Chronic hyperbaric oxygen therapy causes only minor ultrastructural changes in the human nasal epithelium

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SUMMARY

Hyperbaric oxygen (HBO) therapy is becoming increasingly popular in the treatment of several pathologies, namely in vascular diseases. It is generally considered to be innocuous, with few restrictions. Thus, patients subjected to HBO therapy breath saturated oxygen at an elevated pressure. Since the respiratory mucosa comes into contact with this altered inhaled air, we decided to use light and electron microscopy to investigate whether chronic HBO therapy causes significant changes in the nasal mucosa. For this, we obtained biopsies of the anterior portion of the lower nasal turbinate from two groups of 9 individuals under direct visual inspection. The first group had a diagnosis of tinnitus and was subjected to 15 sessions of 100 min-long HBO treatments, and the latter group comprised healthy volunteers not subjected to HBO therapy. The samples were processed for light and electron microscopy. We found that the turbinate mucosa of the HBO-treated group showed a moderate infiltration by leukocytes and an increase in the thickness of the epithelial basement membrane. By transmission electron microscopy (TEM), we observed that only a minority of the nasal epithelial cells presented alterations due to the HBO treatment; these alterations were focal and restricted to cilia. We conclude that chronic HBO treatment induces only minor alterations in the nasal mucosa and that these are likely to be reversible when treatment is discontinued.

Key words: Hyperbaric therapy – Oxygen – Nasal mucosa – Basement membrane – Neutrophils – Cilia

INTRODUCTION

The spectrum of hyperbaric oxygen (HBO) therapy in internal medicine has expanded during the last decade. HBO is performed inside a hyperbaric chamber and consists of the delivery of 100% oxygen to patients at pressures that are 2-3 times higher than at sea level (Narozny et al., 2002). The aim of the increased pressure is to enhance the amount of oxygen dissolved in the plasma of the patients. The therapeutic actions of HBO are related not only to the direct physical effects of oxygen on blood and tissues but also to vasoconstriction, angiogenesis, fibroblast proliferation, leukocyte oxidative killing, toxin inhibition and antibiotic synergy (Sahni et al., 2004; Neumeister et al., 2004). Currently, the indications for HBO are consen-

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sual and are determined by medical societies. The Undersea and Hyperbaric Medical Society has approved the use of HBO in the treatment of a number of pathologies, namely in air or gas emboli, carbon monoxide poisoning, gas gangrene, acute traumatic ischemia, decompression sickness, prolonged failure of wound healing, exceptional blood loss, intracranial abcesses, necrotizing soft tissue infections, osteomyelitis, osteoradionecrosis, compromised skin grafts or flaps, and heat burns (Wattel and Mathieu, 2006). Other pathologies have also been successfully treated with HBO, such as sudden hearing loss (Dundar et al., 2007).

Owing to the increased therapeutic uses of HBO in human medicine, we were prompted to investigate whether this type of treatment causes any alterations in the respiratory mucosa since this mucosa is in direct contact with the altered air breathed by patients subjected to HBO. To accomplish this, here we compared the morphology of the nasal epithelium and chorion of HBO-treated and control individuals.

Materials and methods

In the present study, two groups of 9 individuals were formed. The first group of patients was subjected to chronic (15 sessions) HBO treatment for a diagnosis of tinnitus. They were all men, with an age range of 28-68 years, with a mean of 50.89±11.78 years. HBO treatment took place in a multiplace Hyperbaric Chamber (Haux® – Starmed 2200) in the presence of a nurse. All HBO-treated patients concluded 15 sessions of HBO therapy at 2.5 ATA (1 atmosphere absolute – ATA) at 75 minutes per session. They attended one session per day, at the same hour, for 15 days. The total length of time that the patients remained in the chamber for each session was 100 minutes owing to the time needed for compression (10 minutes) and decompression (15 minutes). The pressure was sustained by compressed air, and the patients breathed 100 % humidified oxygen through tightly fitting (nose and mouth) masks, expiring to the space outside the chamber. The second group of 9 men (controls) comprised patients scheduled for ear surgery. They were not subjected to HBO treatment and had an age range of 25-47 years with a mean of 36.89 ± 8.95.

The exclusion criteria to eliminate patients from the study were as follows: all criteria that exclude patients from HBO therapy, anatomical abnormalities of the upper airways, a history of asthma, rhinitis, upper airway infection (less than 6 weeks), previous trauma or nasal surgery, drug addiction, a history of cigarette smoking or tobacco exposure, professional exposure to air pollutants and having indoor pets.

Previous authorization was obtained from the Ethics Committee that oversees clinical investigation at the Portuguese Navy Hospital (Lisbon) where the nasal biopsies were collected. All subjects gave written informed consent.

Two samples of the head of the lower turbinate were obtained with a Hartmann forceps (Karl Storz® 634822) under direct visual inspection, without local anesthesia. Local hemorrhage occured in each case; in 3 patients, compression of the wound was insufficient and the hemorrhage had to be controlled by using silver nitrate. In the case of the HBO-treated patients, the biopsies were harvested immediately after the last HBO session.

One half of the sample was fixed in buffered 10% formaldehyde, decalcified with 10% nitric acid, dehydrated with increasing concentrations of ethanol, and embedded in paraffin. Serial 3-μm thick sections were obtained from each tissue block; paraffin sections were stained with hematoxylin-eosin (H&E), periodic acid-Schiff (PAS) stain and Verhoeff stain. The other half of the sample from the inferior turbinate was fixed in an aldehyde mixture containing 4% formaldehyde, 1.25% glutaraldehyde and 10 nmol/L $CaCl_2$ in 0.05 mol/L cacodylate buffer, pH 7.2 (Silva et al., 1987). Postfixation was performed in ferrocyanide-reduced osmium tetroxide made up in distilled water (Águas, 1982). The specimens were dehydrated in ethanol, embedded in Epon, and thin-sectioned on a LKB (Sweden) ultramicrotome. The thin sections were stained with uranyl acetate and lead citrate. Grids containing sections were examined and photographed under a Jeol 100C transmission electron microscope.

Histopathologic analysis of 3-μm thick hematoxylin-eosin (H&E) stained sections included an evaluation of epithelial shedding, necrotic cell numbers, basement membrane thickness, goblet cell hyperplasia, squamous metaplasia, mononuclear and polymorphonuclear infiltration, wide intercellular spaces and shortened or absent cilia.

H&E samples and transmission electron micrographs were examined blindly by two of the authors. Series of electron micrographs were obtained from each sample from the two cohorts at magnifications of 2800x to 16000x. Ciliar areas were examined at a final magnification of 16000x.

Electron micrographs of the nasal epithelium were assessed for the following criteria: (i) intracellular alterations, namely mitochondrial damage and changes in nuclei, endoplasmic reticulum and Golgi apparatus, (ii) the presence of squamous metaplastic cells, (iii) alterations in the distribution of ciliated and goblet cells and (iv) alterations in the ultrastructure of cilia.

Results

The microscopic morphology of the nasal mucosa was studied in humans subjected to chronic HBO therapy and in controls. Comprehensive light microscopy screening of mucosal biopsies of the lower nasal turbinate revealed that the individuals that had been subjected to chronic HBO therapy exhibited a moderate leukocyte infiltration of the chorion and epithelium, as well as an enhancement of the thickness of the basement membrane (Fig. 1). These alterations were absent in samples from the control group.

We also used transmission electron microscopy (TEM) to compare the fine structure of the nasal epithelial cells from the biopsy samples from HBO-treated and control individuals. We found no morphological differences between the two groups of samples as regards the ultrastructure of the intracellular components; namely nuclei, mitochondia, endoplasmic reticulum and Golgi apparatus, or of intercellular junctional complexes. In a minority of nasal epithelial cells (less than 10%) from samples from HBO-treated patients there were scattered changes in the morphology of some cilia, and these alterations were not detected in control samples. The alterations of the cilia consisted of membrane swelling or fusion between the membrane of adjacent cilia, and changes in microtubules (either a lack of central microtubules or additional microtubules) (Fig. 2).

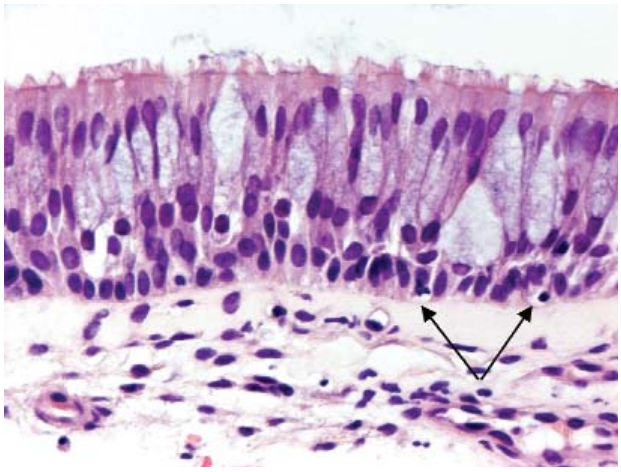

Fig. 1. Respiratory epithelium with leukocyte infiltration (arrows) and increased basement membrane thickness. (H&E staining, x 400).

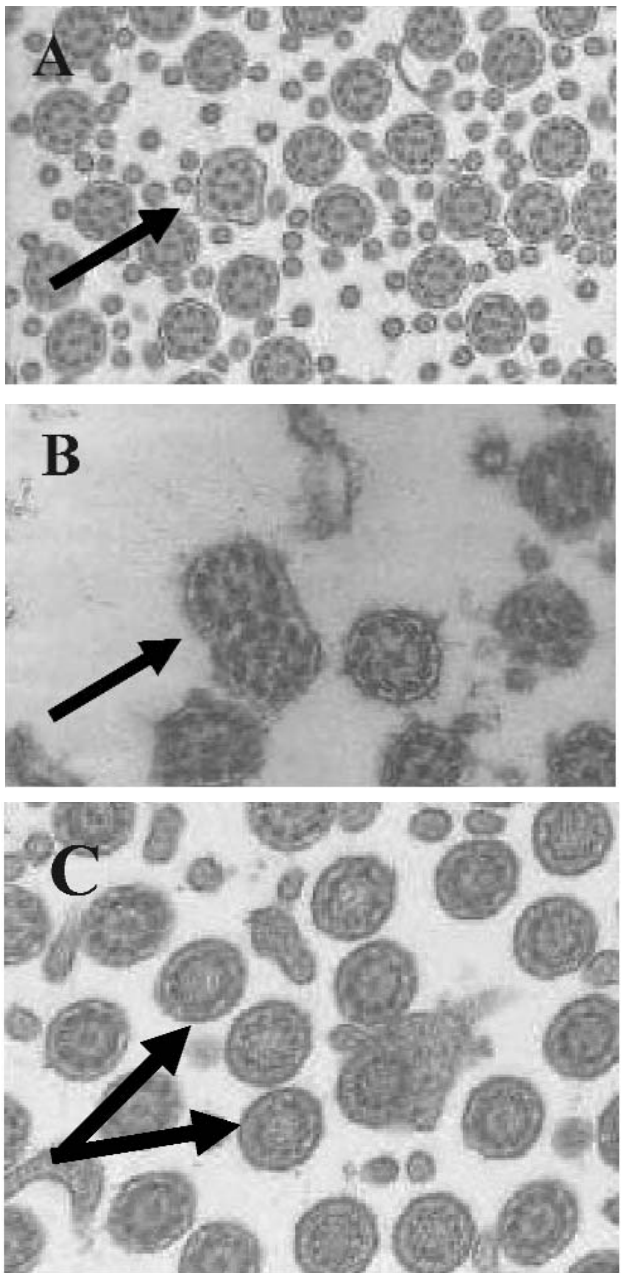

Fig. 2. Transmission electron microscopy micrographs showing ciliar swelling (**A**, x 5300), compound cilia (**B**, x 13000) and absent central microtubules (**C**, x 8000).

Discussion

HBO is an established therapy with significant advantages in the treatment of several pathologies that are ameliorated when the availability of oxygen to body tissues is enhanced (Wattel and Mathieu, 2006). However, it must be kept in mind that increasing the oxygen concentration and air pressure may potentially harm the lining of the respiratory tract, from the nose down to the alveoli (Capellier et al., 1997). Most such potential changes are related to a pro-inflammatory effect of O_2 on the mucosa (Fildissis et al., 2004). It was these putative harmful effects of HBO therapy that prompted us to perform the investigation described here.

We observed that chronic HBO therapy only caused minor inflammatory alterations of the mucosa and a few isolated changes in the ultrastructure of epithelial cilia. The latter changes in the ultrastructure of cilia due to HBO treatment are in keeping with data from previous studies. For example, albino guinea pigs subjected to middle ear barotrauma showed a minor loss of cilia and cell vacuolization (Sato et al., 1997). In the nasal cavity, HBO has been associated with increased mucociliary transport (Narozny et al., 2002). The fact that increased air pressure is able to change the nasal mucosa has been demonstrated in patients treated with continuous positive airway pressure (CPAP). After a 3-10 month CPAP-therapy all patients showed alterations in the shape of epithelial cells and conglutination and clumping of the microvilli. Mucociliary clearance was distinctly prolonged in all of these cases (Constantinidis et al., 2000). The effects of oxygen in the nasal mucosa have mainly been established taking into account the effects of the ozone molecule. It has been reported that excess oxygen may also cause DNA damage to the epithelial cells of the nasal mucosa (Pacini et al., 2003).

In conclusion, the present investigation reinforces the current prevailing view that HBO is a relatively safe method for the treatment of human patients, with negligible side effects.

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