Effects of obstructive sleep apnea on circulating ICAM-1, IL-8, and MCP-1

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Obstructive sleep apnea syndrome (OSAS) is related to inflammatory process induced by activation of proinflammatory mediators, including adhesion molecules (11) and cytokines (10, 31). To induce leukocyte migration to inflamed tissue, it is essential for leukocytes to adhere to microvascular endothelium (32). Potential mediators responsible for leukocyte attachment to endothelium include intercellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1). It has been reported that ICAM-1, a member of the immunoglobulin superfamily, is required for leukocyte migration into inflamed area (3, 6, 35) and plays an important role in inflammatory disease, including bronchial asthma, lung injury, and ischemic heart disease (18–22). IL-8, a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, is a potent angiogenic factor that may play a substantial role in atherosclerosis (4, 31). Increased expression of IL-8 has been reported in atherosclerotic lesions and circulating macrophages from patients with atherosclerosis (31). MCP-1 is upregulated in human atherosclerotic plaques, suggesting a role for MCP-1 in the development of early atherosclerotic lesions (5, 10).

Hypoxic stress increases the adherence of neutrophils to endothelial cells, and this increased adherence is mediated by proinflammatory mediators, including ICAM-1 (2) and IL-8 (13, 30). Furthermore, it has been reported that hypoxia induces the synthesis and expression of both ICAM-1 and IL-8 via the activation of nuclear transcription factor (NF)-κB (7, 36, 37).

In the treatment of OSAS, the efficacy of nasal continuous positive airway pressure (nCPAP) has been reported (8, 27). nCPAP improves sleepiness and quality of life in patients with OSAS, probably because nCPAP intervention removes sleeping upper airway collapse and decreases apnea episode (27). Although it is expected that nCPAP may ultimately improve the prognosis of various disorders associated with OSAS, its exact mechanism is not yet proven.

RECENTLY, IT HAS BEEN SHOWN that obstructive sleep apnea syndrome (OSAS) is related to obesity, insulin resistance, and diabetes mellitus (17, 28, 33). Moreover, OSAS could be one of the most important risk factors of cardiovascular disorders, including hypertension, ischemic heart disease, and cerebrovascular events (12, 15, 23, 25), whereas hypoxic stress elicited by OSAS may be involved in the development of cardiovascular disorders. However, the exact mechanism remains to be elucidated.

One of the potential mechanisms is that OSAS-induced hypoxic stress increases circulating inflammatory mediators, leading to cardiovascular lesions. It has been recently suggested that atherosclerosis is related to inflammatory process induced by activation of proinflammatory mediators, including adhesion molecules (11) and cytokines (10, 31). To induce leukocyte migration to inflamed tissue, it is essential for leukocytes to adhere to microvascular endothelium (32). Potential mediators responsible for leukocyte attachment to endothelium include intercellular adhesion molecule-1 (ICAM-1), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1). It has been reported that ICAM-1, a member of the immunoglobulin superfamily, is required for leukocyte migration into inflamed area (3, 6, 35) and plays an important role in inflammatory disease, including bronchial asthma, lung injury, and ischemic heart disease (18–22). IL-8, a CXC chemokine that induces the migration and proliferation of endothelial cells and smooth muscle cells, is a potent angiogenic factor that may play a substantial role in atherosclerosis (4, 31). Increased expression of IL-8 has been reported in atherosclerotic lesions and circulating macrophages from patients with atherosclerosis (31). MCP-1 is upregulated in human atherosclerotic plaques, suggesting a role for MCP-1 in the development of early atherosclerotic lesions (5, 10).

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In the present study, we hypothesized that nCPAP may decrease OSAS-induced hypoxic stress and the generation of proinflammatory mediators. To examine this hypothesis, we measured circulating ICAM-1 and IL-8 before and after nCPAP therapy in OSAS patients.

**METHODS**

**Subjects.** Among patients diagnosed as OSAS in our department, 20 male subjects participated in the present study. As age-matched controls, 10 male subjects were chosen and studied. No subjects had any history of cardiovascular, pulmonary, metabolic, or neuromuscular diseases. All subjects were in a stable condition for 1 mo before the study. The characteristics of the subjects in the OSAS and normal groups are shown in Table 1. There were no significant differences in age and body mass index (BMI) between the two groups, whereas apnea index (AI) in the OSAS group was markedly greater than that in the control.

After the polysomnography study, the patients with OSAS underwent therapeutic nCPAP treatment, and eight subjects continued to receive nCPAP successfully for 8–18 mo.

**Polysomnography.** The subjects underwent polysomnography for 2 consecutive nights. The polysomnography included an electroencephalogram, an electrooculogram, an electromyogram of the chin, and an electrocardiogram (DG Compact32, Medelec, Surrey, UK). We monitored ventilation and airflow using inductive plethysmography (Respitrace, Ambulatory Monitoring, Ardsley, NY) and thermistors (Fukuda-Sangyo, Chiba, Japan) placed at the nostril and mouth. Arterial oxygen saturation (SaO2) was continuously measured via pulse oxymeter (Datex, Helsinki, Finland). Data acquisition was performed overnight from 9:00 PM to 6:00 AM the next morning.

**Assessment of hypoxic episodes.** To assess OSAS-induced hypoxia, we applied desaturation magnitude (DM) in this study. Desaturation episodes were defined as hypoxia of SaO2 <90%. We defined DM as

\[
DM = \sum (90 - SaO2)t
\]

where \( t \) is time of desaturation (in h). As shown in the equation, DM expresses the severity of hypoxic stress quantitatively.

**Measurements of circulating ICAM-1, IL-8, and MCP-1.** We obtained peripheral blood from the subjects at 9:00 AM before and after the nCPAP treatment. The blood samples were centrifuged at 250 g and 4°C for 10 min. The serum samples were then stored at −80°C until measurements. The concentrations of ICAM-1, IL-8 and MCP-1 in the serum were measured by ELISA method. The data are defined as

### Table 1. Characteristics of the subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Age, yr</th>
<th>Body Mass Index</th>
<th>Apnea Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSAS</td>
<td>20</td>
<td>47.8 ± 2.2</td>
<td>29.4 ± 1.4</td>
<td>38.9 ± 3.1*</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>48.9 ± 2.9</td>
<td>28.4 ± 2.9</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. OSAS; obstructive sleep apnea syndrome. *\( P < 0.001 \) vs. control group.
circulating ICAM-1 (cICAM-1), circulating IL-8 (cIL-8), and circulating MCP-1 (cMCP-1), respectively.

**Data analysis.** Comparisons of data between each experimental group were carried out with Student’s t-test. Data are expressed as means ± SE. P values <0.05 were taken as significant.

**RESULTS**

**Assessment of hypoxic episodes.** There were significant differences in baseline DM between the OSAS and normal groups (2.01 ± 0.66 and 0.02 ± 0.01, respectively; P < 0.001), suggesting that the OSAS patients were exposed to significantly greater degree of hypoxia compared with the control subjects.

**Baseline measurements of cICAM-1 and cIL-8.** Figure 1 summarizes the cICAM-1 and cIL-8 levels in the baseline measurements. The levels of both cICAM-1 and cIL-8 in the OSAS group were significantly greater than those in the normal group.

Figure 2 demonstrates the relationships between cICAM-1 and AI and between cICAM-1 and DM. As shown, significant correlations are observed between cICAM-1 and apnea episodes. Similarly, significant correlation between cIL-8 and DM is detected, whereas the positive correlation is suggested between cIL-8 and AI (Fig. 3).

As indicated in Fig. 4, cICAM-1 is significantly correlated with cIL-8.

**Effects of nCPAP on physiological parameters and circulating mediators.** After nCPAP, the improvement in sleepiness was observed in all of the OSAS patients who successfully received therapeutic nCPAP. Consequently, nCPAP significantly decreased apnea and desaturation (Fig. 5).

Figure 6 summarizes the effects of long-term nCPAP on cICAM-1 and cIL-8 levels. As shown, nCPAP longer than 8 mo significantly decreased the levels of both cICAM-1 and cIL-8 in the treated OSAS group.

Figure 7 summarizes the cMCP-1 level in the OSAS group and normal group. The level of cMCP-1 in the
OSAS group was significantly greater than that in the normal group.

DISCUSSION

The results of the present study demonstrate that nCPAP decreased apnea, desaturation, and the circulating ICAM-1 and IL-8 levels in the OSAS patients. In the baseline measurements, the levels of both ICAM-1 and IL-8 in the OSAS group were significantly greater than those in the control group. These observations suggest that nCPAP therapy could reduce OSAS-induced hypoxia and generation of inflammatory mediators, leading to the possible prevention of cardiovascular disorders.

Several issues warrant consideration before the results are discussed. First, we measured circulating ICAM-1 and IL-8 to assess the expression of cell-associated adhesion molecule and chemokine. Whereas this approach has been widely used (11, 20), it remains unclear whether the circulating levels of these mediators might precisely reflect the real expression of molecules attached to the endothelium or leukocytes. Second, the number of subjects in this study is relatively low, although the characteristics of the subjects were well matched. Increasing the number of subjects may be required to confirm the interpretation of the present results, and we should acknowledge this point.

It has been recently postulated that inflammatory process has a crucial role in the pathogenesis of atherosclerosis, leading to the various cardiovascular disorders (1, 9). To promote migration of leukocytes from circulation to inflamed areas, it is essential for leukocytes to adhere to vascular endothelium via adhesion molecules (32). Especially, ICAM-1 has been reported to play important roles in leukocyte migration to inflamed area (2, 3, 29). ICAM-1 is an 80- to 110-kDa glycoprotein consisting of five immunoglobulin-like domains and a ligand for LFA-1α (18, 32). It has been demonstrated that the ICAM-1/LFA-1α pathway evolves to function in cell-cell adhesion (33) and mediates various inflammatory diseases (16, 19, 21, 22, 35). Recently, it has been reported that the circulating ICAM-1 levels are higher in patients with ischemic heart disease than those in controls (20). Moreover, the circulating ICAM-1 level may indicate a risk of future myocardial infarction, suggesting that antiadhesion therapies can be considered as a novel therapeutic means of cardiovascular disease (26). In the previous study, we have demonstrated that the circulating ICAM-1 level is significantly increased compared with the control group, suggesting that OSAS-induced hypoxia may induce the activation of ICAM-1 and the inflammation of endothelium in patients with OSAS (24). This observation may give rise to a hypothesis that the therapy for OSAS might be a potential approach to prevention of cardiovascular disorders via antiadhesion mechanism.

Recently, it has been demonstrated that IL-8 may play an important role in the development of atherosclerosis (4, 10, 31). Although monocytes contribute to the development of atherosclerotic lesions, IL-8 is a powerful trigger for firm adhesion of monocytes to vascular endothelium (10). It has been shown that hypoxia induces expression and generation of IL-8 (13, 30), indicating that OSAS-associated desaturation could lead to upregulation of IL-8 expression. In addition, one could presume that the effective therapy
for OSAS may attenuate hypoxic stress, which may prevent the development of vascular lesions via the reduction of IL-8 production.

To treat patients with OSAS, nCPAP therapy is widely used, because nCPAP reduces excessive daytime sleepiness and improves quality of life (8, 27). Based on the recent studies, beneficial effects of nCPAP on the prognosis of OSAS-associated diseases are anticipated, but there exists little evidence to prove this notion. We therefore performed this study to address the question whether nCPAP could affect physiological phenomena and production of proinflammatory mediators. We observed that long-term nCPAP was effective to improve sleepiness, nocturnal apnea, and desaturation and that the levels of circulating mediators were reduced after nCPAP. One of the possible explanations is that nCPAP decreases hypoxic episodes, resulting in the reduction of hypoxia-induced inflammation and expression of ICAM-1 and IL-8.

Considering the proinflammatory effects of ICAM-1 and IL-8, the attenuated production of these mediators elicited by nCPAP may suggest a novel approach to manage OSAS and prevent OSAS-associated inflammatory diseases.

To assess the severity of hypoxia induced by OSAS, we used DM. Possibly, this parameter may reflect OSAS-induced hypoxic stress more directly than AI. The usual way to assess the degree of OSAS includes the number of apnea episodes, but DM could reflect both decreases in SaO₂ and time spent below 90%. However, to accurately analyze the hypoxic stress, exploring other indexes of hypoxic stress may be important and helpful.

We observed that there was a significant correlation between circulating ICAM-1 and IL-8 in the population studied. It has been demonstrated that nuclear transcription factor (NF)-κB regulates the synthesis and expression of both ICAM-1 and IL-8 (26, 27). In addition, NF-κB is upregulated by hypoxia, leading to the increased expression of both ICAM-1 and IL-8 (7, 37). These reports may explain the present findings that there were significant correlations between desaturation and mediators measured.

We further investigated the level of circulating MCP-1 in the normal and OSAS groups. Recently, it has been reported that the level of MCP-1 is increased in patients with coronary heart disease (14). In the present study, we observed that the level of MCP-1 in the OSAS group was increased compared with that of the normal group. Possibly, the increases in the circulating chemokines, including MCP-1, may play an important role in the pathogenesis in OSAS patients complicated with cardiovascular disease.

In summary, the circulating ICAM-1, IL-8, and MCP-1 levels increased in the OSAS patients compared with the normal subjects. After nCPAP therapy, significant decreases in the levels of ICAM-1 and IL-8 were observed in the OSAS group. Taken together, OSAS-induced hypoxia activates ICAM-1 and IL-8, resulting in the important risk factor of cardiovascular disorders. Treatment of OSAS with the use of nCPAP can be, therefore, a potential approach to decrease risk of the progression of OSAS-associated disorders.

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REFERENCES


