Effects of Nasal Positive Airway Pressure Treatment on Oxidative Stress in Patients With Sleep Apnea-Hypopnea Syndrome

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OBJECTIVE: To analyze whether nasal continuous positive airway pressure (CPAP) reduces oxidative stress in patients with sleep apnea-hypopnea syndrome (SAHS).

PATIENTS AND METHODS: Thirty-six patients with SAHS requiring nasal CPAP treatment and 10 controls in whom SAHS was ruled out were enrolled. Oxidative stress was evaluated by measuring plasma malondialdehyde (MDA) concentrations to assess lipid peroxidation at the beginning of the study and then again after a mean (SD) of 2.9 (0.6) months of nasal CPAP. Plasma MDA concentrations were determined by measuring thiobarbituric acid reactive substances. We controlled for the following factors known to influence oxidative stress: age, sex, use of vitamin supplements, smoking habit, body mass index (kg/m²), ischemic cardiopathy, hypertension, diabetes, and hypercholesterolemia.

RESULTS: The mean age of patients with SAHS was 51.4 (9.9) years and the mean body mass index was 32.9 (5.3) kg/m². Nasal CPAP was titrated to a mean pressure of 8.9 (3.4) cm H₂O. The mean score on the Epworth sleepiness scale was 10.2 (4.3) before treatment and 4.2 (2.8) after treatment (P < .001). The apnea-hypopnea index decreased from 43.7 (22.6) before treatment to 4 (3.5) after treatment (P < .001). Mean MDA concentrations in patients with SAHS were 2.0 (1.1) µmol/mL before treatment and decreased significantly to 1.6 (0.07) µmol/mL after treatment, whereas MDA concentrations remained unchanged in control subjects.

CONCLUSIONS: Nasal CPAP treatment significantly reduced oxidative stress in patients with SAHS in our study.

Key words: Oxidative stress. Sleep apnea-hypopnea syndrome. Nasal positive airway pressure.

Efectos del tratamiento con CPAP nasal en el estrés oxidativo en pacientes con síndrome de apnea del sueño

OBJETIVO: Analizar si el tratamiento con presión positiva continua de la vía aérea nasal (CPAPn) reduce el estrés oxidativo (EO) en pacientes con síndrome de apneas-hipopneas durante el sueño (SAHS).

PACIENTES Y MÉTODOS: Se incluyó en el estudio a 36 pacientes con SAHS que requirieron tratamiento con CPAPn y a 10 controles en quienes se excluyó dicho síndrome. Se realizó una primera determinación del EO mediante las concentraciones de malondialdehído (MDA) en sangre para conocer la peroxidación lipídica, y una segunda tras una media ± desviación estándar de 2.9 ± 0.6 meses de seguimiento con CPAPn. Las concentraciones plasmáticas de MDA se midieron como sustancia reactiva al ácido tiobarbitúrico. Se controló para los siguientes factores, que se sabe que influyen en el EO: edad, sexo, suplementos vitamínicos, consumo de tabaco, índice de masa corporal (kg/m²), cardiopatía isquémica, hipertensión, diabetes e hipercolesterolemia.

RESULTADOS: La edad media de los pacientes con SAHS fue de 51,4 ± 9,9 años y el índice de masa corporal, de 32,9 ± 5,3 kg/m². La CPAPn se regló a una presión media de 8,9 ± 3,4 cmH₂O. La puntuación en la escala de Epworth antes del tratamiento fue de 10,2 ± 4,3, frente a 4,1 ± 2,8 después del tratamiento (p < 0,001). El índice de apneas-hipopneas/h descendió de 43,7 ± 22,6 antes del tratamiento a 4 ± 3,5 después de la CPAPn (p < 0,001). En los pacientes con SAHS las concentraciones de MDA antes del tratamiento con CPAPn fueron de 2,0 ± 1,1 µM y descendieron significativamente a 1,6 ± 0,7 µM después del tratamiento, mientras que no se modificaron en los sujetos controles.

CONCLUSIONES: El tratamiento con CPAPn reduce de forma significativa el EO en los pacientes SAHS de nuestro estudio.

Palabras clave: Estrés oxidativo. Síndrome de apneas-hipopneas durante el sueño (SAHS). Tratamiento con CPAPn.

Introduction

Free radicals are metabolic byproducts of aerobic cellular respiration and the function of antioxidant systems is to eliminate excess free radicals. Oxidative...
stress and damage to cells and tissues occur when the generation of free radicals exceeds antioxidant capacity. The interaction of oxygen reactive species with the unsaturated fatty acids of the biomembrane leads to the formation of lipid hydroperoxides as primary products, as well as a series of secondary products, including malondialdehyde (MDA). Of the reactive aldehydes that can result from the decomposition of lipid peroxides, MDA is the one that is most often used as a marker of oxidative stress. The derivatization of MDA with thiobarbituric acid is the method most often used to assess lipid peroxidation and free radical activity in biologic samples.

It is well known that certain cardiovascular risk factors such as hypertension, hypercholesterolemia, and diabetes predispose to endothelial dysfunction and that oxidative stress is the common denominator of all the processes that lead to such dysfunction. In addition, endothelial dysfunction is considered a subclinical indicator of vascular or myocardiac dysfunction prior to the appearance of signs and symptoms of vascular disease.

There is an increased prevalence of cardiovascular diseases in patients with sleep apnea-hypopnea syndrome (SAHS) and, although the pathophysiological mechanisms are not altogether clear, it can be affirmed that episodes of hypoxia-reoxygenation, such as those associated with SAHS, predispose to arteriosclerosis through the production of free radicals.

The literature provides evidence for an increase in oxidative stress in patients with SAHS, although other studies are not in agreement. Increases in oxidative stress may produce a degree of endothelial dysfunction that would predispose SAHS patients to increased cardiovascular morbidity, even in the absence of cardiovascular risk factors. Nasal continuous positive airway pressure (CPAP) treatment has been shown to have a beneficial effect on the course of cardiovascular risk and heart disease.

The objective of the present study was to show that nasal CPAP reduces oxidative stress in patients diagnosed with SAHS and to shed light on the pathophysiologic mechanisms involved in order to help explain the prevalence of cardiovascular disease in such patients.

Patients and Methods

We studied patients who had consulted for symptoms of SAHS and who, according to established criteria, required nasal CPAP as determined by diagnostic polysomnography carried out in our hospital’s sleep unit. We enrolled only SAHS patients with cardiovascular risk factors (specifically, hypertension, hypercholesterolemia, and diabetes) and/or ischemic cardiopathy. Patients with other diseases or those taking vitamin supplements were excluded.

We also studied a group of 10 controls selected from among our hospital’s health care staff. SAHS had been ruled out in these subjects, as they presented neither snoring, apnea, nor daytime sleepiness.

Study Protocol

SAHS Patients. All patients completed a structured interview to collect data on age; sex; whether or not they had hypertension, hypercholesterolemia, diabetes, and/or ischemic cardiopathy; frequency of smoking; and body mass index (BMI). The Epworth test was also administered in order to quantify daytime sleepiness.

Patients underwent spirometry according to the guidelines of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) and arterial blood gases were measured with patients breathing room air. A venous blood sample was collected at 8 AM in order to determine plasma MDA concentrations, an indicator of oxidative stress. Patients also underwent diagnostic polysomnography with a Somnoman Alpha (SensorMedic Corp, Yorba Linda, California, USA) that recorded 6-hour electroencephalogram, electro-oculogram, submental electromyogram, electrocardiogram, thoracic and abdominal movements using bands with piezoelectric sensors, airflow using nasal cannulae and a Ptit Lite pressure transducer (Pro-Tech Services Inc, Mukilteo, Washington, USA), and oxygen saturation with a Nonin 8600 pulse oximeter (Nonin Medical Inc, Plymouth, Minnesota, USA).

Sleep stages were analyzed manually at 30-second intervals in accordance with standard criteria and total sleep time was calculated. Apnea was defined as the absence of airflow for at least 10 seconds and hypopnea as a reduction in airflow of at least 50% for 10 seconds or more associated with a decrease in oxygen saturation equal to or greater than 4% or with transitory arousal. The apnea-hypopnea index (AHI) was calculated by dividing the total number of apneas and hypopneas by the number of hours of sleep. The criteria of the American Sleep Disorders Association were used to define transitory arousals. A diagnosis of SAHS was established when the AHI was 10 or more.

Patients later underwent polysomnography with nasal CPAP titration for a full night. Once the patient had fallen asleep, pressure was initiated at 4 cm H₂O and gradually increased. Nasal cannulae were used to record airflow.

After 2 to 3 months of nasal CPAP treatment, objective adherence was recorded by taking the time counter reading and patients had an interview during which a second Epworth test was administered. We also checked that there had been no variation in BMI, smoking habit, other cardiovascular risk factors, or ischemic cardiopathy. A second blood sample was collected at 8 AM in order to quantify MDA concentrations.

Controls. A first blood sample was taken at 8 AM in order to determine MDA concentrations and another was taken at the same time 2 months later. When the blood samples were collected, control subjects were interviewed and age, sex, BMI, smoking habit, cardiovascular risk factors (hypertension, cholesterol, high glucose levels), ischemic cardiopathy, and history of other diseases were noted.

Obtaining Plasma Samples

After a night-long fast, blood samples were drawn from the antecubital vein at 8 AM and collected in tubes containing ethylenediaminetetraacetic acid dipotassium salt in order to obtain plasma. They were then centrifuged at 3000 g for 10 minutes at 4°C. The plasma thus obtained was stored at –70°C until it was analyzed.
Analysis of Lipid Peroxidation (Plasma MDA Concentrations)

Plasma MDA concentrations were determined by measuring thiobarbituric acid reactive substances according to the analytic method described by Kikugawa et al. We added 0.2 mL of phosphoric acid (0.2 mol/L) to 0.2 mL of plasma and the color reaction was started by adding 0.025 mL of a 0.11 mol/L thiobarbituric acid solution. The samples were then heated to 90°C for 45 minutes. Then, once the samples had cooled, the pink complex was extracted into 0.4 mL of n-butanol for 30 minutes. The aqueous and butanol phases were separated by centrifugation at 6000 g for 10 minutes. Absorbance of the butanol phase was measured at 535 nmol using a Benchmark Plus (Bio-Rad Laboratories, Inc, Hercules, California, USA) microplate spectrophotometer. The standard quantity of MDA (0-20 µmol) was used to prepare the calibration line. The intra- and interanalysis variation coefficients were 1.82% and 4.01%, respectively. The limit of detection was established at 0.079 µmol (mean blank measurements plus 3 SDs).

Statistical Analysis

Quantitative variables are expressed as means (SD) and qualitative variables as percentages. The Kolmogorov-Smirnov test was used to check the normal distribution of MDA concentrations, Epworth scores, and AHI.

The Mann-Whitney test was used for between-group comparisons for the following categorical variables: smoking habit, hypertension, ischemic cardiopathy, hypercholesterolemia, and diabetes.

For the comparisons of MDA concentrations before and after treatment, we selected those variables that had a likelihood ratio less than 0.20 (smoking habit, hypercholesterolemia, and diabetes).

Mean MDA concentrations at baseline and after treatment were compared using a 3-way multivariate analysis of variance controlling for diabetes, hypercholesterolemia, and smoking habit. Patients who did not comply with treatment for at least 4 hours per night were excluded from the analysis.

Statistical analysis was carried out using the SPSS 12.0.1 statistical software package (SPSS, Inc, Chicago, Illinois, USA). A P value less than .05 was considered significant.

RESULTS

Thirty-nine patients with SAHS requiring nasal CPAP treatment were studied. Of these, 3 were excluded because they complied with treatment for less than 4 hours per night. The mean AHI of the patients excluded was 20.7 (11.7) (compared to 43.7 [22.6] for compliant patients) and their mean nasal CPAP pressure was 6.7 (2.3) cm H2O (compared to 8.9 [3.4] for compliant patients).

Table 1 shows the characteristics of the 36 SAHS patients and the 10 controls. None of the SAHS patients showed an obstructive spirometric pattern (forced expiratory volume in 1 second/closing volume, 79.7 [4.8]) or daytime hypoxemia (PaO2, 95.9 [14.3] mm Hg). We checked that there had been no variation in BMI, smoking habit, medication, ischemic cardiopathy, or cardiovascular risk factors among SAHS patients or controls between the first and second blood sample collections. Mean duration of nasal CPAP treatment before the second blood sample collection was 2.9 (0.6) months. The second blood sample was collected from controls 2 months after the first.

The mean AHI assessed by diagnostic polysomnography was 43.7 (22.6) and it decreased...
SAHS is characterized by repeated episodes of apnea during sleep associated with oxygen desaturations followed by episodes of reoxygenation. In animal models, intermittent hypoxia followed by reoxygenation, which resembles ischemia-reperfusion episodes, has been associated with increased oxidative stress. The decrease in oxidative stress can be attributed to the elimination of episodes of hypoxia-reoxygenation achieved with the use of nasal CPAP.

In SAHS patients there is a decrease in sleep quality. In some animal studies, sleep deprivation has been associated with lipid peroxidation, although other studies have shown discrepant results. An inverse relationship between REM sleep and oxidative stress in SAHS patients has been observed. Nasal CPAP treatment improves sleep quality in such patients, thereby reducing oxidative stress.

Finally, in SAHS patients there is an increase in levels of catecholamines, which may undergo oxidation. This is one of the reasons why an excess of catecholamines may lead to heart disease and contribute to cardiovascular complications in such patients.

There are multiple factors that can modify oxidative stress. Obesity, old age, smoking habit, and male sex have been shown to increase oxidative stress, and for this reason we controlled for all these factors in our study.

Some of the studies showing a reduction in oxidative stress in SAHS patients treated with nasal CPAP included patients with no cardiovascular risk factors and thus failed to control for variables that might confound the relation between SAHS and oxidative stress. As the frequency of cardiovascular risk factors in our series was similar to that found in daily clinical practice, external validity was ensured. In the statistical analysis we controlled for confounding factors that might influence oxidative stress, thereby increasing internal validity. We also ensured that no patient took vitamin supplements and excluded patients with chronic obstructive pulmonary disease, which is often associated with SAHS and can increase lipid peroxidation.

Patients with any disease other than those specified above were also excluded. We also checked that there had been no changes in the previously mentioned factors that might influence oxidative stress between the first and second blood sample collections. We believe that these strengths allowed us to confirm that the significant decrease in MDA obtained in these patients was due to nasal CPAP treatment.

The follow-up period in our study was 3 months. Other studies had longer follow-up periods, with a greater likelihood, therefore, that other factors that contribute to oxidative stress would undergo changes. Still other studies, however, had even shorter follow-up periods, in which variation in oxidative stress was measured after only a single night, with inconclusive results.

**TABLE 2**

<table>
<thead>
<tr>
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<th>SAHS (n=36)</th>
<th>Controls (n=10)</th>
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<tbody>
<tr>
<td><strong>Systolic/diastolic BP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mm Hg†</td>
<td>156 (21)/95 (8)</td>
<td>110 (19)/80 (15)</td>
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<tr>
<td>Glucose, mg/dL†</td>
<td>98.7 (7.6)</td>
<td>89.2 (8.3)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL†</td>
<td>212.5 (29.7)</td>
<td>182.3 (19.5)</td>
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<tr>
<td>MDA, µmol</td>
<td></td>
<td></td>
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<tr>
<td>First measurement</td>
<td>2.1 (1.2)</td>
<td>0.7 (0.5)</td>
</tr>
<tr>
<td>Second measurement</td>
<td>1.6 (0.7)</td>
<td>0.6 (0.4)</td>
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*Results are expressed as means (SD).
†Baseline values.
Finally, to the best of our knowledge, the number of patients in whom there was a demonstrated reduction in oxidative stress with nasal CPAP treatment was greater than in any other study published to date.10,11 and we believe that this lends further support to our findings.

The present study included a control group of subjects without symptoms of SAHS in whom MDA measurements did not vary during the 2-month period. MDA concentrations were significantly higher in patients with SAHS than in controls, but this finding is of limited value, as controls had fewer cardiovascular risk factors than SAHS patients.

Nasal CPAP has been shown to be effective in increasing survival in patients with SAHS.12 The reduction of oxidative stress with nasal CPAP treatment in such patients could prevent the development or progression of cardiovascular morbidity and, consequently, reduce mortality in patients with SAHS.

The greatest limitation of our study lies in the fact that, while oxidative stress was measured by one of the most validated techniques, we did not determine the level of antioxidant activity, which also forms a part of the balance of all redox systems.

It would be interesting to carry out studies with long-term monitoring of oxidative stress in such patients and to study its relationship to the appearance of cardiovascular diseases, as well as to measure antioxidative enzyme capacity.

In conclusion, oxidative stress measured by MDA plasma concentrations decreased significantly in the SAHS patients studied following nasal CPAP treatment.

REFERENCES