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Histopathological evaluation of the effect of intranasal phototherapy on nasal mucosa in rabbits

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ABSTRACT

Allergic rhinitis is a high-incidence allergic inflammation of the nasal airways that impacts quality of life. Of the numerous therapies used to treat allergic rhinitis, intranasal phototherapy has emerged as a promising new treatment modality for inflammatory airway disease. Phototherapy is widely used for the treatment of immune-mediated skin diseases because its profound immunosuppressive effect inhibits hypersensitivity reactions in the skin. Intranasal phototherapy using a combination of Ultraviolet-A (UVA) and Ultraviolet-B (UVB) plus Visible light (VIS) has been shown to suppress the clinical symptoms of allergic rhinitis, but limited data regarding its adverse effects on the nasal mucosa currently exists. In this study, we demonstrate that UV displays no harmful effects on the nasal mucosa cells of rabbits following 2 weeks of intranasal phototherapy.

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1. Introduction

Phototherapy has been successfully used in the treatment of a variety of immune-mediated skin diseases, including atopic dermatitis and psoriasis. Its beneficial effects have been attributed to its profound immunosuppressive effect [1]. In the last decade, Ultraviolet (UV) light has been used to treat oral mucosal diseases, lichen planus, and graft versus host disease [2,3]. The major mechanisms producing the beneficial effects of phototherapy involve the reduction of Langerhans cell number and function, induction of apoptosis in infiltrating T cells, and induction of immunomodulatory cytokines such as TNF- α and IL-10 [4,5].

Allergic rhinitis is an allergen-induced IgE-mediated inflammatory disease of the nasal mucosa; it is developed and maintained by eosinophils, T and B lymphocytes, dendritic cells, and mast cells [6]. Recently, intranasal phototherapy using a combination of UVA and UVB plus VIS was proposed as a therapeutic alternative for patients with allergic rhinitis, as UV has been shown to significantly suppress the clinical symptoms [4,7].

Although allergic rhinitis has been successfully treated with phototherapy, limited data are available regarding the potentially harmful effects of UV light on the nasal mucosa. In this study, we histopathologically evaluated the effect of intranasal phototherapy on the nasal mucosa in a rabbit model.

2. Materials and methods

A total of 12 female New Zealand rabbits (6 months old, 2000–2500 g each) were used. The experimental protocol was conducted in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals (DHEW Publication NIH 85-23, revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD) and was approved by the Committee of Animal Research of Abant Izzet Baysal University, Bolu, Turkey. The study was performed at the Abant Izzet Baysal University Institute for Experimental Medicine Research, Bolu, Turkey. Every effort was made to minimize animal pain and discomfort, and to limit the number of animals necessary to obtain statistical significance. All of the animals had free access to food and water. The rabbits were divided into two groups. Group I was the study group ($n = 7$), and Group II was the control group ($n = 5$). All experiments were performed in the same room at a constant room temperature and environment. During this study, the rabbits were sedated with ketamine (80 mg/kg Ketalar; Pfizer, Ltd., Vienna, Austria) and xylazine (5 mg/kg Rompun; Bayer, Ltd., Leverkusen, Germany) through intraperitoneal injection prior to and during phototherapy. Phototherapeutic illumination of all animals was performed by the same examiner using the same rhinolight device (model Rhinolight III; Rhinolight, Ltd., Szeged, Hungary). The rhinolight devices have compatible tips diameter in different sizes to be applied in to the nostril of the rabbits (Figs. 1 and 2).

Each intranasal cavity was irradiated three times a week for 2 weeks, using the incremental dosage times shown in Table 1. The starting dose of 2 min was equal to 1.6 J/cm². Each consecutive

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Fig. 1. The different size rhinolight device' tip diameters.

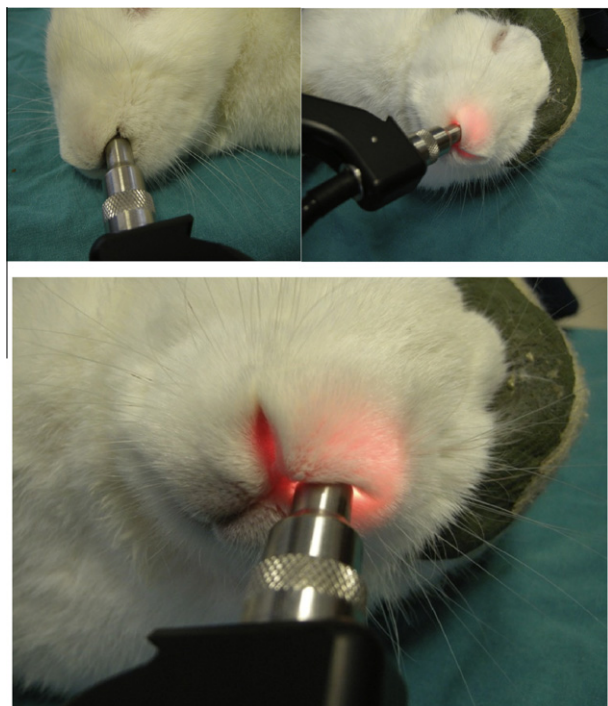


Fig. 2. The rhinotherapy application to the rabbit.

Table 1

Treatment regime of intranasal cavity irradiation.

Week	1	1	1	2	2	2
Treatment number	1	2	3	4	5	6
Treatment time (min)	2:00	2:15	2:30	2:45	3:00	3:00

treatment dose was increased by 0.2 J/cm^2 , reaching the highest dose of 2.6 J/cm^2 at the sixth treatment. At the end of the endonasal phototherapy, the rabbits were sedated by intraperitoneal injection with ketamine (80 mg/kg Ketalar) and xylazine (5 mg/kg Rompun). Nasal mucosal biopsies were obtained from the lower edge of the inferior turbinate and nasal septal mucosa. Biopsy specimens were fixed in paraformaldehyde and embedded in paraffin. For histological analyses, after a routine follow-up, paraffin blocks were prepared from the tissues, and 5- μm sections were cut using a microtome. Hematoxylin–eosin was used to stain the

mucosal structure, anilin blue was used to determine the mast cell number, and TUNEL assays (Gen Script apoptosis kit.) were performed to evaluate epithelial apoptosis.

3. Results

We observed regular nasal mucosa and concha structure was normal (Figs. 3 and 4) in the control group. In study group, normally stained cells and cells with round picnotic nucleus and pale cytoplasm were observed in histological examination of conchal epithelia (Fig. 5). Nasal septal structures were similar in both groups. Ductal structures were more prominent especially in sub-epithelial connective tissue. In the lumen of the some glands cellular debris was observed. In both groups, epithelial structures were similar (Fig. 6). No differences in the structure of the nasal septum hyaline cartilage or the structure or size of chondrocytes were observed between the control and phototherapy groups. In addition, no TUNEL-positive cells were evident following phototherapy. The mast cell distribution in the sub-epithelial connective tissue also did not differ between the control and phototherapy groups.

4. Discussion

Allergic rhinitis, defined as an inflammation of the nasal membranes, is an extremely common condition. Although it is not life-threatening, complications can occur, and the condition can significantly impair the quality of life. Intranasal steroid and antihistamines are the current efficacious topical therapies of allergic rhinitis [8]. But these medications are sometimes contraindicated, as in pregnant or breast-feeding women. Even in the absence of contraindications, patients frequently decline medication to relieve their symptoms [8].

Phototherapy is an alternative treatment modality for inflammatory and immune-mediated mucosal diseases. During the last decade, UV light has been successfully applied to the treatment of oral mucosal diseases, including lichen planus and graft versus host disease [2,3,9]. Despite its obvious clinical benefits, the potential side effects of intranasal phototherapy on the nasal mucosa have not been assessed in detail. The total UV dose in rhinophototherapy is lower than that used in skin phototherapy and no morphological changes or alterations in Langerhans cell number after endonasal phototherapy have been reported [10].

Previous studies have shown that UV did not produce harmful effects on the DNA of nasal mucosa cells and that the nasal mucosa

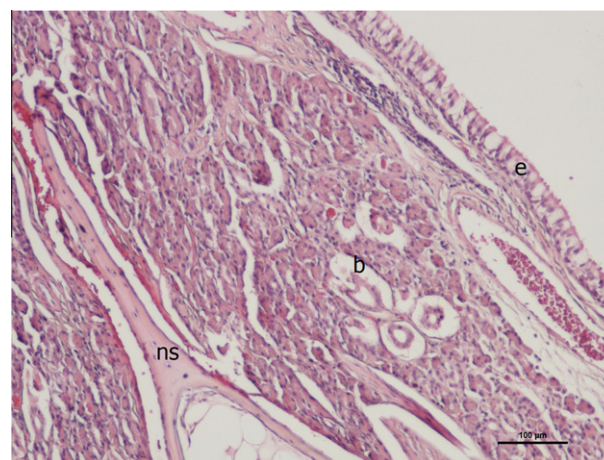


Fig. 3. Nasal septum (ns) and epithelium (e), connective tissue and gland (b) (H&E, bar 100 μm).

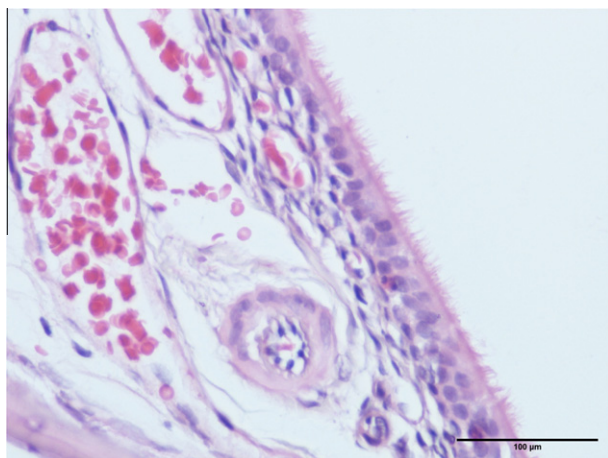


Fig. 4. Epithelial cells with prominent regular cilia in conchal specimen of control group (H&E).

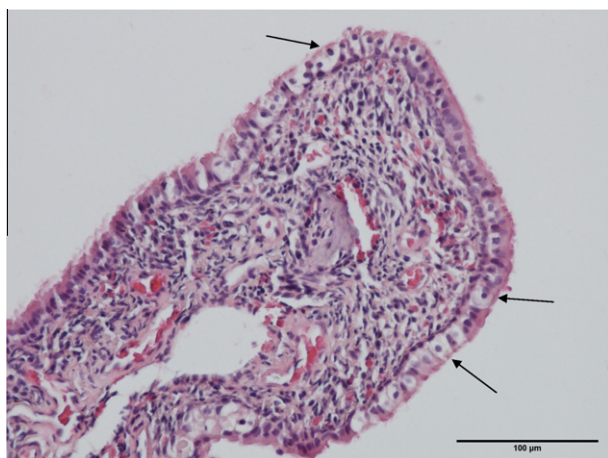


Fig. 5. In study group, normally stained cells and cells with round picnotic nucleus and pale cytoplasm were observed in histological examination of conchal epithelia (→) (H&E).

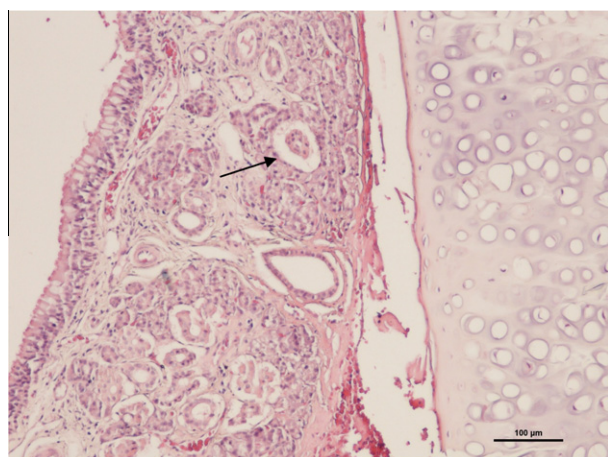


Fig. 6. Ductal structures were more prominent especially in sub-epithelial connective tissue. In the lumen of the some glands cellular debris was observed (→) (H&E, bar 100 μm).

could effectively repair UV induced DNA damage [2,11]. Interleukin (IL)-5 is a cytokine that promotes the maturation, activation, and survival of eosinophils [12]. Irradiation of the nasal mucosa

has been demonstrated to locally decrease the IL-5 concentration. As T lymphocytes are a major source of IL-5, apoptosis in these cells following phototherapy was suggested as the underlying mechanism of the decreased IL-5 production [2,4]. Allergic rhinitis is also accompanied by an elevated level of IL-4. The proapoptotic effects of IL-4 are more dramatic in eosinophils and the quantitative relationship between IL-4 and IL-5 produced during inflammation may determine the apoptotic status of eosinophils at the site of allergic inflammation. No changes in the IL-4 level in nasal lavage samples have been reported following phototherapy. Nevertheless, the reduction of IL-5 in the nasal mucosa together with the persistence of IL-4 may further promote phototherapy-induced eosinophil apoptosis [4].

In addition to eosinophils, T cells, basophils and mast cells possess important roles in the effector phase of the allergic reaction, particularly as a source of histamine [13]. In this regard, it has been shown that UVB light inhibits histamine release from mast cells and human basophils [14].

In this study, the effect of intranasal phototherapy on the nasal mucosa of rabbits was assessed for the first time. A pathological change was not evident in the rabbit nasal mucosa or concha epithelium cells of the rabbits receiving phototherapy. The main limitations of this study include our relatively small sample size due to animal study. Further studies to assess these effects for longer time periods in an allergic rhinitis model would be beneficial.

5. Conclusion

In this pilot study, we demonstrate that after exposure to UV light, the nasal mucosa of rabbits displayed no changes in the mucosa, concha epithelium cells, or nasal septum cartilage structure.

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