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Detection of oxidized low-density lipoproteins in gingival crevicular fluid from dental patients

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Background and Objective: Oxidative modification of low-density lipoprotein (LDL) occurs in various diseased tissues and sites of local inflammation. For example, an increased plasma oxidized low-density lipoprotein (OxLDL) level is a well-known risk marker for cardiovascular diseases. Gingival crevicular fluid, the exudate from gingival tissues into the sulci, can be easily collected in a non-invasive manner. However, the possible presence of OxLDL in gingival crevicular fluid has not been studied. In this study, we established a procedure to measure OxLDL in human gingival crevicular fluid.

Material and Methods: Human gingival crevicular fluid was sampled with paper points or paper strips. The gingival crevicular fluid samples from healthy gingival sulci (pocket depth < 4 mm, $n = 14$) were subjected to western blot and/or sandwich ELISA. The amounts of OxLDL and LDL were measured by sandwich ELISA using an anti-oxidized phosphatidylcholine monoclonal antibody and two anti-apolipoprotein B antibodies. Venous blood samples were analyzed biochemically.

Results: We tested two methods of gingival crevicular fluid collection, namely paper points and paper strips. Gingival crevicular fluid could be collected very safely with paper points and they showed good recovery of LDL and OxLDL throughout the analysis. Apolipoprotein B, the major protein component in LDL, was detected in gingival crevicular fluid by western blot, and OxLDL was found to be present in gingival crevicular fluid by ELISA. The OxLDL/LDL ratio in gingival crevicular fluid was 17.0 times higher than that in plasma.

Conclusion: This is the first report to show the presence of apolipoprotein B and apolipoprotein B– oxidized phosphatidylcholine complex, which correspond to LDL and OxLDL, respectively, in gingival crevicular fluid.

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Gingival crevicular fluid is exuded from the surrounding tissue of periodontal pockets in response to the pathogenic process in periodontitis. Gingival crevicular fluid contains microbial plaque, host inflammatory cells, host tissue and serum-derived factors (1,2). Several biochemical parameters of gingival crevicular fluid are markers of periodontal disease activity. Gingival crevicular fluid volume increases in parallel with gingival inflammation by alteration of vascular permeability. Recently, evidence has indicated that patients with advanced periodontitis have increased systemic inflammation, as demonstrated by raised plasma levels of C-reactive

protein when compared with control subjects (3). Since gingival crevicular fluid can be collected using a non-invasive procedure, it may serve as an expedient biological source of patient information.

It is well known that oxidative stress is involved in a variety of forms of tissue damage and inflammatory response (4,5). Evidence has accumulated that an increase in plasma oxidized low-density lipoprotein (OxLDL) level is a marker for vascular diseases (6–9). In addition, increased plasma OxLDL was observed in patients with renal failure (10) or rheumatoid arthritis. Oxidative stress is closely associated with a systemic and local inflammatory response (4,5). For example, it was reported that patients with rheumatoid arthritis (11) have a higher level of plasma OxLDL. It was also reported that oxidized phospholipids accumulated in cartilage cells in osteoarthritis (12).

Periodontitis is a chronic infectious disease affecting the tissues surrounding and supporting the teeth, caused by gram-negative bacteria in dental plaque. It begins as gingivitis, inflammation of the soft tissue alone, and this may progress to periodontitis, in which destruction of connective tissue attachment and alveolar bone eventually lead to tooth loss. This tissue destruction can be caused and enhanced by free oxygen radicals and active proteases released during the host's immune and inflammatory responses to bacterial infection. The global prevalence of periodontal disease is high, with approximately 10–15% of individuals in many countries being affected by severe forms of chronic periodontitis (13).

The oxidative modification of low-density lipoprotein (LDL) is thought to have an important role in atherogenesis. Apolipoprotein B (ApoB) is the major protein constituent of LDL, where one molecule of ApoB exists in each LDL particle. During oxidation of LDL, various types of modifications can occur in the amino acid residues in ApoB (14). Atherosclerosis is a pathological condition in which arteries undergo gradual intimal thickening, causing decreased elasticity, narrowing and reduced blood supply. The atherosclerotic involvement of these blood vessels brings about the distinct clinical manifestations of cardiovascular diseases. A characteristic histopathological finding of atherosclerosis is the focal appearance of macrophage-derived lipid-laden foam cells. The cholesterol that accumulates in foam cells is derived from circulating lipids, mainly from OxLDL generated by vascular inflammation and oxidative stress from different types of pathological injury (15,16). Oxidized LDL also exhibits a number of cell biological activities, such as enhancement of interaction between leukocytes and endothelial cells, inhibition of endothelial cell migration and induction of endothelin secretion from endothelial cells and macrophages (17). Additionally, Lalla *et al*. (18) reported that inoculation of *Porphyromonas gingivalis* into ApoE-deficient mice, an animal model for spontaneous hypercholesterolemia and atherosclerosis, resulted in enhanced atherosclerosis, providing evidence for the association of periodontitis and atherosclerosis.

Gingival crevicular fluid is a potentially a good substrate from which to estimate both the conditions of local gingival tissues and the systemic status. In this study, as a first approach to characterization of gingival crevicular fluid, we investigated whether LDL and OxLDL are present in gingival crevicular fluid.

Material and methods

Subjects

The subjects of this study were fully informed of the protocol, and their written informed consent was obtained according to the Declaration of Helsinki. The experimental protocol for this human study was approved by the ethical committee of Showa University Dental School, Tokyo, Japan. Blood and gingival crevicular fluid were collected from patients with dental problems at the Department of Periodontology of the Dental Hospital, Showa University, from September 2007 to June 2008. Fourteen patients with no systemic disease were invited to participate in the present study. Exclusion criteria included the following: (1) known systemic disease; (2) history and/or presence of systemic infectious disease; or (3) pregnancy or lactation in females. All the subjects had healthy gingivae and showed no significant inflammation or attachment loss.

The smoking history of the patients was checked according to a standardized questionnaire. Following this questionnaire, patients were classified as either smokers, i.e. regular daily smokers (current smokers), or non-smokers, i.e. patients who had never smoked cigarettes. Former smokers, i.e. patients who had previously been smokers but had stopped the habit, were excluded.

Gingival crevicular fluid sampling

Gingival crevicular fluid samples were collected from one site per subject. A buccal maxillary anterior tooth with a shallow pocket was selected based on the periodontal examination record. Samples were carefully collected from subjects after scaling in the initial preparation or maintenance phase. Note that all the teeth chosen for gingival crevicular fluid sampling had no periodontitis from the initial preparation and throughout the maintenance phase. The site of gingival crevicular fluid collection was isolated with cotton wool rolls, and saliva was gently removed from the adjacent tooth surface and gingiva using an air syringe. Two methods for gingival crevicular fluid collection were compared to determine the best for lipoprotein analysis. In the first method, the gingival crevicular fluid that had accumulated in the site under investigation was sampled using two paper points (Yoshida Co., Tokyo, Japan), gently inserted into the pocket and left there for 1 min, and transferred into 100 μL of ice-cold sterilized phosphate-buffered saline (PBS) containing protease inhibitor cocktail (Sigma, St Louis, MO, USA; final 5% v/v). After 5 min, the same procedure was repeated with new paper points and the gingival crevicular fluid was added to the same tube. In the second method, the gingival crevicular fluid that had accumulated in the pockets was sampled using

paper strips (Periocol®; Yoshida Co.) for 5 s. The volumes of gingival crevicular fluid exuded in the selected sites were estimated using paper strips and a Periotron 8000 (ProFlow, New York, NY, USA), which was calibrated by adding known volumes of water to the paper strips and then recording the number displayed electronically. All samples with bleeding were excluded. When a known volume (0.25–1.25 μL) of 1 mg/mL bovine serum albumin (BSA) solution was applied on the paper strip, a good linear correlation was observed between the volume of the sample fluid and the electro-resistance measured by the Periotron 8000 (data not shown). Gingival crevicular fluid samples were stored at 4°C until analysis of LDL and OxLDL.

Recovery of lipoproteins

In the present study, the recoveries of LDL and OxLDL from two devices for collection of gingival crevicular fluid, namely paper points and paper strips, were evaluated. The LDL or OxLDL was added to fetal bovine serum (FBS) to make FBS containing 100 μg/mL human lipoproteins. One microliter of FBS containing 100 ng lipoprotein was applied onto paper points and paper strips. After the devices were placed in 300 μL PBS, the amount of lipoproteins eluted in the PBS was determined by ELISA (see below).

Blood sampling

Venous blood (10 mL) was drawn from brachial veins of the subjects using a collecting tube containing sodium-EDTA, and 6 mL of the 10 mL blood sample was used to measure the following parameters: total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride in fresh plasma at BML Co. (Tokyo, Japan). The rest of the sample was immediately centrifuged at 15,000 *g* for 15 min to separate plasma for the measurements of LDL and OxLDL. No efforts were made to modify the diets of the subjects in any way prior to blood collection.

The LDL fraction was separated using sequential ultracentrifugation as described previously (19). Phosphate-buffered saline containing 250 μM EDTA (PBS–EDTA) was layered on top of plasma, and the tubes were centrifuged at 600,000 *g* (Himac CS150GX; rotor S100AT6; Hitachi, Tokyo, Japan) for 7 min at 4°C. The superficial layer of the samples was discarded to remove chylomicrons. A further 900 μL of PBS–EDTA was layered onto the remaining 2.7 mL, and the samples were centrifuged at 600,000 *g* for 2.5 h at 4°C to separate very low-density lipoproteins (VLDL). The top 900 μL was discarded again, and 540 μL of 0.5 g/mL KBr added to the remainder to form a mixture (density = 1.019). After centrifugation at 600,000 *g* for 2.5 h at 4°C, the superficial layer (540 μL/tube) was recovered as the LDL fraction. The LDL fraction was dialyzed against PBS–EDTA to remove KBr. Protein concentration was determined using BCA protein assay reagent (Thermo, Rockford, IL, USA) with BSA as standard.

Stability of lipoproteins during storage in freezer

Plasma LDL fraction (200 μg/mL) was mixed with the same volume of FBS. Low-density lipoprotein (100 μg/mL)/50% FBS was diluted 1:1 with a stabilizer (Kyowa Medex Co., Tokyo, Japan) for OxLDL measurement. Low-density lipoprotein concentrations of the mixed samples were measured just after dilution or after storage at −80°C for 10 d by using ELISA for ApoB detection. Gingival crevicular fluid samples were diluted 1:1 with the stabilizer, and OxLDL concentration in the gingival crevicular fluid was measured before and after storage at −80°C for 10 d.

Measurements of ApoB and OxLDL levels

The amount of ApoB was measured by sandwich ELISA (7). Apolipoprotein B is the major protein in LDL particles and mainly distributes to the LDL fraction, although ApoB is also present in VLDL and chylomicron fractions. The microtiter wells were coated with 100 μL of an anti-human ApoB monoclonal antibody (H454640M; Biodesign, Kennebunk, ME, USA) diluted to 10 μg/mL in PBS, and incubated at room temperature with shaking for 2 h. After blocking the plates with 20 mM Tris-buffered saline (TBS) containing 1% BSA, 100 μL of gingival crevicular fluid samples were incubated in the wells at room temperature for 2 h, and then at 4°C overnight. Then the plates were washed three times with 200 μL of TBS containing 0.1% Tween-20 (TBS-T). Sheep anti-human ApoB polyclonal antibody (binding site, PC086; 1:1000 dilution in PBS) was added to the wells and incubated at room temperature with shaking for 2 h. The plates were washed three times with 200 μL of TBS-T. Alkaline phosphatase-conjugated donkey anti-sheep immunoglobulin G polyclonal antibody (1:4000 diluted in TBS containing 1% skimmed milk) was added to the wells and incubated at 37°C for 1 h. The plates were washed five times with 200 μL of TBS-T and then once with Tris–HCl, pH 8.8. The plates were incubated with 100 μL per well of *p*-nitrophenyl phosphate for 30 min at 37°C. The absorbance at 405 and 490 nm was measured using a microplate reader (model 680; Bio-Rad Laboratories, Hercules, CA, USA).

The OxLDL levels were measured using a sandwich ELISA kit MX (Kyowa Medex Co.) according to the manufacturer's instructions (20). The sandwich ELISA kit uses monoclonal antibody against oxidized phospholipid, DLH3. The DLH3 was originally raised by immunizing mice with human atheromatous homogenates (19). The DLH3 binds to OxLDL but not to native LDL or chemically modified LDL. It was shown that DLH3 recognizes oxidized phosphatidylcholine molecules. Briefly, diluted gingival crevicular fluid samples were placed into microtiter wells precoated with anti-OxLDL monoclonal antibody, DLH3. After washing the wells, the bound OxLDL was detected using anti-ApoB polyclonal antibody and horseradish peroxidase-conjugated second antibody.

Western blotting

The presence of ApoB-containing lipoproteins was confirmed by western

blot analysis. Proteins in the gingival crevicular fluid were separated by SDS–PAGE (4–12% gradient gels). After electrophoresis, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane. The blotted PVDF membrane was soaked in PBS containing 2% skimmed milk at 4°C overnight to block the membrane surface. After being washed five times in TBS-T, the membrane was treated with mouse anti-human ApoB monoclonal antibody (1:1000 dilution) and incubated at room temperature with shaking for 1 h. After washing five times with TBS-T, it was treated with horseradish peroxidase-conjugated donkey anti-sheep immunoglobulin G polyclonal antibody (1:50,000 dilution) and incubated at room temperature with shaking for 1 h. Finally, the membrane was visualized using ECL-Plus (GE Healthcare, Buckinghamshire, UK).

Statistical analysis

Results are presented as the means ± SD. Statistical analysis was performed using Student's paired *t*-test and the coefficient of correlation. For analysis, STATVIEW™ version 5.0 (SAS Institute Inc., Cary, NC, USA) was used.

Results

Gingival crevicular fluid and blood samples were collected from 14 subjects. The basic characterization of the subjects is presented in Table 1. To measure LDL and OxLDL concentrations in gingival crevicular fluid accurately, it is important to collect lipoproteins in gingival crevicular fluid samples quantitatively. Two methods to collect gingival crevicular fluid, namely paper points and paper strips, were examined to see whether they were suitable for the measurement of lipoproteins. Onto each paper point or paper strip, 1 μL of FBS containing 100 μg/mL lipoprotein was applied, and then three paper points or three paper strips were placed in 300 μL of PBS to elute lipoproteins. The amounts of the human lipoproteins recovered in the PBS were determined by sandwich ELISA. Paper strips are widely used to analyze gingival crevicular fluid, and are also useful for measuring the volume of gingival crevicular fluid exuding from the periodontal pockets. We found that the recovery of LDL and OxLDL from the paper strips was about 25%, whereas more than 85% of the lipoproteins were recovered from the paper points. The procedure of collecting gingival crevicular fluid with paper points was carried out easily and safely. Therefore, we decided to use paper points rather than paper strips to collect gingival crevicular fluid in this study (Fig. 1).

Table 1. Characteristics of the patients

Parameter	Total
Age (years)	49.5 ± 19.2
Females (%)	50.0
Systolic BP (mmHg)	131.1 ± 16.3
Diastolic BP (mmHg)	79.7 ± 12.2
Pocket depth (mm)	2.6 ± 0.5
Cigarette smokers (%)	28.6
Total cholesterol (mg/dL)	209.4 ± 44.0
HDL-cholesterol (mg/dL)	65.0 ± 15.1
LDL-cholesterol (mg/dL)	118.9 ± 33.4
Triglyceride (mg/dL)	115.6 ± 78.4

Plasma and gingival crevicular fluid samples were collected from dental patients (n = 14). The data obtained for these patients are summarized in this table. Results are presented as the means ± SD.

For the characterization of gingival crevicular fluid, we examined whether ApoB, the major protein present in LDL particles, was present in gingival crevicular fluid. By sandwich ELISA using two anti-ApoB antibodies, ApoB was quantified in all the gingival crevicular fluid and plasma samples from the 14 subjects (Table 2). We found that ApoB was present in all the gingival crevicular fluid samples. It was noted that the concentration of ApoB in gingival crevicular fluid did not correlate with that in the plasma (r = 0.223, p = 0.451).

The presence of ApoB in gingival crevicular fluid was further confirmed by western blotting (Fig. 2). Apolipoprotein B is a huge protein of about 550 kDa. We detected immunopositive bands in gingival crevicular fluid samples from periodontal sites (lanes 3–7), which corresponded to the band detected in the control plasma (lanes 1 and 2).

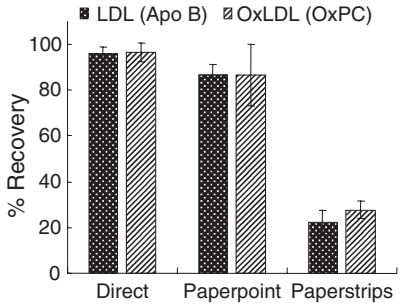

Fig. 1. Evaluation of the recovery of low-density lipoprotein (LDL) and oxidized low-density lipoprotein (OxLDL) from two devices for collection of gingival crevicular fluid, namely paper points and paper strips. One microliter of fetal bovine serum containing 100 ng of either LDL or OxLDL was applied onto paper points and paper strips. The devices were then placed in 300 μL phosphate-buffered saline. Results are presented as the means ± SD. Statistical analysis was performed using Student's paired *t*-test. The amounts of lipoproteins eluted in the PBS were determined (n = 4).

Table 2. Concentrations of apolipoprotein B (ApoB) in gingival crevicular fluid and plasma low-density lipoprotein (LDL) fractions

Patient number	ApoB in gingival crevicular fluid (μg/mL)	Plasma ApoB (mg/mL)
1	46.9	2.42
2	267.8	1.97
3	22.2	2.83
4	54.8	2.65
5	39.8	3.41
6	49.0	4.05
7	183.8	2.83
8	4.2	1.13
9	47.3	1.32
10	25.2	2.65
11	4.2	2.1
12	1.5	1.58
13	0.8	0.8
14	4.7	0.73
Average	53.7 ± 77.3	2.17 ± 0.99
(r = 0.233, p = 0.45)		

Amounts of ApoB in gingival crevicular fluid samples and plasma LDL fractions were measured by a sandwich ELISA that uses two anti-ApoB antibodies. The volume of gingival crevicular fluid collected from each tooth was estimated by the data from the Periotron 8000. Results are presented as the means ± SD. Statistical analysis was performed using coefficient of correlation.

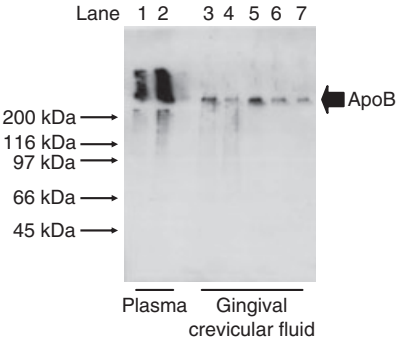

Fig. 2. Detection of apolipoprotein B (ApoB) in gingival crevicular fluid by western blotting. After gingival crevicular fluid samples were subjected to SDS–PAGE, proteins transferred onto a PVDF membrane were reacted with anti-ApoB antibody. Lanes 1–2, 0.03 and 0.06 μg LDL, respectively (control); lanes 3–5, 15 μL of gingival crevicular fluid from periodontally diseased sites; and lanes 6 and 7, 15 μL gingival crevicular fluid samples from healthy sites.

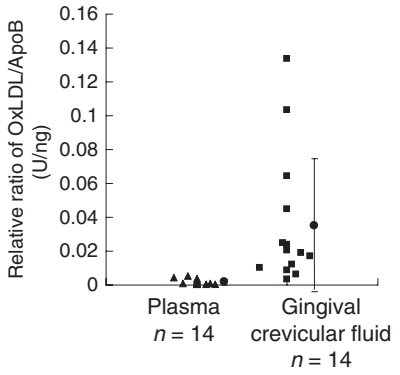

Fig. 3. The OxLDL/ApoB ratios in gingival crevicular fluid and plasma samples collected from 14 subjects. Symbols used are: triangle, each value for OxLDL/apoB ratio in plasma LDL fraction; square, each value for OxLDL/apoB ratio in gingival crevicular fluid; circle and error bar, the mean ± SD. Statistical analysis was performed using Student's paired *t*-test.

We measured the OxLDL levels present in human plasma and gingival crevicular fluid by a sandwich ELISA. When the ELISA kit MX for OxLDL measurement was applied to gingival crevicular fluid samples, we found that there were detectable amounts of OxLDL in gingival crevicular fluid. Since the volume of gingival crevicular fluid collectable from each tooth varies from 0.2 to 1.0 μL, the OxLDL values themselves cannot be directly compared. Thus, the ratio of OxLDL/ApoB was calculated to evaluate the OxLDL levels of gingival crevicular fluid samples. The mean OxLDL/ApoB ratio of the gingival crevicular fluid samples was 0.03 units/ng, which was 17.0 times higher than that in plasma samples from the same subjects (Fig. 3). Four of the 14 subjects were smokers, and the all smokers on average consumed 20 cigarettes/d and had been smoking for more than 10 years. The mean OxLDL/ApoB ratios for smokers and non-smokers were 0.041 ± 0.037 and 0.022 ± 0.012 ($p = 0.17$), respectively. In the present study, although the OxLDL/ApoB ratio was higher in the smokers, it is not clear whether smoking affects OxLDL levels because of the high *p* value and small number of samples.

It is important to assess whether gingival crevicular fluid samples can be stored in a freezer before measument for future large-scale lipoprotein analysis. When LDL was stored at −80°C for 10 d as a mixture of LDL, FBS and the stabilizer for OxLDL measurement (Kyowa Medex Co.), recovery of LDL was 99.1% of the prestorage value. After storage of gingival crevicular fluid samples mixed with the stabilizer at −80°C for 10 d, the OxLDL values detected were 97.6% of the the prestorage values, suggesting that OxLDL measurements can be applied when gingival crevicular fluid samples are properly stored at −80°C.

Discussion

Gingival crevicular fluid can be collected using a non-invasive procedure and could provide a useful source of biochemical markers for systemic and or local inflammation. In the present study, we applied a sandwich ELISA method to measure OxLDL in human gingival crevicular fluid samples and found considerable amounts of OxLDL in gingival crevicular fluid from dental patients. To our knowledge, this is the first report to show the presence of OxLDL in human gingival crevicular fluid.

Oxidized LDL is a well-known marker for atherosclerosis, and measurement of plasma OxLDL levels could provide predictive information for secondary prevention of patients with cardiovascular diseases (17). In contrast, it was demonstrated that serum OxLDL levels decreased significantly after periodontal treatment (21). It will be interesting to clarify OxLDL behavior in periodontal disease. Gingival crevicular fluid is a tissue exudate at the site of periodontal pockets and is thought to be derived from circulating plasma; however, precise characterization of lipoprotein components in gingival crevicular fluid has not been reported.

We found that ApoB, the major protein component in plasma LDL, is present in gingival crevicular fluid. In fact, the amount of ApoB in gingival crevicular fluid correlates with the volume of gingival crevicular fluid exuding from the pockets (data not shown). We also found that OxLDL is present in gingival crevicular fluid. The OxLDL/LDL ratio in gingival crevicular fluid does not correlate with that of plasma, and the OxLDL/LDL ratio is 17.0 times higher in gingival crevicular fluid despite the fact that the LDL concentration is lower in gingival crevicular fluid. This provides evidence for the hypothesis that the oxidative modification of LDL can occur locally. The oxidation reagent responsible for OxLDL formation *in vivo* has not yet been clarified. One possibility is that exposure of LDL to oxygen in the atmosphere might cause oxidative modification when LDL is exuded into gingival crevicular fluid. Another plausible candidate is myeloperoxidase released from neutrophils or macrophages. Myeloperoxidase not only generates reactive oxygen species such as hypochlorous acid but also modifies amino acid residues by chlorination and nitration reactions, which are not inhibited in the presence of serum (22). Our results suggested that local oxidative stress developed in gingival tissue, which is in agreement with previous reports (23–26). Recently, Akalin *et al.* (27) reported that thiobarbituric acid-reactive substances, which are widely used to detect lipid peroxidation products such as malondialdehyde, increased in gingival crevicular fluid. They pointed out that the total amount of malondialdehyde in gingival crevicular

fluid, rather than its concentration, displayed a strong relation with periodontal status. Several other studies reported an increase in oxidative markers in gingival crevicular fluid from periodontally diseased sites compared with healthy sites (23–26). Our preliminary data also indicated that the increased amount of OxLDL in gingival crevicular fluid, but not the ratio of OxLDL/LDL, significantly correlates with the pocket depth. It is possible that lipid oxidation products in gingival crevicular fluid could enhance the inflammatory reaction in periodontitis. A number of studies have detected the presence of OxLDL in atherosclerotic lesions in the aorta, and close co-localization of OxLDL and inflammatory cells such as macrophages were observed (8). Considering these findings together with our results, it is likely that the local inflammatory responses in gingival tissues could cause OxLDL generation.

In this study, we used antibodies recognizing oxidized phosphatidylcholine and ApoB. Not only LDL but also VLDL and chylomicrons contain ApoB as their protein components. Although the amounts of VLDL and chylomicrons are much smaller than LDL in normal human plasma, the occurrence of oxidized VLDL or oxidized chylomicrons cannot be ruled out. Unfortunately, the sample volume of gingival crevicular fluid was too small to permit analysis of the lipid composition of the gingival crevicular fluid, but this will be examined in a future study.

It is interesting that the OxLDL/LDL ratio in gingival crevicular fluid does not correlate with that in plasma. Plasma OxLDL is thought to originate from atherosclerotic lesions and would reflect the severity of plaque vulnerability and inflammatory activity. However, we speculate that OxLDL in gingival crevicular fluid does not originate from circulating plasma, but is rather generated in local periodontal tissue. It is possible that local OxLDL in gingival crevicular fluid facilitates the inflammatory response in periodontal lesions. We are now preparing a manuscript indicating that the inflamed response of human gingival epithelial cells was increased in the presence of OxLDL *in vitro* (K. Suzuki, Y. Sakiyama, M. Usui, T. Obama, R. Kato, H. Itabe and M. Yamaoto unpublished data). Since gingival crevicular fluid samples can be stored in the freezer, OxLDL measurements can be applied in large-scale epidemiological studies in the future to elucidate their clinical relevance.

Recently, a number of studies suggested that periodontitis is associated with the progression of cardiovascular diseases (28–37). In contrast, it is indicated that several systemic disorders, such as diabetes, obesity, hypertension and hyperlipidemia, are risk factors for periodontitis. Although an association between periodontitis and lipid metabolism has been reported, the precise biochemical mechanism has not yet been clarified. Some studies have focused on the tissue oxidative stress, examining total oxidant status and lipid peroxidation (23–26), leading us to consider the possible occurrence of OxLDL in periodontal tissues.

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