ORIGINAL CONTRIBUTION

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Chronic hyperbaric oxygen therapy and the human nasal mucosa: increased thickness of epithelium basement membrane and moderate neutrophilic infiltration*

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SUMMARY

Objective: *We aimed to identify potential morphologic changes induced in the nasal mucosa by hyperbaric oxygen (HBO) treatment.*

Study Design: *Biopsies were obtained from two groups of 9 individuals: the first group had a diagnosis of tinnitus and was submitted to 15 sessions of 100 min-long HBO treatments, and the latter group consisted of healthy volunteers not submitted to HBO therapy.*

Methods: *Small biopsies of the anterior portion of the lower nasal turbinate were collected with the help of a Hartmann forceps under direct visual inspection. The samples were processed for light microscopy and morphometric analysis. Inflammatory infiltration (neutrophils and lymphocytes) was evaluated by a semiquantitative method. Unpaired t test and Bernoulli distribution were applied to evaluate statistical differences between data from the two groups of samples.*

Results: *Samples of the turbinate mucosa of the HBO-treated group showed a significant increase in the thickness of the epithelial basement membrane and a moderate enhancement in infiltrating neutrophils when compared with the samples from the control group.*

Conclusions: *Chronic HBO treatment causes only minor changes in the architecture of the nasal mucosa that may represent the response of the respiratory tract to the increase in pressure and in oxygen content induced by this type of therapy.*

Key words: hyperbaric therapy, oxygen, nasal mucosa, basement membrane, neutrophils

INTRODUCTION

Hyperbaric oxygen therapy (HBO) consists of the delivery of 100% oxygen to patients at pressures that are 2-3 times higher than sea level atmospheric pressure [(1)]. The patients are kept inside a hyperbaric chamber and the increased pressure aims at enhancing the amount of oxygen dissolved in the plasma of the patients. The therapeutic action of HBO is related to the direct physical effects of oxygen on blood and tissues and also with a number of secondary physiological and biochemical benefits [(2,3)]. The Undersea and Hyperbaric Medical Society has approved the use of HBO in the treatment of the following situations: air or gas emboli, carbon monoxide poisoning, gas gangrene, acute traumatic ischemia, decompression sickness, prolonged failure of wound healing, exceptional blood loss, intracranial abscess, necrotizing soft tissue infections, osteomyelitis, osteoradionecrosis, compromised skin grafts or flaps and thermal burns [(4-10)]. Other pathologies have also been successfully treated with HBO, like sudden hearing loss [(11-14)].

It has been reported that the HBO treatment alters nasal mucociliary transport [(1)], probably due to the high oxygenation of blood plasma and enhancement the metabolism of ciliated cells and to a decrease in substance P, seen in cluster headache patients [(15)]. In spite of this evidence, there are no papers on putative morphological changes of the nasal mucosa associated with HBO treatment. The current study aimed to evaluate the microanatomy of the mucosa of the lower nasal turbinate in patients submitted to HBO treatment.

MATERIALS AND METHODS

Hyperbaric Oxygen (HBO)

The HBO treatment took place in a multiplace Hyperbaric

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Chamber (Haux – Starmed 2000) in the presence of a nurse. All HBO-treated patients concluded 15 sessions of HBO therapy at 2.5 ATA (1 atmosphere absolute – ATA) for 75 minutes per session. They made one session each day, at the same hour, for 15 days. The total length of patient's stay in the chamber for each session was 100 minutes because of the time needed for compression (10 minutes) and decompressing (15 minutes). The pressure was obtained by compressed air and the patients breathed 100 % humidified oxygen through tightly fitted (nose and mouth) masks, expiring through valves connected to the space outside the chamber.

Patients

Two groups of 9 individuals were chosen for this study. The first group of patients was submitted to chronic (15 sessions) HBO treatment because of the diagnosis of tinnitus. They were all male patients, with age ranging 28-68 years, with a mean of 50.89 ± 11.78 years. The second group of 9 men (controls) comprised patients that were scheduled for ear surgery. They were not submitted to HBO treatment and presented with an age range of 25-47 years with a mean of 36.89 ± 8.95. This research project had obtained previous authorization from the Ethics Committee that oversees clinical investigation at the Portuguese Navy Hospital (Lisbon) where nasal biopsies were collected. Exclusion criteria to eliminate patients from the study were the following: all criteria that exclude patients from HBO therapy, upper airways anatomical abnormalities, history of asthma, rhinitis, upper airway infection (shorter than 6 weeks), previous trauma or nasal surgery, drug addiction, cigarette smoking or professional exposure to air pollutants.

Nasal Biopsies and Light Microscopy

Samples of the head of lower turbinate were obtained with a Hartmann forceps (Karl Storz® 634822) under direct visual inspection, without local anesthesia. Local haemorrhage occured in every case; in 3 patients, compression of the wound was not enough and the haemorrhage had to be controlled with cautherization using silver nitrate. With regards to HBO-treated patients, the biopsies were harvested immediately after the last HBO session. The samples were fixed in buffered 10% formaldehyde, decalcified with 10% nitric acid, dehydrated with increasing concentrations of ethanol, and then embedded in paraffin. Serial 3μmicron-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 [16]. The epithelium and basement membrane thickness were evaluated using a calibrated eyepiece at original magnification x400. Standard morphometric methods were used to obtain light microscopy measurements [17]. In each H&E slide, three points where the epithelium was perpendicularly cut were measured for epithelial and basement membrane thickness, in order to minimize tangential section artifacts. The mean values of each variable were used for all specimens.

Table 1. Quantitative comparison of epithelial and basement membrane thickness between samples of the lower nasal turbinate of HBO-treated and control patients.

Thickness in micra of the epithelium and basement membrane.			
	Control group	HBO exposed group	p-value
Epithelium	77.5 ± 27.7	65 ± 11.2	0.16
Basement membrane	8.9 ± 2.7	12.1 ± 4.1	< 0.05

The epithelium and the chorion were assessed for the presence of inflammatory cell infiltrate (lymphocytes and polymorphonuclear [PMN] leukocytes), and the presence of submucosal seromucinous glands was recorded. The inflammatory cell infiltrate (lymphocytic and PMN) was classified as mild (+ : scattered inflammatory cells in the epithelium or chorion, with less than 5 leukocytes / high power field [HPF] 400x), moderate (++ : inflammatory cell infiltrate in the epithelium or chorion, with 5-20 cells/ HPF) or intense (+++ : dense inflammatory cell infiltrate in the epithelium or chorion, with 21 or more cells / HPF). These three categories were used in order to simplify the statistical analysis and because in other organs where inflammatory infiltration and its consequences are best studied, such grouping is also employed [18].

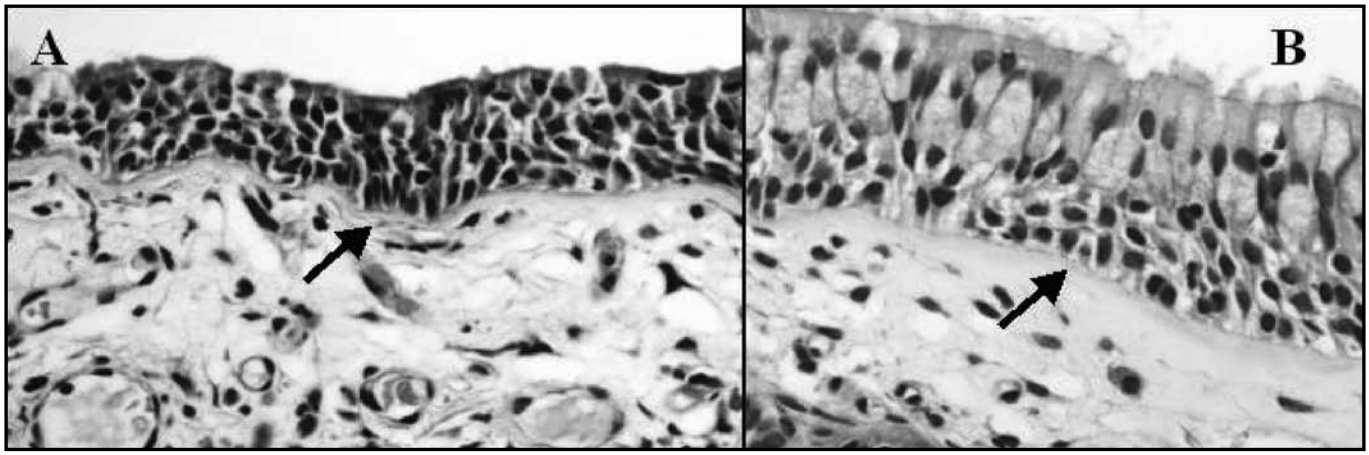

Figure 1. Light microscopy micrograph of paraffin section of human lower nasal turbinate mucosa showing the basement membrane (arrow) in the control group (A) and increased in a sample from HBO-treated group (B). H&E staining, x400.

Table 2. Semiquantitative scoring of inflammatory infiltrates by neutrophilic leukocytes of the epithelium and chorion of the lower nasal turbinate in HBO-treated and in control patients.

Epithelial and chorion polymorphonuclear infiltration							
Epithelial inflitration				Chorion infiltration			
Control group		HBO exposed group		Control group		HBO exposed group	
Control 1	0	Patient 1	0	Control 1	0	Patient 1	0
Control 2	0	Patient 2	+	Control 2	0	Patient 2	++
Control 3	0	Patient 3	0	Control 3	0	Patient 3	+
Control 4	0	Patient 4	+	Control 4	0	Patient 4	+
Control 5	0	Patient 5	0	Control 5	0	Patient 5	+
Control 6	0	Patient 6	+	Control 6	0	Patient 6	+
Control 7	0	Patient 7	++	Control 7	0	Patient 7	+++
Control 8	0	Patient 8	0	Control 8	0	Patient 8	+
Control 9	0	Patient 9	0	Control 9	0	Patient 9	+

Statistical analysis

Statistical comparison between data from the two groups of samples (HBO-treated and control individuals) was performed using the Microsoft Excel® program. Histological and morphometric differences between control and HBO treated patients were tested with the unpaired t test. Measurements were expressed as mean ± SD. A p-value of < 0.05 was considered to indicate a significant statistical difference between the two groups. The Bernoulli distribution was used to evaluate the statistical significance of the presence of inflammation.

RESULTS

Comprehensive screening by light microscopy of nasal biopsy samples of HBO-treated and control individuals showed no dramatic changes in the architecture of the epithelium and mucosa of the lower turbinate. In the majority of the samples (70%), a squamous metaplastic epithelium was observed, as is expected from the anterior portion of the lower turbinate in a human adult population. There were no age-related differences in the control and HBO-treated individuals.

We also found that the HBO treatment did not increase the frequency of squamous metaplastic epithelium in the nasal turbinate.

Two major differences were found between samples from HBO-treated and controls individuals: (i) the thickness of the epithelial basement membrane was increased in HBO-treated patients (Figure 1); and (ii) the epithelium and chorion of samples from HBO-treated patients, but not from controls, showed leukocyte infiltrates (Figure 2). Numerical data on the thickness of the turbinate epithelial basement membrane is presented in Table 1; the difference between the two groups was statistically significant ($p < 0.05$).

Polymorphonuclear neutrophils (PMN) were the dominant leukocytes that were observed in the inflammatory infiltrates found in the epithelium and chorion of the lower nasal turbinate of HBO-treated patients (Figure 3). In contrast, inflammatory infiltrates were rare or absent in control samples. We have applied a semiquantitative scale (0 to +++) to compare the two groups of samples with regards to PMN or lymphocyte infiltration. Comparison of these data revealed a statistically significant difference ($p < 0.05$) in PMN infiltration of

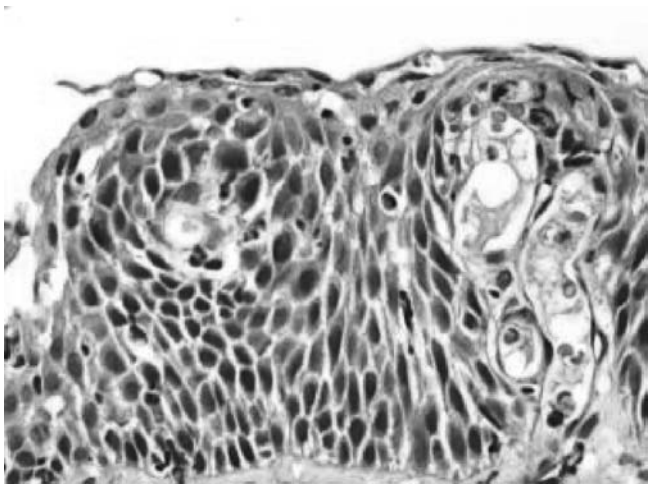

Figure 2. Light microscopy micrograph of paraffin section of human lower nasal turbinate mucosa showing neutrophils infiltrating the epithelium in a sample from the HBO-treated group of patients. H&E staining, x400.

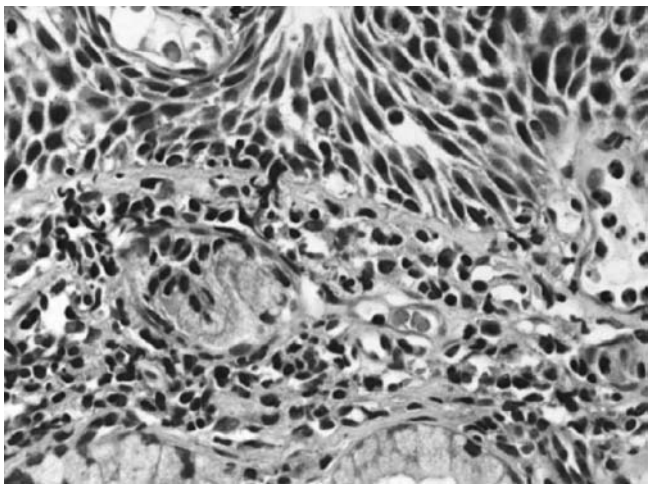

Figure 3. Light microscopy micrograph of paraffin section of human lower nasal turbinate mucosa showing infiltration by inflammatory cells (neutrophilic leukocytes) of the chorion of a patient from the HBO-treated group of patients. H&E staining, x400.

both epithelium and chorion between samples from HBO-treated and control individuals (Table 2). Regarding lymphocytes, comparison of the numerical data failed to reveal a significant difference ($p = 0.27$) between the two groups of samples, although qualitative analysis of paraffin sections had suggested that, at least in the chorion, a moderate lymphocyte infiltration was present in the HBO-treated patients and absent in controls.

DISCUSSION

The current investigation documents that chronic HBO therapy is associated with moderate inflammation of the nasal mucosa, expressed by its mild infiltration by neutrophilic leukocytes, and with enhanced thickness of the epithelial basement membrane. Our patients were submitted to fifteen treatments of HBO that was used at 100% O_2 and 2.5 ATA. This type of treatment consisted in submitting the patients to daily periods of 75 minutes of high pressure and high concentration of O_2. A limitation of the design of our study is the lack of nasal biopsies before the HBO treatment was started, as well some time after its conclusion. Data from these additional biopsies would ascertain whether the herein described changes are permanent or reversible. However, we were not allowed by the Ethics Committee of our hospital to perform these two additional biopsies in the patients.

To understand the HBO-associated changes of the nasal mucosa, it is pertinent to recall the effects on the respiratory tract of the two major factors involved by HBO therapy: physical stimulation of the mucosa by enhanced atmospheric pressure and chemical stimulation of the tissues by 100% O_2. Increase in atmospheric pressure is known to cause decrease in mucociliary transport time, namely under air oscillations of 16 Hz and pressure differences of 200 mmHg [19]. Ventilatory support with continuous positive airway pressure (nCPAP) is a common clinical situation that involves changes in the atmospheric pressure reaching the nose. Three months after nCPAP therapy, the architecture of the nasal mucosa appears to be restored [20] and after 6 months, the mucociliary clearance is normal [21].

Enhancement in oxygen concentration is known to affect the whole lining of the respiratory tract, from the nose down to the alveoli. Most of these changes are related to a pro-inflammatory effect of O_2 on the mucosa. At normobaric conditions, O_2 applied to one nostril enhances nasal mucociliary transportation, and also increases the number of PMN and cylindric cells and of IL-6, IL-8 and ICAM-1 expression [22]. In vitro experiments have shown that increasing O_2 content will accelerate the nasal ciliary beat frequency. At high O_2 concentrations this effect is reversed, possibly due to oxygen toxicity [23]. Animals exposed to hyperoxia develop nasal epithelium cell replication, hypertrophy of non-ciliated cuboidal cells, and increased activity of glucose-6-phosphate dehydrogenase and of glutathione peroxidase [24]. Hyperoxia is also known to induce lesions in lung endothelial cells and in type I pneumocytes [24]; additionally, DNA damage is also seen in type II pneumocytes [25] and increase in Fas/FasL gene expression promoting apoptosis of these cells [26]. Oxygen in the form of ozone (O3) has also been studied because of the current importance of respiratory lesions due to air pollution. Major abnormalities caused by ozone are related with inflammatory effects [27], DNA damage [28], release of neuropeptides [29], cell death [30] and mucinous metaplasia [31,32].

Taking into account the above described actions of increased atmospheric pressure and of enhanced concentration of O_2 on the nasal mucosa, it is plausible to consider that the inflammatory and basal membrane changes that we have found in patients submitted to chronic HBO therapy are more likely derived from the action of O_2 on the nasal mucosa, rather than from the increased atmospheric pressure. Since nasal mucosa shares many common responses with the lower airway of the respiratory tract [22], it is pertinent to consider whether similar changes occur along the respiratory tract and in the lung tissue after chronic HBO therapy.

CONCLUSION

Treatment of patients with hyperbaric oxygen in repeated 15 sessions of 100 minutes causes minor, but nevertheless significant, changes of the nasal turbinate mucosa, namely granulocyte infiltration and increased thickness of basal membrane. Whether these changes of the nasal mucosa extend to the whole respiratory tract is a question that deserves further investigation.

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