Increased lipid peroxidation in adult periodontal patients: Reactivity of their sera with thiobarbituric acid

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[Accepted for publication : January, 14, 1986]

Key words: lipid peroxidation / adult periodontitis / thiobarbituric acid / malondialdehyde

Introduction
Damage induced by the oxygen radical has been implicated in many pathological processes1-3). Also, unsaturated lipids are peroxidated by oxygen radicals released from phagocytic cells4-7). The experimental observation of oxygen radicals in periodontal disease are limited8). The lipid peroxidated products may also play a significant role in tissue destruction in inflamed sites. To ascertain a possible correlation between lipid peroxidation in adult periodontal patients and the severity of their disease, we looked for a correlation between lipid peroxidation and the status of the disease by measuring the amount of thiobarbituric acid (TBA)-reactive materials in sera from adult periodontal patients having varied degrees of severity of the disease.

Materials and Methods
Periodontal subjects (age range: 20-64 years) at Josai Dental University Hospital were characterized by Russell's periodontal index (PI)9). Control subjects (PI≤0.1) were healthy individuals who had not received dental treatment for at least 7 months before this study. Forty-two subjects were divided into healthy subjects (PI≤0.1, n=13), PI-1 group (0.1<PI≤2.0, n=10), PI-2 group (2.0<PI≤4.0, n=7), and PI-3 group (4.0<PI≤8.0, n=12). All sera were stored at −70°C for 4 months.

The amount of TBA-reactive malondialdehyde, a by-product of lipid peroxidation, was estimated by the method of Yagi10). Twenty μl of serum was added to 4 ml of 41.7 mM H₂SO₄ and mixed gently. Then, 0.5 ml of 10% phosphotungstic acid was added, and the mixture was centrifuged at 1,000×g for 10 min at room temperature. The precipitate was suspended to 2 ml in 41.7 mM H₂SO₄; and subsequently 0.3 ml of 10% phosphotungstic acid was added, after which centrifugation was carried out at 1,000×g for 10 min. The precipitate from this centrifugation was then suspended in 4 ml of distilled water, followed by the addition of 1 ml of 0.335% TBA in 50% acetic acid. Four ml of distilled water or 4 ml of 0.5 nmole 1, 1, 3, 3-tetramethoxypropane was added to 1 ml of 0.335% TBA in 50% acetic acid and used as negative or positive controls. All samples were incubated in boiling water for 10 min and then cooled in tap water. Next, 5 ml of n-butanol was added to each sample. The samples were mixed well and centrifuged at 1,000×g for 10 min. Finally the upper layer was taken, and its fluorescence at 553 nm was measured with 515 nm excitation by fluorometer (JASCO model FP-550).

Results
Fig. 1 shows that the level of TBA-reactive materials in serum of subjects tended to increase as the disease advanced. The levels in sera of patients with the disease were significantly higher than that of healthy subjects. The high TBA-reactive materials levels in
Fig. 1 Relationship between TBA-reactive materials in serum and severity of adult periodontitis. TBA-reactive materials are expressed as the amount of malondialdehyde (MDA). Open circles and bars indicate means and standard errors, respectively.

*,**Significant differences from healthy subjects (* p < 0.025 and ** p < 0.01) estimated by Student's t-test.

Serum from a few PI-3 patients were investigated. When the content of TBA-reactive materials was plotted against each PI value of subjects, the correlation coefficient was 0.44 (p < 0.05) (data not shown).

Since it is well known that the level of serum lipid oxidized products elevates with advancing age, Table 1 presents the relationship between age and the content of TBA-reactive materials of healthy and periodontal subjects (n = 42). The serum malondialdehyde levels of 30 to 39 and 40 to 64 year-old groups were higher than that of 20 to 29 year-old group. However, no significant positive correlation between age and the degree of severity of the disease was found in 29 periodontal patients (Fig. 2).

**Discussion**

Oxygen radicals are involved in tissue damage. Lipid peroxides and hydroperoxides in the body are considered to be formed by the peroxidation of unsaturated lipids with oxy-

<table>
<thead>
<tr>
<th>Age range</th>
<th>Malondialdehyde (nmole/ml serum)</th>
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<tr>
<td>20-29(n=20)</td>
<td>2.47±0.14</td>
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<tr>
<td>30-39(n=12)</td>
<td>3.22±0.26*</td>
</tr>
<tr>
<td>40-64(n=10)</td>
<td>3.62±0.35*</td>
</tr>
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Values represent mean±SEM.

* Significant difference from 20-29 year-old subjects (p < 0.01) estimated by Student's t-test.
gen radicals produced by phagocytic cells\textsuperscript{4-7}). Since phagocytic cells are known to infiltrate actively into inflamed sites in periodontal patients and then to be activated with some pathogens, it is very interesting to examine whether the amount of lipid peroxides and hydroperoxides become elevated with the advancement of the periodontal disease.

The present study suggests that the amount of lipid peroxides and hydroperoxides in the sera of adult periodontal patients is significantly higher than that of healthy subjects. The level of serum TBA-reactive materials was examined for a correlation with both age and PI. However, no significant correlation between age and PI of periodontal patients was observed. Thus, the increased serum TBA-reactive materials level with PI seems to be distinct from the increase of serum TBA-reactive materials that occurs with advancing age.

Although TBA-reactive materials do not always reflect the amount of lipid peroxides and hydroperoxides, the TBA reaction has been used generally to assay malondialdehyde which is a by-product in the peroxidation process of unsaturated fatty acids\textsuperscript{11}). Therefore, we consider that the elevated level of the TBA-reactive materials in sera from these patients may provide supportive evidence for the participation of lipid peroxides and hydroperoxides in periodontal disease.

Acknowledgements

We thank Dr. K. Okutsu (Department of Chemistry, Josai Dental University) for his guidance regarding fluorometric measurements. Also, we wish to thank Dr. L. D. Frye for reviewing the manuscript.

This work was supported by grants from the Scientific Research Funds (No. 60570876 and No. 60771538) of the Ministry of Education, Science and Culture of Japan.

References


