of the junctional epithelium, extension of blood vessels and inflammatory cell infiltrate (*) (B) and hepatic steatosis (D). CEJ, cemento-enamel junction; JE, junctional epithelium. Bar = 25 μm.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Pathological changes in the rat periodontal tissue (mean ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>Control group</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 6)</td>
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<tr>
<td>CEJ-ABC (μm)</td>
<td>560 ± 61</td>
</tr>
<tr>
<td>CEJ-JE (μm)</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Polymorphonuclear leukocytes (numbers/0.1 mm × 0.1 mm)</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>Ratio of HEL-positive fibroblasts to total fibroblasts</td>
<td>0.15 ± 0.05</td>
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CEJ-ABC: the distance between the cemento-enamel junction and the alveolar bone crest, CEJ-JE: the distance between the cemento-enamel junction and the most apical portion of the junctional epithelium

*P < 0.05, compared to the control group, using Mann-Whitney U-test.
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Perglycemic state induced hepatic oxidative damage more pronoucedly than the brain, kidney and heart (11).

Previous studies suggested that periodontal inflammation could influence general health through elevated blood concentration of LPS (20, 27). On the other hand, the present periodontal inflammation model did not increase the serum LPS level. We selected the experimental period of 4 weeks, because this period was enough to induce the increased serum HEL level following periodontal inflammation (28). However, it may be too short to enhance circulating LPS. In addition, the level of serum CRP was increased by periodontal inflammation. CRP is an inflammatory molecule, which is produced by hepatocytes during an acute-phase inflammatory response (2). It is possible that periodontal inflammation could indirectly induce low-grade systemic inflammation through liver injury.

The current findings show that a close link exists between periodontal inflammation and systemic oxidative damage, with increased lipid peroxide suggested as the common denominator. Therefore, the reduction of circulating lipid peroxide by antioxidi-
tant therapy may be effective to prevent systemic oxidative damage following periodontal inflammation. In fact, it is reported that vitamin C intake attenuates the degree of oxidative aorta damage induced by periodontal inflammation in the rat by decreasing serum HEL level (8). However, further studies are needed to clarify the effects of antioxidant therapy on systemic oxidative damage following periodontal inflammation.

In this study, we induced periodontal inflammation by topical application of E. coli LPS and S. griseus proteases to the gingival sulcus. This model mimics several features of human periodontal inflammation (6). However, the present results would have more validity if LPS and proteases from periodontal pathogens (i.e., Porphyromonas gingivalis) had been applied to induce periodontal inflammation. This is a limitation of our study.

In conclusion, periodontal inflammation caused oxidative DNA damage of the brain, heart, liver and kidney, with increasing levels of circulating lipid peroxide. Our results support the hypothesis that tissue oxidative damage following increased blood lipid peroxide may play a key role in systemic diseases induced by periodontal inflammation.

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REFERENCES


