THE HISTOMORPHOLOGIC EFFECTS OF HYPERBARIC OXYGEN THERAPY ON SUBMANDIBULAR, THYROID GLANDS AND TRACHEA

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ABSTRACT
The aim of this experimental study was to investigate the efficacy of hyperbaric oxygen (HBO) therapy in the neck region of rats based on morphologic changes induced in the exocrine and endocrine glands and trachea. Samples of submandibular glands, thyroid gland and trachea were collected following sacrifice and neck dissection of the animals. The samples were processed for light microscopy and morphometric analysis. HBO administered rats were placed into a large pressure chamber and received 100 % oxygen at 253.3125 kPa (2.5 ata) for 60 min per day for a period of seven days. HBO-treated rats showed no statistically significant differences from the control group, according to the analysis of the histopathological scores (p = 0.930). HBO could probably be considered as neither helpful, nor harmless, on the submandibular glands, thyroid glands and trachea in rats.


Keywords: hyperbaric oxygen, rat, submandibular glands, thyroid glands and trachea

Introduction
Hyperbaric oxygen (HBO) therapy is inhalation of 100 % oxygen at pressures more than 1.4 times greater than the atmospheric pressure. Hyperbaric oxygen therapy (HOT) is based on inhalation of 100 % oxygen under pressures above 101.325 kPa (14). HOT has important antioxidant effects, which is why, since it was first introduced in 1953 by Gray et al. (3), it is being increasingly used in a range of medical practice areas to treat patients with various disorders. The Undersea and Hyperbaric Medical Society has approved the use of HBO in the treatment of the following conditions: air or gas embolism, carbon monoxide poisoning, gas gangrene, crush injury, decompression sickness, arterial insufficiencies, severe anemia, intracranial abscess, necrotizing soft-tissue infections, refractory osteomyelitis, delayed radiation injury, compromised grafts and flaps, acute thermal burn injury, and idiopathic sudden sensorineural hearing loss (2, 6, 8, 10).

For example, irradiation causes xerostomia by effect of submandibular or parotid glands, which can sometimes be irreversible. Another big problem due to these traditional radiation therapies is tracheomalacia. Not to mention, the thyroid gland is important in metabolism. Because of this, we need therapies with anti-toxic and regenerating effects that specifically target the thyroid without harmful side effects. HBO therapy is one potential method. This method is non-toxic and can more specifically regenerate cellular activity. HBO has been used in sudden hearing loss, prolonged failure of wound healings in the head and neck, and also prevention and treatment of mandible osteoradionecrosis after irradiation in otolaryngology practice. All in all, HBO can be used as an alternative therapy.

On the other hand, as with all medical procedures and treatments, some potential adverse effects may result from exposure to HBO. Moreover, it is an intervention whose unique mechanism of action is not well understood.

HBO is administered via inhalation, and may contribute to adverse or beneficial effects on the submandibular, thyroid and tracheal surface. However, to the best of our knowledge, no studies to date have investigated the effects of HBO on the neck region, including the submandibular glands, the thyroid gland, and the trachea. The available knowledge is based on reports about animal and human adverse effects from HBO therapy. The main types of potential adverse effects are barotraumatic lesions (middle ear, nasal sinuses, inner ear, lung and teeth), oxygen toxicity (central nervous system and lung), ocular effects (myopia and cataract) and anxiety (7).

The aim of this study was to evaluate the histopathologic effects of HBO on the submandibular glands, the thyroid gland, and trachea, in rats.

Materials and Methods
Wistar albino rats were randomly divided into two groups as follows: Control group (n = 8) and HBO group (n = 8). All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (4). The study was also approved by the Ethical Committee of our institution under permit 2012/09. Sixteen male adult Wistar albino rats with normal weight between 195 g and 260 g were used in this study. They were maintained according to the standard guidelines. All animals were housed reasonably in cages under standard environmental conditions (room temperature between 22 °C and 24 °C and 50 relative humidity within a 12 h light/12 h dark cycle...
Fig. 1. The experimental hyperbaric treatment chamber where HBO treatment was given to the rats.

Fig. 2. Normal view of submandibular gland histological sections from control (A) and HBO-treated rats (B); of thyroid histological sections from control (C) and HBO-treated rats (D); of trachea histological sections from control (E) and HBO-treated rats (F) (H–E staining, 200X magnification).
photoperiod). All the animals had free access to water and conventional laboratory diet until sacrifice. The rats from the HBO group were placed into a large pressure chamber with a volume of 20 L, separately (Fig. 1) and received 100% oxygen at 253.3125 kPa (2.5 ata) for 60 min per day for a period of seven days. Anesthesia was not performed, so the animals breathed spontaneously during HBO treatment. The rats from the control group did not receive HBO.

**Operation procedure**

All procedures were performed under clean but non-sterile conditions. After they were anesthetized, the experimental rats were decapitated. The neck of the animals was cut in the midline and each submandibular and thyroid gland was dissected by scalpel and a pair of scissors. Hands-on dissections were performed with a haemostatic clamp. Bilateral submandibular glands and thyroids were dissected and removed en bloc along with the trachea. Biopsy specimens from each organ were fixed in a 10% formaldehyde solution and stored for histopathological analysis.

**Anesthesia**

Rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Ketalar®) at a dose of 60 mg/kg and 2% xylazine hydrochloride (Rompun®) at a dose of 10 mg/kg by intramuscular injection (IM) before sacrifice.

**Tissue preparation for light microscopy**

Animals were sacrificed by decapitation approximately 2 h after the end of the seventh-day HBO exposure. The sections were stained with haematoxylin and eosin (H–E), Periodic acid–Schiff (PAS) and Trichrome–Masson in order to be observed under a light microscope (Nikon Eclipse 80i). All morphological changes, including inflammatory leukocyte infiltration and cellular hyperplasia, and basal membrane thickness, were noted. Semi-quantitative scaling of inflammatory leukocyte infiltration and cellular hyperplasia was performed. The intensity of these changes was graded from 0 to 3 (0: no infiltration or thickness, 1: faint, 2: moderate, 3: intense). Examinations were performed by experienced pathologists and statistically scored in a blind manner.

**Statistical analysis**

Statistical evaluation was carried out with the Statistical Package for the Social Sciences for Windows (version 15.0, SPSS Inc., Chicago, IL, USA). The histopathological variations in the HBO-treated group and in the group without treatment (control) were evaluated and compared to each other by the Mann–Whitney U-test. The results were expressed as mean ± SD. The level of statistical significance was set at \( p < 0.05 \).

**Results and Discussion**

According to our light microscopy observations, the histopathological examination with H–E staining showed that deterioration was not observed in the submandibular glands, the thyroid gland, or the trachea, in the HBO and control group. Additionally, H–E staining showed no distinctive difference between the control group and the HBO group. No significant differences on average were noted between the histopathological scores of the HBO and control groups (Fig. 2).

Churchill-Davidson et al. (1) started to use hyperbaric rooms for curative medicine for the first time in 1955. Today, HBO therapy is used around world with the aim to increase the quality of life.

All studies of HBO therapy to date have been targeted at preventive or regenerative treatment against disease or toxic agents that negatively affect the body. A growing number of HBO therapy papers have been published in the past decade, especially from studies conveyed in the USA, where there are about 259 hyperbaric facilities with 344 single-occupant hyperbaric-oxygen chambers (5). The most relevant reports, based on statistics about top-cited articles, focus on treatment of stroke, radiation injury, carbon monoxide poisoning, and wounds (5). Lee et al. (5) reviewed the mechanisms of action, evidence of clinical efficacy, and risks of HBO therapy. The treatment efficacy of HBO is based on two effects: mechanical effect on bodily gases and incremental effect on blood partial oxygen pressure (9). Some studies focused on the effects HBO therapy on the nasal and sinus regions. For example, Yorgancilar et al. (15) and Vera-Cruz et al (11) studied the effect of HBO on the rat’s nasal mucosa. Yorgancilar et al. reported that HBO treatment was found to cause mild inflammation and increasing thickness of the pseudostratified columnar epithelial layer of the nasal mucosa. Morphologic changes in the rat nasal mucosa after acute and chronic HBO therapy were also observed by Vera-Cruz et al (11). In a human study, Vera-Cruz et al. (12) demonstrated that samples of the turbinate mucosa from the HBO-treated patients 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. They concluded that chronic HBO treatment causes 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.

Our study showed no statistically significant differences between HBO-treated and control group rats according to the analysis of the histopathological scores. This result may be attributed at least in part to the session and/or dosage of HBO applied, since different protocols for HBO treatment have been used in some studies (13). For example, one difference is the pressure applied during food supplementation (HBO at 253 kPa to 304 kPa applied for diabetic food may give different results). In our study, we applied 253.3125 kPa for 60 min per day for a period of seven days. Moreover, HBO therapy may affect different mechanisms associated with the pathology of oxidative tissue damage to normal tissue or tissue resistance to HBO like that observed in the submandibular glands, thyroid gland, and trachea. Our results indicate that, under our experimental conditions, other organs (submandibular glands, the thyroid gland, and trachea) were not harmed by HBO.
This could be relevant for HBO applications in the medical treatment of different diseases.

Conclusions

The aim of this study was to evaluate the histopathologic effects of HBO (100 % oxygen at 2.5 atm for 60 min per day for a period of seven days) on the submandibular glands, the thyroid gland, and trachea, in rats. Based on the obtained results, we could conclude that HBO has no harmful effects on submandibular glands, parotid glands, thyroid glands, and trachea, when used properly for medical care and treatment.

REFERENCES