Sleep apnea is associated with bronchial inflammation and continuous positive airway pressure–induced airway hyperresponsiveness

Gilles Devouassoux, MD, PhD,[a,d] Patrick Lévy, MD, PhD,[a,b] Eliane Rossini, MSc,[a,b] Isabelle Pin, MD, PhD,[a] Michèle Fior-Gozlan, MD,[c] Mireille Henry, MSc,[c] Daniel Seigneurin, MD, PhD,[c] and Jean-Louis Pépin, MD, PhD[a,b] *Grenoble and Lyon, France*

Background: Obstructive sleep apnea syndrome (OSA) is associated with systemic and upper airway inflammation. Pharyngeal inflammation has a potential role in upper airway collapse, whereas systemic inflammation relates to cardiovascular morbidity. However, the presence of an inflammatory involvement of lower airway has been poorly investigated.

Objective: The aim of the study was to demonstrate an inflammatory process at the bronchial level in patients with OSA and to analyze effects of continuous positive airway pressure (CPAP) application and humidification on bronchial mucosa.

Methods: The study was conducted by using sequential induced sputum for cell analysis and IL-8 production, nitric oxide exhalation measurement, and methacholine challenge before and after CPAP.

Results: Bronchial neutrophilia and a high IL-8 concentration were observed in untreated OSA compared with controls (75% ± 20% vs 43% ± 12%, $P < .05$; and 25.02 ± 9.43 ng/mL vs 8.6 ± 3.7 ng/mL, $P < .001$, respectively). IL-8 in sputum supernatant was correlated to apnea hypopnea index ($P < .01$; $r = 0.81$). After 1 month of CPAP, this inflammatory pattern remained unchanged, and an increase in airway hyperresponsiveness (AHR) was observed ($P < .001$).

Conclusion: Obstructive sleep apnea syndrome is associated with bronchial inflammation. Our data demonstrate CPAP effect on the development of AHR, possibly facilitated by the pre-existing inflammation. Both issues should be evaluated during long-term CPAP use.

Clinical implications: Results showing a spontaneous bronchial inflammation in OSA and the development of a CPAP-related AHR require a long-term follow-up to evaluate consequences on chronic bronchial obstruction. (J Allergy Clin Immunol 2007;119:597-603.)

Key words: *Obstructive sleep apnea syndrome, bronchial inflammation, neutrophil, IL-8, NO exhalation, airway hyperresponsiveness, continuous positive airway pressure*

Repeated pharyngeal collapses occurring during sleep that are characteristic of obstructive sleep apnea syndrome (OSA) are explained by a permanent reduction in upper airway size and an increased pharyngeal collapsibility.[1] Local upper airway inflammation and edema[2] probably participate in the pharyngeal narrowing. Whereas there are robust and concordant data regarding local inflammation occurring at the nose, pharyngeal, and laryngeal levels, there is only 1 preliminary study reporting bronchial inflammation in patients with apnea.[3] In addition, oxidative stress and proinflammatory cytokines production in breath condensates have been demonstrated as increased in patients with apnea compared with controls, and this increased production was not related to obesity but rather to sleep apnea severity.[4] Thus, a positive correlation was demonstrated between both IL-6 and 8-isoprostane concentrations and the apnea hypopnea index (AHI).[4] However, this study did not document whether changes in differential cell counts at the bronchial level and in airway hyperresponsiveness (AHR) occurred concurrently.

Systemic inflammation has also been reported in association with sleep apnea with significant increase in plasmatic ultrasensitive C-reactive protein[5] and cytokines.[6] This cytokine production might contribute to the sleepiness experienced by patients with OSA.[7] On the other hand, systemic inflammation is proposed as one of the main intermediary mechanisms explaining the increased risk of cardiovascular diseases in sleep apnea.[8]

Among the interleukins affecting atherosclerosis progression, IL-8 mediates the accumulation of macrophages in atherosclerotic lesions.[9] It has been repeatedly demonstrated in chronic obstructive pulmonary disease (COPD) that increased neutrophilic infiltration of the airway is associated with a significant release of IL-8 by airway cells.[10] If persistent bronchial inflammation occurs in patients with apnea, this chemokine might be one of the contributors to systemic inflammation and might facilitate atherosclerosis occurrence during the course of the disease.

From [a]the Sleep Laboratory, Exploration Fonctionnelle Cardio-Respiratoire University Hospital Grenoble; [b]the HP2 Laboratory (Hypoxia: Pathophysiology), Institut National de la Santé et de la Recherche Médicale INSERM ESPRI 0017 Joseph Fourier University, Grenoble; [c]the Department of Cytology, University Hospital Grenoble; and [d]the Unit of Respiratory Diseases, CHLS, Hospices Civils Lyon, Lyon.

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Reprint requests: Jean-Louis Pépin, MD, PhD, Laboratoire du sommeil, EFCR, CHU de Grenoble, BP 217X, 38043, Grenoble Cedex 09, France. E-mail: JPepin@chu-grenoble.fr.

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Abbreviations used
AHI: Apnea hypopnea index
AHR: Airway hyperresponsiveness
COPD: Chronic obstructive pulmonary disease
CPAP: Continuous positive airway pressure
GER: Gastroesophageal reflux
NO: Nitric oxide
OSA: Obstructive sleep apnea syndrome
VEGF: Vascular endothelial growth factor

Continuous positive airway pressure (CPAP), the first-line therapy of sleep apnea,[11] is at least partly able to improve upper airway inflammation[12] and to normalize plasmatic levels of C-reactive protein and proinflammatory cytokines.[13] On the other hand, nasal intolerance is a very frequent minor side effect occurring with CPAP, affecting as many as 50% of treated patients with OSA.[14] This side effect might be related to persistent inflammation at the airway level in CPAP-treated patients. No data are currently available on sputum-induced airway cells analysis before and after CPAP.

Therefore, the principal objectives of this investigation were 3-fold. First, we sought to determine whether there is bronchial inflammation in patients with apnea by performing sputum induction in patients with OSA in comparison with healthy subjects. Second, we wished to ascertain the consequences of this bronchial inflammation by measuring nitric oxide (NO) exhalation and nonspecific airway responsiveness to methacholine as well as IL-8 release in supernatant of sputum cells. Our third objective was to evaluate the effects of 1 month of nasal CPAP treatment on bronchial inflammation and airway responsiveness to methacholine.

METHODS

Subjects

Fifty-seven consecutive patients (43 males and 14 females) with OSA were included in the study (Table I). OSA was confirmed by using nocturnal polysomnography. Patients were nonsmokers, and none had a previous OSA diagnosis or CPAP therapy. A control group of 13 healthy nonsmoking subjects was simultaneously analyzed. Atopy was investigated by using skin prick tests to a panel of 7 usual aeroallergens. Allergic sensitization was defined by the presence of at least 1 positive skin test (mean wheal diameter $\geq$3 mm). Finally, respiratory medical data were collected, with a special focus on the presence of an allergic history. The review board of our institution (Comité Consultatif de Protection des Personnes en Recherche Biomédicale, Grenoble, France) approved the protocol, and informed consent was obtained from each subject.

Study design

Once the diagnosis of OSA was established, patients underwent pulmonary function tests, bronchial inflammation using induced sputum for cellular analysis and IL-8 production, exhaled NO measurement, and methacholine challenge test to determine airway responsiveness. Patients with OSA were further rapidly treated by nasal CPAP. Control subjects underwent the same tests at baseline but were not treated by CPAP. One week after the initiation of CPAP treatment, patients were asked for the presence or the absence of nasal side effects related to CPAP use. Occurrence of symptoms consistent with rhinitis and coughing were noted. Again, all patients with OSA were evaluated with pulmonary function tests, bronchial inflammation tests, and methacholine tests. In the presence of symptoms of rhinitis and/or coughing, humidification was then added to CPAP. Three weeks later, at the end of the study, all CPAP-treated patients were tested in a similar manner.

Standard polysomnography procedures and scoring were performed[15] (see this article's Online Repository at www.jacionline.org for additional information about sleep study methodology and definitions of events).

TABLE I. Characteristics of OSA and control groups

	OSA group	Control group
n	57	13
Sex (male/female)	43/14	7/6
Age (y)	54 ± 11	45 ± 7
Body mass index (kg/m^2)	28.7 ± 5.4	18.2 ± 3.7
Epworth index	15 ± 4	5 ± 3
AHI (n/h)	41 ± 14.3	Not available
Atopy (n)*	4	4
Allergic history (n)†	4	1
FEV_1 (% of predicted value)	102 ± 17	110 ± 19
Forced expiratory flow at 25% to 75% of forced vital capacity (% of predicted value)	93 ± 23	94 ± 23

*Atopy was defined by the presence of at least one skin test larger than 3 mm (OSA group, 1 ragweed + skin test, 3 mite + skin test; control group, 2 mite + skin test, 1 ragweed + skin test, and 1 cockroach + skin test).
†OSA, 3 rhinitis, 1 conjunctivitis; control group, 1 rhinitis.

Sputum induction and processing

Induction and analysis of sputum were performed according to previous recommendations[16] (see this article's Online Repository at www.jacionline.org for details).

Measurements of IL-8 production

IL-8 in sputum supernatant was measured in duplicate using ELISA methods (R&D Systems, Minneapolis, Minn) with a lower limit of detection of 1.6 ng/mL. Briefly, each sample was incubated in microtiter wells coated with an antibody anti–IL-8. After washing, a peroxidase-labeled antihuman IL-8 antibody was added, and the plates were incubated. IL-8 concentration was measured by spectrophotometry after addition of enzyme substrate. Recombinant IL-8 was used as a standard.

Exhaled NO measurement

Exhaled NO was assessed by means of a NO/NOx chemiluminescence analyzer with a detection limit of 1 ppb (model Topaze 2020; Cosma, Igny, France). Calibration was performed before each experiment by using a gas mixture of known concentration (100 ppb; Air Liquide, Paris, France). According to previous recommendations,[17] after inhalation of synthetic NO-free air to total lung capacity, bronchial NO measurement was performed during a constant expiratory maneuver of 8 $L.min^{-1}$ performed against an added expiratory resistance of 10 cm H_2O.