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Platelet Activation in Patients with Obstructive Sleep Apnea Syndrome and Effects of Nasal-Continuous Positive Airway Pressure

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***Objective*: Our study was undertaken to determine whether increased platelet activation occurs in patients with obstructive sleep apnea syndrome (OSAS) and whether a therapy with nasal-continuous positive airway pressure (N-CPAP) alters this activation. *Methods*: We measured the positive rate of activated platelets using activation-dependent monoclonal antibodies (MoAb) and flow cytometry in whole blood from 94 patients with OSAS, and from 31 age-matched controls. Thrombotic vascular diseases were surveyed as a background of alternative of platelet activation.**

***Results*: The positive rate for activated platelets was significantly higher in patients with OSAS (PAC1 52.6 ± 22.9 %, CD62P 6.8 ± 7.1 %, mean ± SD), as compared with healthy control subjects (PAC1 16.7 ± 8.6 %, CD62P 0.7 ± 0.5 %, $p < 0.001$). The activation indexes were significantly reduced after 1 month with N-CPAP treatment as a whole (PAC1; from 52.6 ± 22.9 to 44.2 ± 22.4, $p < 0.05$, CD62P; from 6.8 ± 7.1 to 5.3 ± 5.5, $p < 0.05$). In nearly 60 % of patients, platelets activation remained high despite significant improvement of sleep apnea-episodes after N-CPAP. These patients had significantly higher incidence of previous myocardial infarction and/or cerebral infarction and abnormalities of head MRI and carotid sonograpy; indicating that the platelet activation appears to be induced by existing atheroma plaque and not by sympathetic activity in OSAS. *Conclusion*: In conclusion, patients with OSAS have increased percentages of activated platelets as assessed by flow cytometrical analysis of activation dependent surface markers, and were divided into two groups, one group with response to N-CPAP treatment in the reduction of platelet activation and the other without. One possible reason of no response to N-CPAP treatment in the reduction of platelet activation was suggested to be thrombotic diseases.**

Key words : Obstructive sleep apnea syndrome, Platelet activation, Thrombotic disease, PAC1, Nasal-Continuous Positive Airway Pressure

INTRODUCTION

In is well known that patients with obstructive sleep apnea syndrome (OSAS) have an increased risk of cardiovascular complications including myocardial infarction (MI) and stroke [1–3]. The mechanism underlying increased risk was suggested to be platelet activation in OSAS. On the other hand, in thrombotic vascular diseases platelet are activated probably by the contact with atheromatous plaques and by high shear stress at narrowed vessels [4, 5].

Recently we found significant platelet activation in patients with OSAS [6], which was consistent with previous suggestions [7, 8]

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and supported the notion of an increased sympathetic tone during hypoxic period at night. Thus the effect of nasal continuous positive air pressure (N-CPAP) treatment on platelet activation is expected. However, its effect has not been established yet.

The purposes of this study are to see, 1) whether platelet activation regresses after N-CPAP treatment, 2) whether patients with platelet activation has an increased risk for thromboembolic events, and 3) whether effect of N-CPAP treatment on platelet activation in OSAS can differentiate the OSAS-induced platelet activation from atheromatous plaque-induced activation.

SUBJECTS AND METHODS

Patients

We enrolled 94 patients (87 males and 7 females, mean age 48 ± 12 years) who were diagnosed as OSAS in this study. Thirty-one normal volunteers (26 males and 5 females, mean age 53 ± 15 years) served as control. Patients under anti-platelet therapy were excluded from this study. Informed consent was obtained from all patients before entry into the study.

OSAS was diagnosed by clinical history and polysomnography using criteria made by American Thoracic Society [9] [apnea-hypopnea index (AHI) > 20 /h, percentage of time during sleep with an oxygen saturation below 90 % (t < 90 %) > 5 %]. In each patient a polysomnography was performed at study entry, in order to confirm and quantify the OSAS and to rule out any other sleep disturbances.

The polysomnography included electroencephalograms (EEG) and electromyogram (EMG) that were recorded according to guidelines of American Thoracic Society .

The oronasal airflow was also recorded via thermistors mounted over the nose and mouth, and the thoracic and abdominal respiratory movements were recorded by impedance plethysmography. Arterial oxgen saturation was measured continuously via a noninvasive infrared finger probe. Apnea was defined as a cessation of airflow lasting at least 10 seconds, accompanied by a drop of saturated oxygen (SaO_2) by more than 2 % below the immediately preceding baseline. For hypopnea scoring we applied a combination of criteria widely used in American Sleep Disorders Association (ASDA) [10].

To evaluate whether the patients with OSAS have thrombotic complications such as cardiovascular diseases and/ or cerebrovascular diseases, we carefully checked the medical history of all patients. Head MRI and carotid sonography were carried out for patients who agreed to take them.

N-CPAP treatment

Patients were subjected to polysomnographic study during which N-CPAP was applied and regulated at the proper level in order to prevent apneas [11]. For N-CPAP treatment, patients were requested to stay two nights in the hospital. In Tokai University Hospital, a diagnostic reassessment with the Auto Set device (Auto Set, Res Med, Australia) was performed without N-CPAP in the first night. N-CPAP titration with the Auto Set device was performed using N-CPAP in the second night.

Then, N-CPAP treatment in each patient has been continued for 1 month at home.

Sample collection

Blood sampling was done at 10 a.m. on the first day and 1month after N-CPAP treatment for OSAS patients and also at 10 a.m. for normal volunteers. Blood was obtained from antecubital vein with a light tourniquet. After the first 2 ml of blood was discarded, 4.5 ml of blood was collected into a plastic syringe (TERUMO, Tokyo, Japan) containing 0.5 ml of 3.13 % sodium citrate.

Staining

Within 15 minutes after blood collection, 2.5 μl aliquots of the blood were added to 12 × 15-mm polystyrene tubes containing a cocktail of 10 μl each of three kinds of fluorescence labeled monoclonal antibodies (MoAb). One fluorescent reagent, peridinin chlorophyll protein (PerCP)-MoAb-CD61 was used to identify the platelets, fluorescein isothiocyanate (FITC)-PAC1 and phycoerythrin (PE)-MoAb-CD62P were used to detect activated platelets (three color flow cytometry). PAC1 is a MoAb specific for fibrinogen receptors expressed on GPIIb/IIIa of platelet surface membranes when platelets are activated [12]. CD62P is a glycoprotein on the α-granule membranes and is exposed on platelet surface membranes when platelets are activated. The samples were incubated

for 15 minutes at room temperature in the dark, and then fixed with CELL-FIX (Becton Dickinson Biosciences, San Jose, CA) for 2 hours in the refrigerator.

To assess extent of nonspecific staining by antibodies, 2.5 μL of whole blood is incubated with 0.62 μL of mouse IgG1 PE isotype control, 10 μL of FITC-PAC-1 with 10 μL of 5 mg/mL RGDS solution, and 10 μL of PerCP-MoAb-CD61.

Flow Cytometry

Flow cytometric analysis was performed on a FACS Calibur (Becton Dickinson Biosciences, San Jose, CA) with standard 488-nm excitation. Instrument setting was performed by FACSComp software (Becton Dickinson Biosciences, San Jose, CA).

Platelets were identified by both side scatter profiles and platelet specific antibody, MoAb-CD61. PAC1 and CD62P positive platelets were counted and expressed as a percentage of platelets positive for antibody. Antibody-positive cells were defined as those platelets with a fluorescence intensity > 99.0 % of platelets that were determined in the presence of isotype IgG or fibrinogen receptor – blocking tetrapeptides, RGDS as negative control.

Statistical Analysis

The total platelet populations were displayed, including any light scatter gated subpopulation, as two-color dot plots and was analyzed statistically by nonparametric analysis and expressed as mean ± SD. And p-value of < 0.05 was considered to be significant. Chi- square test was used for incidence of vascular diseases.

RESULTS

1. Characteristic of platelet activation in patients with OSAS compared to controls

Table 1 shows control and patients. All patients tolerated. The percentages of platelets positive for PAC1 and CD62P positive platelets were significantly higher in patients with OSAS (PAC1 52.6 ± 22.9 % and CD62P 6.8 ± 7.1 %, respectively), as compared with healthy control subjects (PAC1 16.7 ± 8.6 % and CD62P 0.7 ± 0.5 %, respectively, $p < 0.001$) (Fig. 1).

2. Effects of N-CPAP treatment

After N-CPAP treatment both AHI and t < 90 % (%) by polysomnography improved significantly (Table 2). Pre- vs post- data were 43.1 ± 25.4 vs 6.01 ± 9.5 in AHI, $p < 0.001$ and 20.9 ± 26.4 vs 0.7 ± 1.6 in t < 90 % (%), $p < 0.001$. Similarly, percentages of platelets positive (%) for PAC 1 and CD62P were significantly reduced after N-CPAP treatment (PAC1, 44.2 ± 22.4 (%), $p < 0.05$, CD62P, 5.3 ± 5.5 (%), $p < 0.05$) as compared

Table 1 Age and sex of patients and control subjects

Clinical category	n (M/F)	Age (Mean ± SD)
control	31 (26/5)	53 ± 15
OSAS	94 (87/7)	48 ± 12

Table 2 Effect of continuous positive airway pressure therapy on polysomnographic data

	n (M/F)	AHI n/h		t < 90 % (%)	
		pre	post	pre	post
responders	39 (39/0)	43.1 ± 8.5	3.7 ± 2.7	25.0 ± 24.3	0.2 ± 0.3
		*		*	
non-responders[#]	55 (48/7)	44.7 ± 23.51[※]	3.3 ± 3.2[※]	19.2 ± 23.1[※]	0.4 ± 1.1[※]
		*		*	

#: non-responder; patient whose PAC1 and/or CD62P did not reduce.
AHI: apnea/hypopnea index.
t < 90 % : percentage of time during sleep with an oxygen saturation < 90 %.
* : $p < 0.001$, Values presented as mean ± SD by pre- and post- N-CPAP treatment.
※ : Difference in AHI and t < 90 % (%) in responders and non-responders and did not significant.

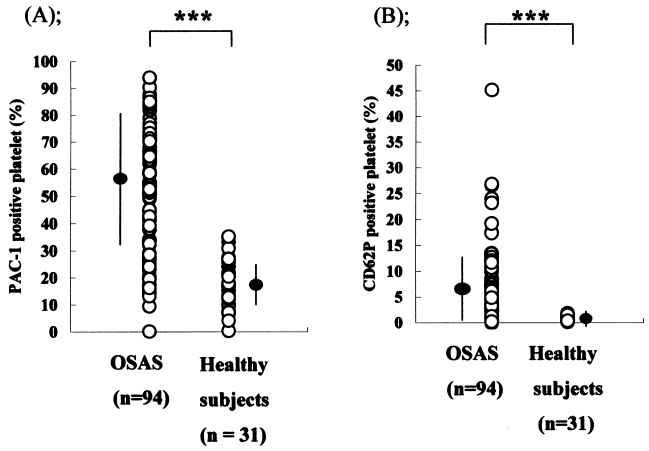

Fig. 1 Distribution aspects of the percentage of PAC1 positive platelet (%) (A) and CD62P positive platelets (%) (B) in patients with OSAS (n = 94) and the normal subjects (n = 31). Increased in vivo platelet activation in patients with OSAS as shown as a higher percentage (%) of PAC1 and CD62P positive platelets compared to healthy controls was observed. Differences in distribution between the two groups were significant according to the Mann-Whitney U test (*** $p < 0.001$).

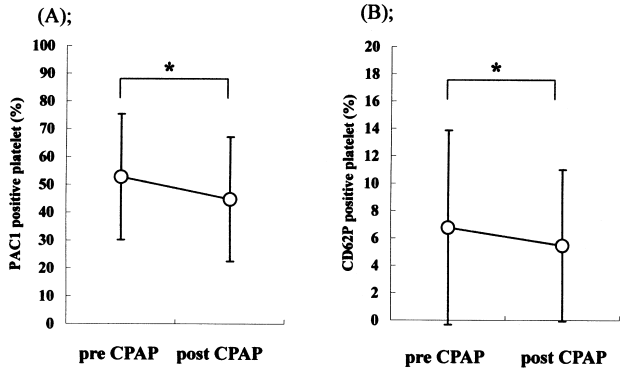

Fig. 2 Effects of N-CPAP treatment on the percentage of PAC1 positive platelets and CD62P positive platelets in patients with OSAS as a whole. Percentages of platelets positive for PAC1 and CD62P were significantly reduced after N-CPAP treatment as with those before N-CPAP treatment. * $p < 0.05$.

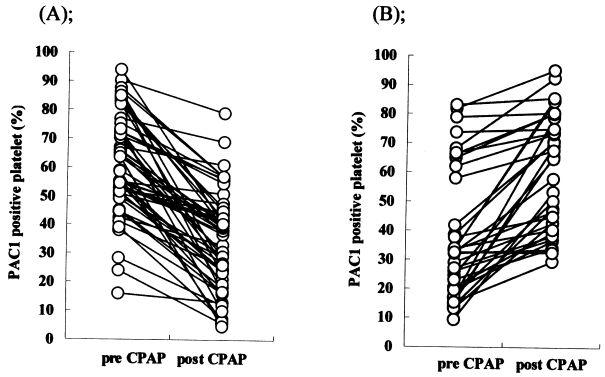

Fig. 3 Effects of N-CPAP treatment on the percentage of PAC1 positive platelets (%) in patients with OSAS. OSAS patients were divided into two groups, (A); with (n = 56) or (B); without (n = 38) response to CPAP treatment.

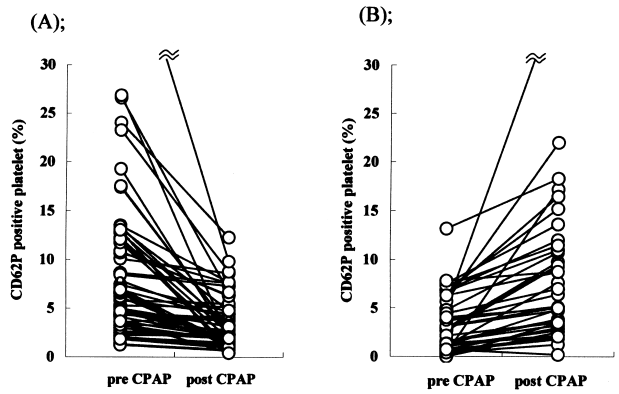

Fig. 4 Effects of N-CPAP treatment for one month on the percentage of CD62P positive platelets (%). OSAS patients were divided into two groups, (A); with (n = 55) or (B); without (n = 39) response to CPAP treatment.

Table 3 Incidence of thrombotic complications in OSAS patients who responded or responded poorly to CPAP treatment

Clinical category	age	Positive History for cerebral and/or myocardial infarction (n = 9)	Positive MRI and/or carotid sonography findings (cerebral infarction and/or carotid atherosclerosis) (n = 11)
responders (n = 7)	50.4 ± 11.3	1	0/6 ※
non-responders (n = 13)	57.9 ± 7.1	8	5/5 ※

※ : patients with positive findings.

with those before N-CPAP treatment in patients with OSAS as a whole (Fig. 2). In order to differentiate between OSAS-induced and atheroscleosis-induced platelet activation, we divided them into N-CPAP responder and non-responder groups. Thirty eight among 94 patients (40.4 %) for PAC1 and 39 among 94 patients (41.5 %) for CD62P did not respond to N-CPAP. In responders PAC1 and CD62P were reduced from 59.8 ± 20.2 to 31.1 ± 14.5 and 9.5 ± 9.0 to 3.1 ± 2.8, respectively (Fig. 3, 4). In non-responders they were 48.0 ± 23.6 and 55.1 ± 22.7, and 4.8 ± 4.5, 7.5 ± 6.2 for prior and after N-CPAP, respectively.

We reviewed the medical history of all 94 patients and found that 9 patients had been suffered from either myocardial infarction and/or cerebral infarction (CI). To further examine the presence of silent athermantors changes, we asked patients to have head MRI and carotid sonography. Then eleven patients agreed to take examinations of MRI and carotid sonography upon our request. Table 3 shows that thromboembolic events determined by medical history, MRI and carotid sonography. In responders there was only one patient who had thrombotic complication, whereas in non-responder groups 8 patients had a history of CI and/or MI. In MRI and carotid sonography none had abnormality in responders but 5 among 5 examined patients had positive findings in the non-responders. Mean age was also higher in non-responders than that in responders.

DISCUSSION

This study revealed a significant increase in PAC1 and CD62P positive platelets in patients with OSAS as compared to healthy controls.

The immediate physiological consequences of OSAS are nocturnal hypoxia, hypertension and sleep disruption, which can lead to an increased sympathetic activity as shown by increased epinephrine levels in patients with OSAS [13, 14]. The major cause of platelet activation in patients with OSAS has been suggested as elevated epinephrine

levels during night through insufficient oxygenation. Our previously study also showed significant correlations between increase in percentages of PAC1 positive platelets and the severity of OSAS as indicated by AHI or oxygenation parameters [6].

In this report we demonstrated the favorable effect of N-CPAP treatment on enhanced platelet activation in patients with OSAS as a whole. However, about 40 % of patients responded poorly to N-CPAP treatment. The failure of N-CPAP treatment to reduce the high percentages of activated platelets could be accounted for by insufficient duration of N-CPAP treatment as reported by Geiser *et al*. [15]. Thus we devided responders and non-responders of platelet activation and compared the efficacy of N-CPAP. As was shown in table 2 the improvement of sleep apnea was comparable in two groups. Improvement of the AHI and percentage of time of oxygen saturation under 90 % by N-CPAP treatments appears to result in the reduction of positive rate of activated platelets after N-CPAP treatment and presumably decrease catecholamine levels [13, 14] through the improvement of sympathetic activity. Therefore, other mechanisms of OSAS to activate platelets were suggested. We consider other possibilities of platelet activations such as arteriosclerosis and high shear stress [16, 17] in patients who do not respond to N-CPAP treatment. Results of medical history and examinations of MRI and carotid sonography showed higher incidence of vascular diseases in patients whose platelet activation was not improved by N-CPAP.

In conclusion, activation of platelets in patients with OSAS can be regressed when the predominant cause of activation is OSAS-induced one. Acivation of platelets in patients who do not respond to N-CPAP may be caused by atheromatous plaque. The latter patients tend to be suffered from thrombolic episodes. The differentiation of these subset of patients by N-CPAP response may have great significance for future evaluation.

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