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Original article

Oxygen administration improves the serum level of nitric oxide metabolites in patients with obstructive sleep apnea syndrome

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Abstract

Objectives and background: Nocturnal apnea and hypoxia are implicated in the pathogenesis of pulmonary and systemic hypertension in obstructive sleep apnea syndrome (OSAS). We have hypothesized that vasodilating factors including nitric oxide (NO) are affected by nocturnal apnea and hypoxia in patients with OSAS.

Method: We examined the serum level of NO production in 24 patients with OSAS (mean age 54.2 ± 7.9 years) and 24 age-matched control subjects (53.4 ± 8.1 years) and tested the effects of oxygen administration on the production of NO in the patients.

Results: The serum level of nitrite/nitrates (NO_x), which are stable metabolites of NO, was lower in patients with OSAS than in control subjects. Administration of 1–2 l/min of oxygen during night increased the patients' NO_x level from 35.6 ± 7.3 to 57.8 ± 11.6 μM. Compressed air administration did not affect the NO_x level in the patients.

Conclusion: These results indicate that systemic NO production is impaired in OSAS patients, possibly due to nocturnal hypoxia.

Keywords: Nitric oxide; Hypoxia; Obstructive sleep apnea syndrome; Oxygen administration

1. Introduction

Obstructive sleep apnea syndrome (OSAS) is now recognized as an important sleep disorder, contributing to excessive daytime sleepiness, cardiovascular dysfunction, and the impairment of health-related quality of life [1-6]. Hypoxia, hypertension, hypoxic pulmonary vasoconstriction, pulmonary hypertension, and altered cardiovascular variability are implicated in the subsequent development of overt cardiovascular diseases, resulting in increased mortality [7,8]. However, the mechanisms underlying the causal relationship between OSAS and cardiovascular diseases are largely unknown. Nitric oxide (NO) is one of the key regulators of vascular physiology [9,10]. Abnormalities of NO productions have been implicated in the pathogenesis of pulmonary hypertension [11,12]. The concentration of NO in the exhaled air appears to be reduced in patients with pulmonary hypertension [13]. Treatment of pulmonary hypertension with NO inhalation reduces pulmonary vascular resistance in patients with pulmonary hypertension [14]. We thus speculated that the production of NO might be impaired in patients with OSAS.

The aim of the present study was to compare the serum level of NO production between OSAS and control subjects. Furthermore, to examine the relationship between NO production and nocturnal hypoxemia in patients with OSAS, we examined the effects of oxygen administration on the production of NO in patients with OSAS.

2. Methods

2.1. Subjects

All patients referred for PSG had daytime fatigue, sleepiness, and/or snoring. From April 2000 to November 2001, we invited all patients who had been referred for

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a diagnostic PSG to participate in this study. Once consent had been received, patients underwent a diagnostic polysomnography (PSG).

Twenty-four patients with OSAS (19 men, 5 women, mean (±SD) aged 56 ± 4 (range 35–66)) and 24 age-matched controls (20 men; 4 women, mean (±SD) aged 53 ± 4 (range 30–68)) were studied. OSAS was defined as the presence on polysomnography of >10 obstructive sleep apneas or hypopneas per hour of sleep in association with a history of snoring and excessive daytime sleepiness. None of the subjects consumed alcohol on a regular basis or took hypnotics, sedatives, analgesics, or medications with known effects on sleep and ventilation. None of the subjects had evidence of chronic obstructive pulmonary diseases or other chronic lung disease. Four of 24 patients with OSAS were taking calcium channel blockers, compared to three of 24 control subjects. No other antihypertensive medications were prescribed for the participants in this study. We found no evidence of cardiovascular disease likely to affect pulmonary hemodynamics. Current and ex-smokers were excluded from this study because endothelial function may be affected by smoking. The demographic data relevant to the study are shown in Table 1. Because we measured the serum level of nitrite/nitrate (NO_x), we avoided the major confounding factors of NO_x measurements. All participants avoided foods rich in nitrites/nitrates, such as cured meat, for a week, and none took drugs known to increase NO_x levels (angitensin-converting enzyme (ACE) inhibitors and nitroglycerin).

Spirometry was performed using standard techniques [15] (CHESTAC-5v, Chest Co., Tokyo, Japan). Forced expiratory maneuvers were performed in triplicate, and the best effort was analyzed. Measurements were made on forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1). All testing was performed with the patient in a seated position. The pulmonary function test data were expressed as a percentage of predicted normal values.

Table 1
Demographic and anthropometric data

	OSAS	CTRL
Number of subjects	24	24
Male: female	19:5	20:4
Age (years)	54.2 ± 3.6	53.2 ± 3.6
Height (cm)	164 ± 6	162 ± 5
Weight (kg)	78 ± 6	75 ± 6
BMI (wt/(ht)^2)	29.0 ± 1.6	28.6 ± 1.7
FVC (l)	2.82 ± 0.26	2.79 ± 0.22
FEV_1(l)	2.42 ± 0.12	2.38 ± 0.11
FEV_1/FVC (%)	85.8 ± 5.6	85.3 ± 5.1
PaO_2 (mmHg)	68.2 ± 2.1	70.2 ± 1.8
$PaCO_2$ (mmHg)	42.2 ± 1.3	41.8 ± 1.2
SBP (mmHg)	138 ± 4.8	132 ± 4.6
DBP (mmHg)	74 ± 2.4	73 ± 2.2

Data were presented as mean ± SD.

OSAS, obstructive sleep apnea syndrome; CTRL, control subjects without OSAS; BMI, body mass index,; FVC forced vital capacity (l); FEV_1, forced expiratory volume in 1 s (l); PaO_2, arterial pressure of oxygen; $PaCO_2$, arterial pressure of carbon dioxide; SBP, systolic blood pressure; DBP, diastolic blood pressure.

3. Sleep study

All subjects were admitted for two or more consecutive nights for polysomnographic study. Polysomnography consisted of 8 h of overnight monitoring using a standard technique [16]. Respiratory effort was measured by respiratory inductance plethymography (Respitrace Corp., USA), and airflow at the nose and mouth was measured with thermistors. Surface electrodes were applied to obtain an electroencephalogram (EEG), electrooculogram (EOG), electrocardiogram, and a record of heart rate. Arterial oxygen saturation (SaO_2) was recorded with a pulse oximeter (502-P, Criticare System Inc., Centrouis, MO, USA). A polygraph was used to record data on both the paper of a 6-channel chart recorder (Nihonkoden, Tokyo, Japan) and a floppy disk via an IBM-compatible personal computer data acquisition system (NEC 9801, NEC, Tokyo, Japan). In subjects who slept for less than 6 h, as determined by EEG and EOG, repeat sleep studies were performed to assess whether poor sleep led to a missed diagnosis or inaccurate estimation of disease severity. Apnea was defined as the cessation of oronasal airflow for more than 10 s. Oxyhemoglobin desaturation was not a criterion for scoring apneas. The hypopneas are further defined by flow reduction and 2% desaturation. Flow reduction was defined as a reduction of 50% or more for at least 10 s in the oronasal flow in relation to prevailing values during preceding normal breathing. In this study, sleep apnea syndrome was determined by apnea + hypopnea index (AHI) values greater than 10/h. We did not measure body position or leg movement in the study.

After nocturnal oxygen administration (1–2 l/min) via nasal prong for 1 week, sleep studies were repeated to assess the effects of oxygen supplement on the severity of nocturnal apneas and arterial oxygen desaturation in OSAS patients. For the control arm of the study, the same group received nocturnal administration of compressed air via nasal prong for a week, and the sleep study was repeated to assess the effects of air administration on the NO_x levels, severity of nocturnal apneas, and arterial oxygen desaturation in these patients. The flow rate of oxygen/air administration was determined by the nadir SaO_2; 2 l/min of oxygen/air was administered when the nadir SaO_2 was smaller than 80%. Oxygen and air were randomly administered using a crossover protocol with a 1 week washout period.

3.1. Serum nitrite/nitrate measurement

Peripheral blood samples were obtained from the OSAS patients at 8.00 AM and 8:00 PM, before and after a week of

oxygen administration. Blood samples were collected in ice-cooled tubes containing lithium–heparin. Samples were deproteinized before analysis with 4% $ZnSO_4$. Serum nitrite/nitrate (NO_x) concentrations were determined using an analyzer employing the Griess method as previously described [17]. Data presented in the tables were obtained by means of addition of the NO_2^- plus NO_3^- concentrations, expressed in μM. In most samples, NO_3^- accounted for more than 90% of the total value.

3.2. Statistical analysis

The Mann–Whitney nonparametric test was used to compare the variables of demographic data and pulmonary function data in OSAS patients and control subjects. An analysis of variance with one fixed factor with repeated measures was used to compare the variables in baseline, air, and oxygen in the two subject groups. The association between serum NO_x levels and other variables was assessed separately. The analyses were performed by a software package using Stat View 4.0 (Abacus Concepts, Inc., Berkeley, CA, USA). The data are presented as mean ± SD. $p < 0.05$ was considered to be statistically significant.

4. Results

In the current study, all the participants in both the OSAS group (body mass index (BMI) = 28.8 ± 2.0) and the control group (BMI = 28.4 ± 3.3) were obese. The control subjects were matched for age and BMI. spirometric indices, and variables of arterial blood gas were within normal range in all subjects. Anthropometric and pulmonary function data are shown in Table 1. The control subjects were also matched for parameters of pulmonary function testing and blood gas analysis. In the OSAS group, all 20 patients had moderate to severe obstructive sleep apnea (obstructive or mixed apneas/hypopneas per hour of sleep were greater than 10) and the mean AHI was 38.6 ± 4.8. No member of the control group had more than five apneas/hypopneas per hour (AHI was less than 5) and mean AHI was 1.8 ± 0.8 (Table 2). Although the baseline value of SaO_2 in patients with OSAS was not different from that in the control subjects, the nadir SaO_2 values were considerably lower in OSAS patients than in control subjects ($p < 0.01$). While the number of apneas among OSAS patients was not significantly reduced by oxygen administration, the 4% arterial oxygen desaturations from baseline SaO_2 was markedly reduced (Table 2). The nadir SaO_2 was improved by the oxygen supplementation but not by air administration. In control subjects, 1–2 l/min of oxygen administration did not affect the number of apneas or the nadir SaO_2 (Table 2).

The serum level of NO_x in OSAS patients was lower than that in control subjects (Figure 1) (Table 3). Oxygen administration significantly increased the serum NO_x levels in every patient, but they did not reach the normal levels of the control subjects. Air administration had no effect on the NO_x levels in the patients. Oxygen administration did not affect the serum NO_x levels among control subjects (Table 3).

We examined the relationship between the NO_x levels and the following parameters: AHI, nadir SaO_2, systolic blood pressure, diastolic blood pressure, and arterial oxygen. There were significant relationships between NO_x levels, nadir SaO_2, and the 4% oxygen desaturations (Table 4). However, AHI and systolic/diastolic blood pressures were not correlated with NO_x levels (Table 4).

5. Discussion

The present study demonstrates that the serum levels of nitrite/nitrate (NO_x), which are stable metabolites of NO,

Table 2
Effects of the supplementation of oxygen or compressed air on the numbers of apneas and arterial oxygen desaturation in OSAS patients and control subjects

Supplementation	OSAS			CTRL		
	Non	Air	Oxygen	Non	Air	Oxygen
Number of subjects	24			24		
AHI (/h)	39. ± 5*	36 ± 6*	36 ± 6*	2 ± 1	2 ± 2	2 ± 2
Baseline SaO_2 (%)	95 ± 2	95 ± 2	96 ± 1	95 ± 2	97 ± 2	96 ± 2
Nadir SaO_2 (%)	70 ± 8*	73 ± 4*	90 ± 3#	93 ± 2	95 ± 2	93 ± 1
N of 4% desaturation	264 ± 5*	246 ± 7*	28 ± 6*	4 ± 2	2 ± 2	3 ± 2

Data were presented as mean ± SD.

OSAS, obstructive sleep apnea syndrome; CTRL, control subjects without OSAS; air: 1–2 ml/min of compressed air was administered during night via nasal prong in for a week.

Oxygen: 1 week oxygen administration (1–2 l/min) during night via nasal prong. AHI, apnea and hypopnea index; baseline SaO_2, stable value of SaO_2 at supine position before sleep (%); nadir SaO_2, the lowest value of SaO_2 during night (%); N of 4% desaturation, number of 4% of arterial oxygen desarutation from baseline value of SaO_2.

*$p < 0.01$ compared with the same value of CTRL.

#$p < 0.01$ compared with the same value without oxygen supplementation.

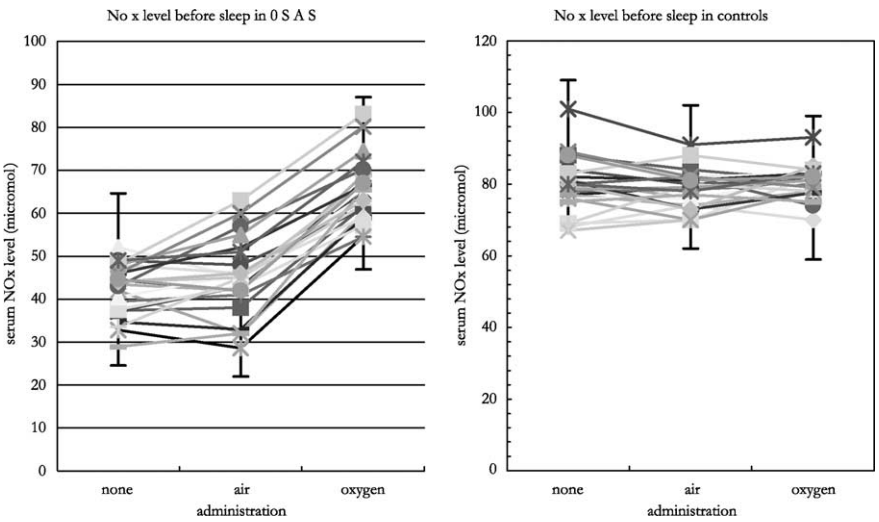

Fig. 1. Serum NO_x levels before sleep in patients with OSAS and in obese controls without OSAS.

were smaller in OSAS patients than in control subjects. It has recently been reported that the early morning serum NO_x levels were significantly lower in OSAS subjects than in control subjects (OSAS = 38.9 μM, control subjects = 63.1 μM) [18]. Schulz and coworkers have reported that NO_x levels were 21.7 μM in OSA patients, compared with 42.6 μM in healthy volunteers and 36.7 μM in control patients without OSA but with a similar spectrum of co-morbidity [19]. Our data support these previous observations.

We report that there is a significant negative correlation between serum nitrites/nitrates and the following parameters: AHI, oxygen desaturation time, and systolic blood pressure. Examination of the relationship between the NO_x level and AHI, nadir SaO_2, systolic blood pressure, diastolic blood pressure, and arterial oxygen desaturation indicates that the level of NO_x is significantly correlated with the number and amount of oxygen desaturations but not with AHI and blood pressure. Repeated episodes of nocturnal hypoxemia may be due to the impaired production of NO. Comparing the effects of nocturnal oxygen supplementation on apneas and NO_x production in OSAS patients with the effects of compressed air supplementation revealed that oxygen (but not air) increased the NO_x level but did not affect the apneas. It is reasonable to assume that repeated episodes of nocturnal hypoxemia are a mechanism of the impaired NO production in patients with OSAS. Because oxygen is a cosubstrate of NO synthase (NOS), OSAS-related nocturnal desaturations might result in depressed synthesis of NO.

The current results are at least supported in part by recent evidence that nasal continuous positive airway pressure (nCPAP) reverses the suppressed NO in OSAS patients [18,19]. However, the nCPAP might reverse both apneas and hypoxemia; there is a possibility that nocturnal apneas themselves are involved in the impaired NO production. Because oxygen supplementation increased the NO production but did not totally reverse the NO_x levels in the current study, we must consider the possibility that nocturnal apnea itself, and other factors, may contribute to endothelium dysfunction in OSAS patients. It has been demonstrated that NOS inhibitors are elevated in OSAS patients and thus might also contribute to lowered NO_x levels [20]. The nitrite/nitrate (NO_x) levels might be influenced by a variety of confounding factors, such as arterial hypertension, cigarette smoking, hypercholesterolemia,

Table 3
Serum concentration of NO_x before and after sleep studies

Supplementation	OSAS			CTRL		
	Non	Air	Oxygen	Non	Air	Oxygen
NO_x before sleep (μM)	43.7 ± 6.2*	44.7 ± 6.4*	65.8 ± 6.4#	79.6 ± 7.3	78.6 ± 5.3	80.6 ± 4.3
NO_x after sleep (μM)	35.6 ± 5.3*	37.6 ± 5.9*	58.8 ± 7.3#	72.6 ± 4.3	75.6 ± 5.2	73.2 ± 4.1

Data were presented as mean ± SD.
Air: 1–2 ml/min of compressed air was administered during night via nasal prong for a week.
Oxygen: 1 week oxygen administration (1–2 l/min) during night via nasal prong.
*$p < 0.01$ compared with the same value of CTRL.
#$p < 0.01$ compared with the same value without air/oxygen supplementation.

Table 4
Relationships between the serum level of NO_x and other variables

	p	*r*
AHI (/h)	(−)	
Baseline SaO_2 (%)	(−)	
Nadir SaO_2 (%)	0.05	−357
N of 4% desaturation	0.01	−401
Systolic BP	(−)	
Diastolic BP	(−)	

BP, blood pressure.

and diabetes mellitus. Thus, we should further determine by direct comparison the effects of CPAP and O_2 on the NO_x levels in patients.

It has been reported, using the measurements of brachial artery diameter under baseline conditions, during reactive hyperemia and after sublingual administration of nitroglycerin (an endothelium-independent vasodilator), that patients with OSAS have an impairment of resistance-vessel endothelium-dependent vasodilation [21]. Although the reduced levels of NO_x were identified in patients with OSAS, the functional impairment of endothelium-dependent vasodilators should be further examined. NO, being one of the mediators, may be involved in the hemodynamic regulation and long-term vascular remodeling in OSAS patients.

In conclusion, these results indicate that systemic NO production is impaired in OSAS patients, possibly due to nocturnal hypoxia.

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