PHOTOTHERAPY IN THE TREATMENT OF ALLERGIC RHINITIS

Ph.D. Thesis

Zsanett Csoma M.D.

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CONTENTS

List of publications related to the subject of the thesis

Original articles

I. **Csoma Zs,** Ignácz F, Bor Zs, Szabó G, Bodai L, Dobozy A, Kemény L: Intranasal irradiation with the xenon chloride ultraviolet B laser improves allergic rhinitis. J Photochem Photobiol B. 75: 137-44, 2004. IF: 2,275

II. Koreck A, **Csoma Zs,** Boros-Gyevi M, Ignácz F, Bodai L, Dobozy A, Kemény L: Inhibition of immediate type hypersensitivity reaction by combined irradiation with ultraviolet and visible light. J Photochem Photobiol B, 77: 93-96, 2004 IF: 2,275

III. Koreck A, **Csoma Zs,** Bodai L, Ignácz F, Kenderessy Szabó A, Kadocsa E, Szabó G, Bor Zs, , Erdei A, Szőny B, B Homey, Dobozy A, Kemény: Rhinophototherapy: a new therapeutic tool for the management of allergic rhinitis. J Allergy Clin Immunol 115: 541-47, 2005 IF: 6,831

IV. Koreck A, **Csoma Zs,** Ignácz F, Bodai L, Kadocsa E, Szabó G, Bor Zs, Nékám K, Dobozy A, Kemény L: Intranasalis fototerápia az allergiás rhinitis kezelésében. Orv Hetil. 146, 965-969 (2005)

List of abbreviations

IgE	Immunglobulin E
Th2 cells	T-helper 2 cells
ECP	eosinophil cationic protein
UV light	ultraviolet light
VIS light	visible light
UVB	ultraviolet-B
BB-UVB	broad-band ultraviolet-B
NB-UVB	narrow-band ultraviolet-B
XeCl	xenon chloride
UVA	ultraviolet-A
PUVA	psoralen plus UVA
IL	interleukin
SPT	skin prick test
MED	minimal erythema dose
mUV/VIS light	mixed ultraviolet and visible light
TNS	total nasal score
8-MOP	8-methoxypsoralen
MPD	minimal phototoxic dose
l-VIS	low-intensity visible light
APC	antigen-presenting cells
GM-CSF	granulocyte/macrophage colony-stimulating factor
RBL	rat basophilic leukemia line

1. Introduction

Allergic rhinitis is one of the most common health problems in many countries, because it is a high-cost, high-prevalence disease, affecting about 15-30% of the population. The number of the patients with allergic rhinitis is still increasing, especially in the well-developed, industrialized countries [1]. Although it is not associated with severe morbidity and mortality, allergic rhinitis has a major effect on the quality of life. Its increasing prevalence, its impact on the individual quality of life and social costs [2, 3] and its role as a risk factor for asthma [4], underline the need for improved treatment options for this disorder.

Allergic rhinitis is an inflammatory disorder of the nasal mucosa characterized by nasal itching, sneezing, nose running, nose blockage, itching of the nasal palate, itching of the eyes and of the external auditory canal, edema of the eyelids and occasionally loss of the sense of smell. In the case of seasonal or transitoric rhinitis pollens of different trees, grasses, flowers and weeds are responsible for developing of the clinical symptoms, which lasts for a few weeks or months depending on the flowering of the plants. The severity of the symptoms mainly depends on the pollen count, but non-specific, environmental factors (such as air pollution), the physical and psychical condition of the patient can also influence it. The pollen season can be divided into three periods in Hungary. The first one is the spring season (March-April), when the trees and bushes bloom. The next one is when the grass and grain bloom (May-June) and, finally, the end of summer and fall, with the blooming of the ragweed and other weeds. This last season is when the symptoms are the most acute and the number of the patients is the highest, too. House-dust mite, animal hair and feather, mould spores are responsible for developing of perennial allergic rhinitis, which exists throughout the entire year. It has the same clinical symptoms such as seasonal allergic rhinitis, but the main symptom is the nasal blocking, and its frequent complication is sinusitis.

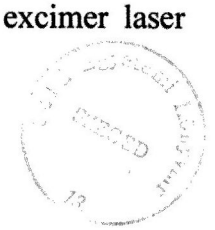
The etiology of allergy is multifactorial, it is genetically determined, but environmental factors also play an important role in the development of the typical symptoms. The inflammation of the nasal mucosa is frequently induces the inflammatory condition of the paranasal sinuses (rhinosinusitis).

The inflammation is a type I, or immediate hypersensitivity reaction of the nasal mucosa that arises in consequence of an allergen-immunoglobulin E (IgE) interaction in sensitized individuals [5]. The development of the disease is characterized by an initial

sensitization phase to a specific allergen, when no clinical symptoms are present. At later time-points the encounter of the same allergen by sensitized individuals is followed by the elicitation of an allergen specific immune response and the activation of effector mechanisms. Previous studies have established that a shift towards T-helper 2 (Th2) cells plays a role in the initiation and maintenance of the disease [6, 7]. Eosinophils, mast cells and basophils are considered the effector cells of hay fever [8, 9]. Following an allergen challenge these cells release inflammatory mediators such as histamine, tryptase, leukotrienes, prostaglandins, cytokines and eosinophil cationic protein (ECP), which are responsible for most of the pathological processes occurring in the nasal mucosa [5, 9-11].

Elimination of the inhalative allergens –which are responsible for developing of hay fever- from the patient's environment is very difficult. For the treatment of the disease, well-established pharmacological therapies are available. Locally and systematically applied antihistamines are widely used to block the released mediators from the increasing number of inflammatory cells in the nasal mucosa. Sodium cromoglycate is used to inhibit mediator release from inflammatory cells; locally and systematically applied corticosteroids are effective in blocking new mediator synthesis. New therapeutic options have recently become increasingly important, including leukotriene modifiers, anti-IgE antibodies, phosphodiesterase inhibitors and intranasal heparin, and there have been developments in appropriate allergen-specific immunotherapy [12]. However, the complete suppression of the clinical symptoms may not be achieved in most of the cases with the currently available drugs. The use of these drugs is controversial in special subsets of patients such as pregnant and breast-feeding women [13], and pharmacotherapy has a numerous side-effects, too. All of these characteristics of allergic rhinitis highlight the need for effective new treatment options.

Phototherapy has a profound immunosuppressive effect [14-18], and the different phototherapeutic methods utilizing both ultraviolet (UV) and visible (VIS) light has been widely used for the therapy of various inflammatory skin diseases, including atopic dermatitis and psoriasis [19-21]. Initially, broadband UV light sources in the 290-320 nm UVB spectrum range (BB-UVB) were used for the treatment of allergic and non-allergic skin diseases. During the last few years, these light sources have been replaced with the more efficient narrow-band UVB light sources operating at 311 nm $\pm$ 2 nm wavelength (NB-UVB) [12]. A new, highly effective laser-based phototherapy has been introduced recently for the treatment of different skin diseases based on the use of the 308 nm xenon chloride excimer laser



radiation. The "super narrow-band" 308 nm xenon chloride (XeCl) excimer laser has been found to be more effective than NB-UVB in inducing T cell apoptosis in vitro and is also clinically more effective for the treatment of an inflammatory skin disease, psoriasis [22-24]. These results now have been confirmed by other groups and the 308 nm excimer laser is currently widely used for the treatment of allergic and non-allergic skin diseases [25, 26]

In addition ultraviolet A (UVA; 315-400 nm), psoralen plus UVA (PUVA), combined UVA-UVB and high-dose UVA1 (340-400 nm) therapies are also essential in the dermatological practice. The major mechanisms of immunosuppression induced by the various forms of phototherapy in the skin involve apoptosis induction in infiltrating T cells, reduction in the number and function of Langerhans cells, and the induction of immunomodulatory cytokines such as IL-10 [16-18, 22, 27-34]

Although the different atopic diseases, e.g. atopic dermatitis and allergic rhinitis, share many common pathogenetic factors, and there are a large number of phototherapeutic modalities for the treatment of atopic dermatitis, the use of UV-based therapies for the treatment of allergic rhinitis has never been reported. Therefore we were interested in whether phototherapy might also be effective for the treatment of allergic rhinitis.

Skin prick test (SPT) is the most frequently used in vivo test for the diagnosis of immediate type allergic reaction identifying an allergen responsible for the development of an allergic disease such as hay fever. To perform SPT a drop of the suspected allergen solution is applied to a skin test area of the forearm. The upper layer of the skin is then pricked using a lancet to promote the penetration of the allergen through the epidermis. After 20 minutes the allergen is removed and the skin reaction is checked. If wheal (urtica) and/or skin reddening (flare reaction) is detected it suggests that the investigated allergen induced an IgE mediated allergic reaction. The size of the urtica/flare reaction reflects the severity of the allergic response. SPT is also used to assess the clinical efficacy of different drug treatments for allergic diseases, such as hay fever or asthma, since there is a good correlation between the suppression of the reaction in SPT and inhibition of the clinical symptoms [35, 36]. Based on these facts, it seemed reasonable to use SPT in order to test the efficacy of the different forms of phototherapy for the treatment of allergic rhinitis. We assumed that if phototherapy suppresses SPT reactions it may also be effective in inhibiting clinical symptoms of allergic rhinitis.

2. Aims

2.1. In the first series of our study we tested the capacity of different wavelengths to inhibit the wheal formation in the SPT reaction.

2.2. According to these results in the second series of our study we sought to investigate the effect of these different light sources in the treatment of allergic rhinitis and thus to identify the clinically most effective wavelengths, which combine the advantages of high efficacy and few side effects.

3. Methods

3.1. Investigation of the effect of different wavelengths on the immediate type hypersensitivity reaction in the skin

3.1.1. Patients

The study protocol was approved by the Human Investigation Review Board of the Szeged University. Informed consent was obtained from 51 patients with a history of at least two years of allergic rhinitis or allergic rhinoconjunctivitis (f:m=31:20, mean age=34.17 years) with skin types II/III. Patients discontinued taking any antiallergic treatment 2 weeks prior to the performance of SPTs. All patients had a positive SPT with a wheal formation of at least 10.0 mm in diameter.

3.1.2. Irradiation protocol

Patients in group 1 (n = 7, f:m = 5:2, mean age: 21.85 years) received UVB irradiation. UVB source was 308 nm XeCl excimer laser (Lambda Physics LPX 105 E). The energy density of each light impulse was 5,5 mJ/cm^2 with a duration of 15 ns, the frequency of the laser was 10 Hz. The individual minimal erythema dose (MED) was determined by irradiating the skin in the gluteal region with increasing doses of XeCl excimer laser. We read the MED values 24 h after the irradiation. The mean MED was 364 mJ/cm^2. After the MED measurements 4 cm^2 test areas on the volar forearm of each patient were irradiated with the XeCl laser, in dosages of 0.5 x MED, 1.0 x MED and 2.0 x MED. Twenty-four and 48 hours after the irradiation, SPTs were performed with the same antigen on both the irradiated and non-irradiated skin areas.

Patients in group 2 (n=5, f:m=4:1, mean age: 39.4 years) received UVA irradiation. UVA source was Waldmann PUVA 4000 (Waldmann, Villingen, Germany, range:320-400 nm). On

the forearms of the patients 4 cm^2 areas were irradiated in dosages of 0,5 J/cm^2, 1 J/cm^2, 2 J/cm^2 respectively. 24, 48 and 72 h after the irradiation, SPTs were performed with the same antigen on both the irradiated and non-irradiated skin areas.

The volar forearm of the patients in group 3 (n=5, f:m=4:1, mean age: 39.4 years) was treated with a 0.15% solution of 8-methoxypsoralen (8-MOP) for 15 min prior to UVA irradiation (Waldmann, Villingen, Germany, range:320-400 nm). 24, 48 and 72 h after the irradiation, SPTs were performed with the same antigen on both the irradiated and non-irradiated skin areas.

In group 4 (n=10, f:m=1:1, mean age: 38.3 years) we used combined UVA-UVB irradiation (Rhinolight-UVA-UVB, Hungary, range: 300-400 nm), the irradiation doses were 0.214 J/cm^2, 0.428 J/cm^2, 0.642 J/cm^2 and 0.856 J/cm^2 with a duration of 45 s, 90 s, 135 s and 180 s respectively, the test areas were 15 mm in diameter. 24, 48 and 72 h after the treatment SPTs were performed on the irradiated and on non-irradiated, control skin areas.

In group 5 (n=10. f:m=1:1, mean age: 38.3 years) we applied photosensitization with a 0.15% solution of 8-MOP for 15 minutes prior to UVA-UVB irradiation (Rhinolight UVA-UVB, Hungary, range: 300-400 nm), then the test areas of the volar forearm were irradiated with dosages of 0.214 J/cm^2, 0.428 J/cm^2, 0.642 J/cm^2 and 0.856 J/cm^2, respectively. 24, 48 and 72 h after the treatment SPTs were performed on the irradiated and on non-irradiated, control skin areas.

In group 6 seven patients were irradiated with increasing doses of visible (VIS) light (Rhinolight-VIS, Hungary, range: 395-600 nm) in dosages of 2, 4, 6, 8 J/cm^2, respectively.

24 hours after the irradiation, SPTs were performed with the same antigen on both the irradiated and non-irradiated skin areas.

In group 7 in 7 patients irradiation was performed with increasing doses of mixed UVB (5%), UVA (25%) and VIS (70%), referred to as mUV/VIS light (2, 4, 6 and 8 J/cm^2) (Rhinolight-mUV/VIS, Hungary, range: 310-600 nm). Measurements were performed with Scientech Vector H410 (Scientech Inc., Boulder, Colorado) and Jobin-Yvon H-20UV (Ocean Optics, RK Duiven, The Netherlands). 24 and 48 hours after the irradiation, SPTs were performed with the same antigen on both the irradiated and non-irradiated skin areas.

3.1.3. Skin prick test

The SPT is a method that is widely used to investigate the immediate hypersensitivity reaction to a specific allergen in the skin [35]. SPTs were performed with recombinant allergens (Soluprick-Epipharm Allergie-Service GmbH). Ragweed, muggle, cat and dog hair antigens were used for the investigations. Twenty-microlitre aliquots of the test solutions were placed on the patients' forearms, with a distance of more than 3 cm between individual application points. Sterile 0.9% sodium chloride solution and histamine hydrochloride were used for control purposes. Reactions were recorded 20 min after testing and the wheal size was measured by digital planimetry. After determination of the individual sensitivity of each patient, for each individual one allergen was chosen that induced an "optimal" wheal of approximately 10 mm in diameter. SPT reactions were then examined with this antigen on the irradiated and non-irradiated control skin areas 24, 48 and 72 hours after irradiation.

3.1.4. Statistical analysis

To evaluate the effect of different wavelength irradiations on the development of allergen-induced wheals, the size of the wheals that developed on the irradiated areas were compared with those on the non-irradiated control test areas. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test. If parametric ANOVA was not applicable we used Friedman repeated measures ANOVA, followed by Dunnett's post-hoc test. Differences were considered to be statistically significant at $p < 0.05$.

3.2 Intranasal phototherapy

3.2.1. Assessment of tolerability and efficacy of intranasal XeCl laser therapy in hay fever

3.2.1.1. Patients

Eighteen patients (f: m = 7:2, mean age: 44.89 years) were enrolled into the XeCl laser study. All of the patients suffered from severe, ragweed-induced hay fever that did not respond well to conventional antiallergic treatment, all had a history of rhinitis of at least 2 ragweed seasons. The diagnosis of allergic rhinitis was confirmed by positive SPT results to ragweed

(a wheal greater than 5 mm) and by measurement of the ragweed-specific IgE antibody level. Patients with significant nasal structural abnormalities, bronchial asthma, an upper respiratory tract infection within the past 2 weeks or a lower respiratory infection within 4 weeks prior to the start of the study were excluded. Further exclusion criteria were treatment with systemic corticosteroids within the previous 4 weeks, topical corticosteroids or cromolyn sodium within 2 weeks, antihistamines and decongestants within 1 week prior to the beginning of the study or immunotherapy in the past 2 years. During the study patients were not allowed to use any drugs. The patients were inrolled into two groups, and an open-label study was performed to assess the tolerability and the efficacy of the XeCl excimer laser in allergic rhinitis. The investigation was performed between 15 August and 20 September 2001, and the ragweed pollen counts were above 50/m^3 in Szeged area throughout the study. Informed consent according to the Institutional Review Board of the Albert Szent-Györgyi Medical Center at the University of Szeged, was obtained from each individual before the start of the study.

3.2.1.2. Low-dose XeCl laser treatment group

The patients in group 1 (n = 10) received low-dose XeCl laser irradiation. The light source was a 308 nm XeCl excimer laser (Lambda Physics LPX 105 E). The energy density of each light impulse was 15.11 mJ/cm^2 with a duration of 15 ns, with a laser repetition rate of 10 Hz. The MED of each patient was determined first by irradiating the skin in the gluteal region with different dosages (100-600 mJ/cm^2) of the XeCl laser. The MED values were read off 24 hours after the irradiation. The mean MED was 285 mJ/cm^2. The treatment of the nasal mucosa was performed by means of a special instrument for targeted phototherapy (Rhinolight handpiece, Rhinolight, Hungary) (Fig.1.), and started with a fluence of 0.25 × the individual MED. The handpiece of the instrument was introduced into the nasal cavity of the patients, and its distal end was carefully moved continuously in order to be able to irradiate homogenously large area of the nasal mucosa. Two treatments were given weekly for two weeks. After the first treatment, the dosage of the UV light was increased in steps of 0.125 × MED up to 0.625 × MED. The patients scored the severity of their clinical symptoms (sneezing, nasal itching, nose running, nasal blockage) on a 4-point scale once a day in a diary; 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe symptoms. The total nasal score (TNS) was calculated as the sum of the severity scores. All side effects observed during

the treatment were recorded. In the course of the study, the patients did not use any antiallergic drugs.

3.2.1.3. Medium-dose XeCl laser treatment group

The patients in group 2 (n = 8) received medium dose XeCl laser irradiation. The treatment of the nasal mucosa started with a fluence of 0.4 x MED, increased in steps of 0.125 x MED or to the individual tolerance level. Four treatments were given weekly for two weeks. The patients scored the severity of their clinical symptoms in the same way as in group 1. Again, all side effects were recorded during the treatment.

3.2.2. Assessment of tolerability and efficacy of intranasal PUVA therapy in hay fever

3.2.2.1. Patients

Seventeen patients (f:m=13:4, mean:41.7 years) with severe ragweed-induced hay fever were enrolled into the study. Previously none of the subjects had responded well to any conventional antiallergic treatment and all had a history of rhinitis of at least 2 ragweed seasons. The diagnosis of allergic rhinitis was confirmed by positive SPT results to ragweed (a wheal greater than 5 mm) and by measurement of the ragweed-specific IgE antibody level. Patients with significant nasal structural abnormalities, bronchial asthma, an upper respiratory tract infection within the past 2 weeks or a lower respiratory infection within 4 weeks prior to the start of the study were excluded. Further exclusion criteria were treatment with systemic corticosteroids within the previous 4 weeks, topical corticosteroids or cromolyn sodium within 2 weeks, antihistamines or decongestants within 1 week prior to the beginning of the study or immunotherapy in the past 2 years. During the study patients were not allowed to use any drugs. An open study was performed to assess the efficacy and tolerability of the PUVA therapy in allergic rhinitis. Informed consent according to the Institutional Review Board of the Albert Szent-Györgyi Medical Center at the University of Szeged was obtained from each individual before the start of the study.

3.2.2.2. PUVA treatment of the nasal cavity

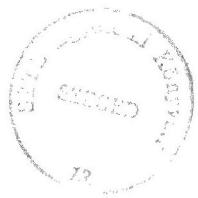
PUVA treatment (n=17) was performed with a nasal spray containing 8-MOP 2 min before irradiation of the nasal cavity with UV light (Rhinolight-PUVA, Szeged, Hungary). The

spectrum of this light source was between 305 and 440 nm with maximum at 365 nm. To determine the initial treatment dose, the minimal phototoxic dose (MPD) was determined on the patients' gluteal skin. The MPD is the lowest UV dose that induces erythema on the previously photosensitized skin. The MPD was determined by applying the photosensitizing drug (a 0.15% solution of 8-MOP) to the test areas 15 minutes prior to different dosages of UVA irradiation. The first UV dosage at which erythema developed 72 hours after UV treatment was regarded as the MPD of that patient. Treatment of the nasal mucosa was performed by means of a special instrument for targeted phototherapy (Rhinolight, Hungary), and started with a fluence of 0.5 x the individual MPD. Four treatments were given weekly for 3 weeks. After the first two treatments the UV light dosage was gradually increased in steps of 0.125 x MPD up to 1 x MPD. The patients scored the severity of their clinical symptoms (sneezing, nasal itching, nose running and nasal blockage) on a 4-point scale once a day in a diary; 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe symptoms. The TNS was calculated as the sum of the severity scores. All side-effects observed during the treatment were recorded. In the course of the study the patients did not use any antiallergic drugs.

3.2.3. Intranasal phototherapy with mUV/VIS light

3.2.3.1. Patients

We conducted a randomized, double-blind study in 49 patients with a history of at least 2 years of moderate to severe ragweed-induced allergic rhinitis that was not controlled by antiallergic drugs. Positive SPTs and an elevated level of ragweed-specific IgE antibody confirmed the diagnosis. The Human Investigation Review Board of the Szeged University had approved the protocol. All patients gave their written informed consent. We excluded potential subjects from the study if they had any significant nasal structural abnormalities; had asthma, perennial rhinitis or upper or lower respiratory infection within 4 weeks prior to the beginning of the study, or had used any of the following drugs: systemic corticosteroids within 4 weeks, topical corticosteroids within 2 weeks, membrane stabilizers within 2 weeks, antihistamines within one week, nasal decongestants within 3 days or immunotherapy 5 years prior to the beginning of the study.



The patients were enrolled after the beginning of the ragweed season, when the pollen counts were above 50/m^3 in Szeged area. Seventy-two patients with allergic rhinitis were recruited to participate in the study. After the screening visit 23 patients were excluded because they did not meet the inclusion criteria. Forty-nine patients were randomly assigned to receive either mUV/VIS irradiation in the active treated group (25 patients) or low intensity visible light (l-VIS) in the control group (24 patients).

3.2.3.2. Treatment protocol with mUV/VIS light

Each intranasal cavity was irradiated 3 times a week for 3 weeks either with increasing doses of mUV/VIS (starting dose 1.6 J/cm^2) or with l-VIS light (starting dose 0.06 J/cm^2). Irradiations were performed with the same device (Rhinolight-mUV/VIS lamp, Hungary, range: 310-600 nm). l-VIS irradiation was obtained by using a Schott FG13 filter (Schott AG, Mainz, Germany). In the mUV/VIS group, the patients were treated with the same dose for two consecutive dates, every third treatment-day the dose was raised by 0.25 J/cm^2, the top dose was 2.6 J/cm^2. During the course of the investigation, the only rescue medication allowed was cetirizine. Each patient kept a diary of daily symptoms on a scale of 0 to 3 (0 indicating no symptoms and 1, 2 and 3 indicating mild, moderate and severe symptoms, respectively) for nasal obstruction, nasal itching, rhinorrhoea and sneezing. An independent investigator examined the patients weekly and performed nasal lavages. At these weekly visits patients also scored their symptoms. TNS, a sum of scores for sneezing, rhinorrhoea, nasal itching and nasal obstruction, which is considered the most common and best established parameter for the clinical assessment of allergic rhinitis, was also calculated. Nasal obstruction was also evaluated by using acoustic rhinometry. At the end of the protocol, the overall efficacy of the therapy was assessed on a scale from 1 to 4 (with 1 corresponding to significant, 2 moderate, 3 slight and 4 no global improvement of symptoms).

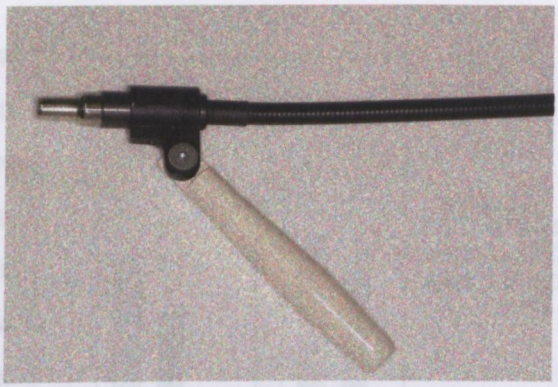


Fig. 1. Instrument used for the intranasal irradiation

3.2.4. Statistical analysis

The effects of intranasal phototherapy on the clinical symptoms were analyzed by the Wilcoxon's Sum of Ranks test, comparing the clinical scores at the beginning with those at the end of the treatment period. The all-available data approach was applied. All analyzed data correspond to pollen counts over $50/m^3$. A probability level $p < 0.05$ was considered to be a statistically significant difference.

4. Results

4.1. Effects of the different wavelength irradiations on the immediate type hypersensitivity reaction in the skin

4.1.1. Effects of XeCl laser irradiation on the SPT reaction

The XeCl excimer laser induced a dose-dependent inhibition of the allergen-induced wheal formation. When the SPT was performed 24 hours after the XeCl laser irradiation, the size of the wheal induced by the allergen decreased by 7.8% at 0.5 x MED, by 35.2% at 1 x MED and by 55.3% at 2 x MED, as compared with that on the non-irradiated, control side. The inhibition was statistically significant at dosages of 1 x MED and 2 x MED ($p<0.05$). 2x MED caused a significantly higher decrease of the wheal size than 0.5xMED ($p<0.05$). When the SPT was performed 48 hours after the XeCl laser treatment, the inhibition of wheal formation was less than that obtained 24 hours after treatment, but on the 2 x MED laser irradiation, the wheal size was approximately 40% less than the size of the wheal on the non-irradiated control areas (Fig.2.). The XeCl laser irradiation had no effect on histamine-induced wheal formation (data not shown).

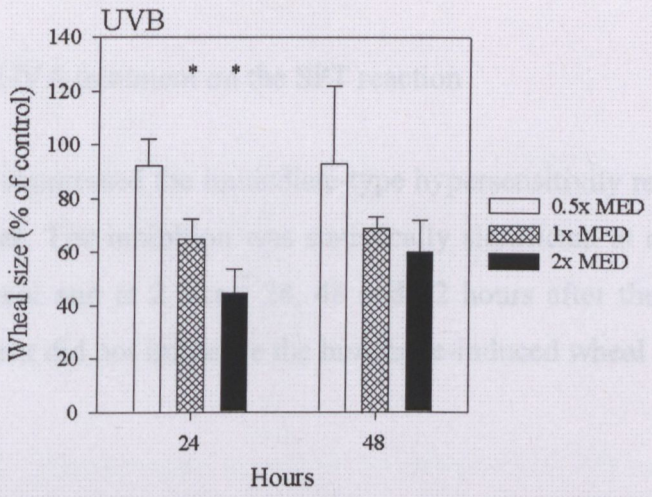


Fig. 2.: Effect of the 308 nm XeCl UVB irradiation on the SPT reaction.

UVB irradiation suppresses the allergen induced wheal size. Values represent wheal size 24 and 48 hours after irradiation with different doses of UVB expressed as a percentage of the untreated control. Error bars represent the standard error of mean. Significant decreases were observed 24 hours after irradiation at 1xMED and 2xMED. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test (n=7). Differences were considered to be statistically significant at $p< 0.05$.

4.1.2. Effects of the UVA irradiation on the SPT reaction

There was a tendency for decreasing of the wheal size in case of UVA irradiation. The strongest, statistically significant inhibition developed 72 h after the UVA treatment, it was 20,36% at 0.5 J/cm^2, 23,3% at 1 J/cm^2 and 21,8% at 2 J/cm^2 ($p<0.05$) (Fig.3.). No correlation was found between the inhibition of allergen-induced wheal formation and the UVA dose.

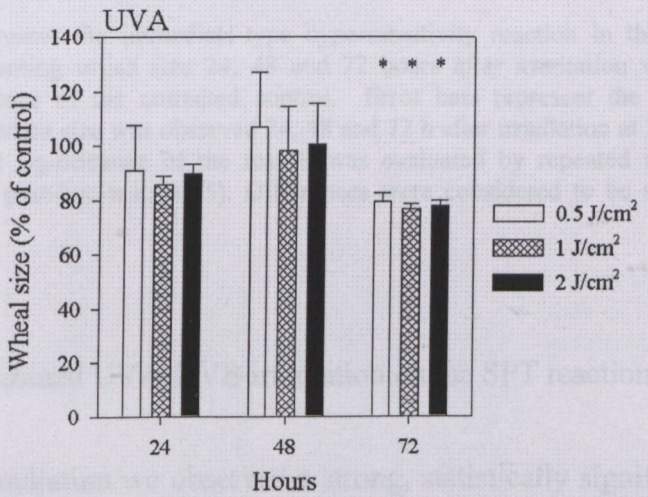


Fig. 3.: Effect of the UVA irradiation on the SPT reaction

Values representing urtica size 24, 48 and 72 hours after irradiation with 0.5, 1 and 2 J/cm^2 are expressed as a percentage of the untreated control. Error bars represent the standard error of mean. A significant decrease in urtica size was observed 72 h after the irradiation at 0.5 J/cm^2, at 1 J/cm^2 and at 2 J/cm^2. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test. If parametric ANOVA was not applicable we used Friedman repeated measures ANOVA, followed by Dunnett's post-hoc test (n=5). Differences were considered to be statistically significant at $p< 0.05$.

4.1.3. Effects of the PUVA treatment on the SPT reaction

The PUVA treatment suppressed the immediate-type hypersensitivity reaction in the skin in a dose-dependent manner. The inhibition was statistically significant at dosages of 1 J/cm^2 48 hours after the treatment and at 2 J/cm^2 24, 48 and 72 hours after the irradiation ($p<0.05$). (Fig.4.) PUVA treatment did not influence the histamine-induced wheal formation.

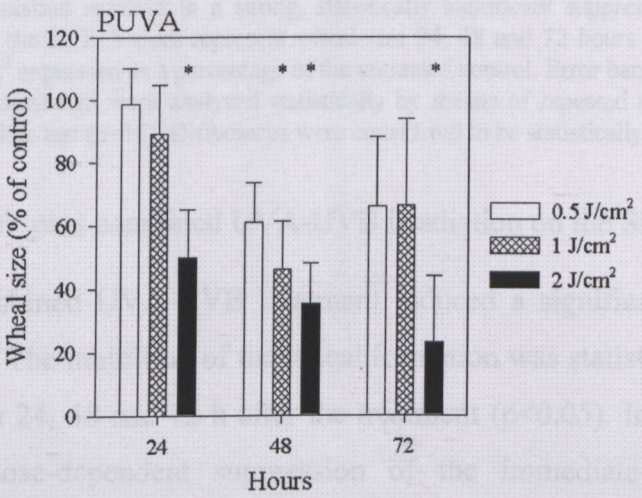


Fig. 4.: Effect of the PUVA treatment on the SPT reaction

PUVA treatment suppresses the immediate-type hypersensitivity reaction in the skin in a dose-dependent manner. Values representing urtica size 24, 48 and 72 hours after irradiation with 0.5, 1 and 2 J/cm^2 are expressed as a percentage of the untreated control. Error bars represent the standard error of mean. A significant decrease in urtica size was observed 24, 48 and 72 h after irradiation at 2 J/cm^2 and also after 48 h at 1 J/cm^2. The statistical significance of the results was evaluated by repeated measures one-way ANOVA followed by Dunnett's post-hoc test; (n=5). Differences were considered to be statistically significant at $p< 0.05$.

4.1.4. Effects of combined UVA-UVB irradiation on the SPT reaction

After UVA-UVB irradiation we observed a strong, statistically significant suppression of the immediate-type hypersensitivity reaction in the SPT. 24, 48 and 72 hours after the irradiation the inhibition of the wheal formation was statistically significant in case of any doses ($p<0.05$). Higher UV doses tended to cause a significantly stronger inhibition of the urtica formation than lower doses. (Fig.5.)

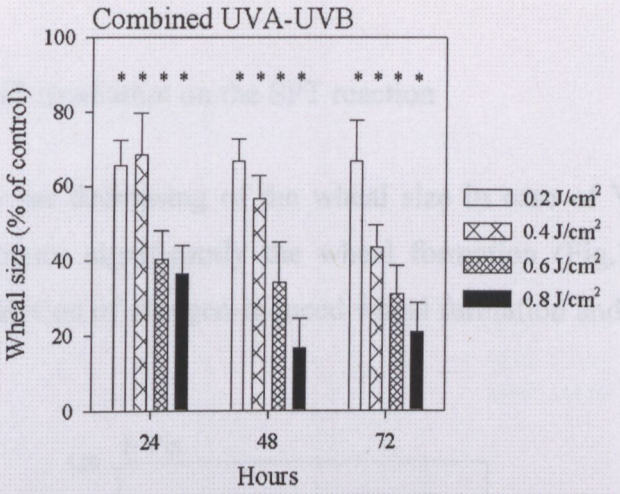


Fig. 5.: . Effect of combined UVA-UVB irradiation on the SPT reaction

Combined UVA-UVB irradiation resulted in a strong, statistically significant suppression of the immediate-type hypersensitivity reaction in the SPT. Values represent wheal size 24, 48 and 72 hours after irradiation with 0.214, 0.428, 0.624 and 0.856 J/cm^2 expressed as a percentage of the untreated control. Error bars represent the standard error of mean. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test (n=10). Differences were considered to be statistically significant at $p< 0.05$.

4.1.5. Effects of 8-MOP plus combined UVA-UVB irradiation on the SPT reaction

The Geroxalen + combined UVA-UVB treatment induced a significant suppression of the urtica size in the SPT. The inhibition of the wheal formation was statistically significant at all of the applied dosages 24, 48 and 72 h after the treatment ($p<0.05$). In this case we couldn't observe so strong dose-dependent suppression of the immediate-type hypersensitivity reaction, but the strongest inhibition developed at 0.856 J/cm^2 (Fig.6.). UV irradiation didn't influence the histamine induced wheal formation.

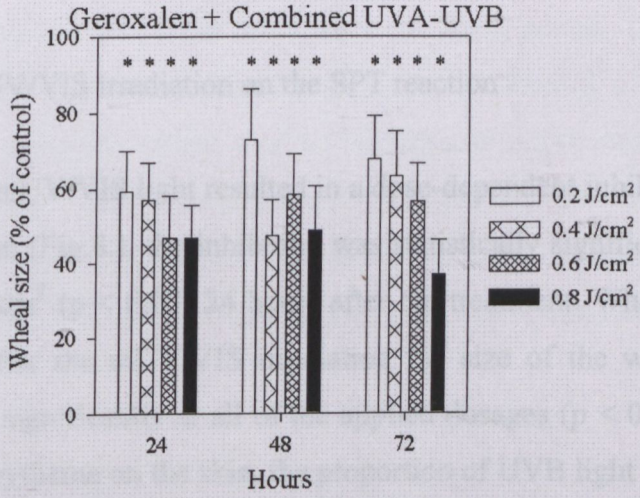


Fig. 6.: Effect of 8-MOP plus combined UVA-UVB irradiation on the SPT reaction

The Geroxalen + combined UVA-UVB treatment induced a significant suppression of the urtica size in the SPT. Values represent wheal size 24, 48 and 72 hours after irradiation with 0.214, 0.428, 0.624 and 0.856 J/cm^2 expressed as a percentage of the untreated control. Error bars represent the standard error of mean. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test (n=10). Differences were considered to be statistically significant at $p< 0.05$.

4.1.6. Effects of the VIS irradiation on the SPT reaction

There was a tendency for decreasing of the wheal size in case of VIS irradiation, but VIS irradiation didn't influence significantly the wheal formation (Fig.7.). No correlation was found between the inhibition of allergen-induced wheal formation and the VIS dose.

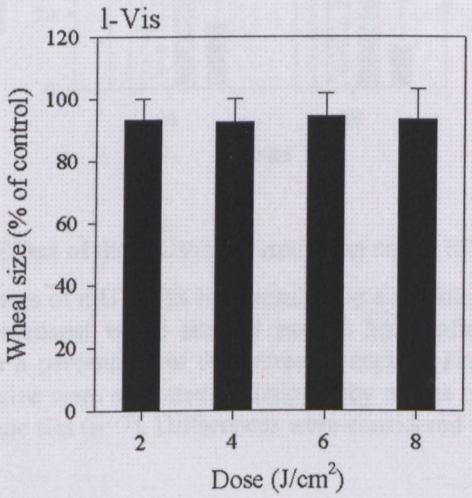


Fig. 7.: Effect of the VIS irradiation on the SPT reaction

Irradiation with increasing doses of VIS light resulted in a slight, not significant inhibition of wheal formation. Values representing urtica size after irradiation with 2, 4, 6 and 8 J/cm^2 are expressed as a percentage of the untreated control. Error bars represent the standard error of mean. The statistical significance of the results was evaluated by repeated measures one-way ANOVA followed by Dunnett's post-hoc test (n=7).

4.1.7. Effects of the mUV/VIS irradiation on the SPT reaction

Mixed irradiation with mUV/VIS light resulted in a dose-dependent inhibition of the allergen-induced wheal formation (Fig.8.), the inhibition was statistically significant at the doses of 4 J/cm^2, 6 J/cm^2 and 8 J/cm^2 ($p < 0.05$) 24 hours after the treatment. When the skin prick test was performed 48 h after the mUV/VIS irradiation the size of the wheal induced by the allergen was decreased significantly at all of the applied dosages ($p < 0.05$). The two lowest doses did not produce erythema on the skin, the proportion of UVB light in mUV/VIS was 0.1 J/cm^2 for 2 J/cm^2 and 0.2 J/cm^2 for 4 J/cm^2. The use of higher, erythematosus doses of mUV/VIS light (0.4 J/cm^2 UVB for 8 J/cm^2 mUV/VIS) led to almost complete inhibition of wheal formation.

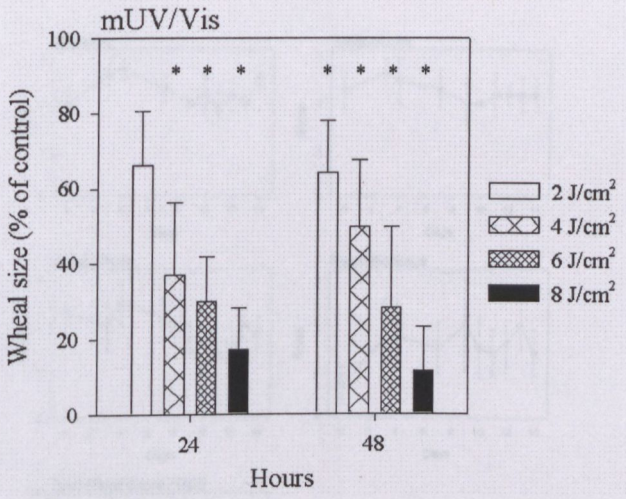


Fig. 8.: Effect of the mUV/VIS irradiation on the SPT reaction

Irradiation with increasing doses of mUV/VIS light resulted in a significant, dose-dependent inhibition of wheal formation in SPT. Values represent wheal size 24 and 48 hours after irradiation with increasing doses of mUV/VIS light expressed as a percentage of the untreated control. Error bars represent the standard error of mean. Differences in wheal size were analyzed statistically by means of repeated measures one-way ANOVA followed by Dunnett's post-hoc test (n=7). Differences were considered to be statistically significant at $p< 0.05$.

4.2. Effects of phototherapy on the clinical symptoms of allergic rhinitis

4.2.1. Effects of the low-dose XeCl laser treatment on the clinical symptoms of allergic rhinitis

Of the 10 enrolled patients, 7 completed the 2-week treatment period. Three patients dropped out before completing the study, because of the significant worsening of their symptoms (lack of effect). Following treatment, there was no significant improvement in the sneezing, rhinorrhoea, nasal itching, nasal blockage or TNS (Fig.9.). The patients tolerated the treatment well, and no severe side effects were observed. Mild dryness of the nasal mucosa occurred in 2 patients; this did not need any intervention, and it disappeared within few days after the last treatment.

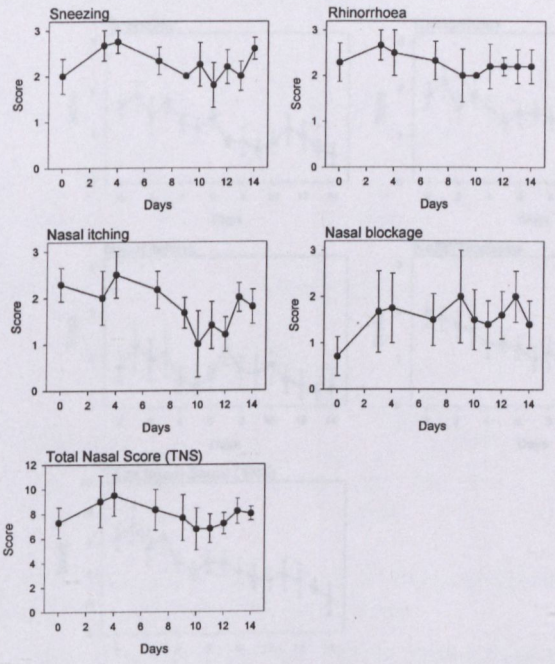


Fig. 9. Changes in the individual clinical symptoms and TNS during the low-dose UVB treatment.

No significant change was found (Wilcoxon's Sum of Ranks Test, n=7) in the nasal scores during the 2-week treatment period.

4.2.2. Effects of the medium-dose XeCl laser treatment on the clinical symptoms of allergic rhinitis

All eight patients enrolled in the medium-dose XeCl laser group completed the study. After the 2-week treatment period, significant improvements were observed in the sneezing, rhinorrhoea and nasal blockage scores, and also in the TNS (Fig.10.). The improved clinical symptoms were usually first noted 4-5 days after the start of therapy, and thereafter the improvement was continuous. At the end of the XeCl laser treatment, the symptom scores were reduced by more than 50%. The XeCl laser treatment also reduced the severity of nasal itching, but the decrease was statistically not significant. No severe side effects occurred, but mild dryness of the nasal cavity was observed in 6 of the 8 patients; this was relieved by application of a vitamin A-containing oil.

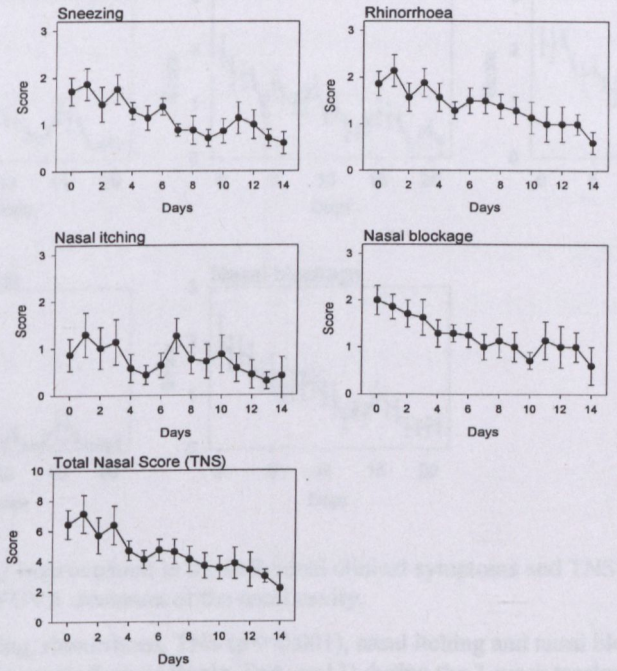


Fig. 10.: Improvement in the individual clinical symptoms and TNS during the medium-dose UVB treatment.

The clinical scores of sneezing, rhinorrhoea, nasal blockage and the TNS decreased significantly($p=0.018$, $p=0.035$, $p=0.013$, respectively; Wilcoxon's Sum of Ranks Test, $n=8$) during the 2-week treatment period.

4.2.3. Effects of the PUVA treatment on the clinical symptoms of allergic rhinitis

Thirteen of the seventeen enrolled patients completed the study. Three subjects dropped out because of noncompliance and one because of a lack of efficacy. All the patients who completed the study responded well to the PUVA treatment. After the 3-week treatment period, significant improvements were observed in all of the nasal symptoms (sneezing, rhinorrhoea, nasal itching and nasal blocking) and also in the TNS (Fig.11.). Fig.12. shows the improvement of the TNS during the PUVA treatment in two patients with the change of the pollen number within the 3-week treatment period. The diagrams represent that the TNS were decreasing continuously due to the treatment, whereas the pollen number was still high. Mild dryness of the nasal mucosa was the only side-effect observed in three patients, this was easily overcome with vitamin A oil.

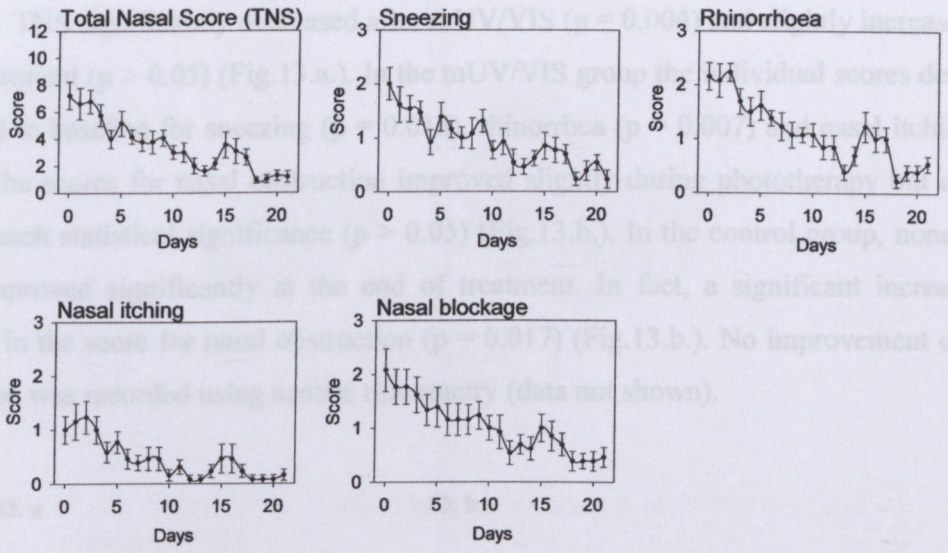


Fig. 11.: Improvement in the individual clinical symptoms and TNS during PUVA treatment of the nasal cavity.

The clinical scores of sneezing, rhinorrhoea, TNS ($p<=0.001$), nasal itching and nasal blockage ($p<=0.01$) decreased significantly (Wilcoxon's Sum of Ranks Test, n=13) during the 3-week treatment period. Error bars represent the standard error of the mean.

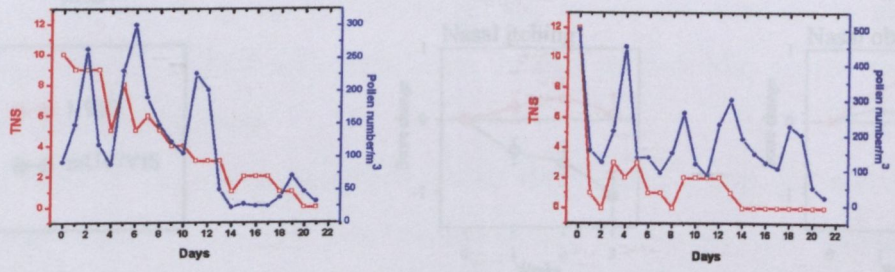


Fig. 12.: Improvement of the TNS during the PUVA treatment of the nasal cavity in two patients.

The diagram also shows the change of the pollen number within the three-week treatment period. The diagrams represent that the total nasal scores were decreasing continuously due to the treatment, whereas the pollen number was still high.

4.2.4. Effects of the mUV/VIS and l-VIS treatment on the clinical symptoms of allergic rhinitis

Forty-nine patients received intranasal irradiation either with mUV/VIS light (n=25, f:m=18:7, mean age: 37.8 years) or l-VIS (n=24, f:m=15:9, mean age:39.3 years). The two

groups did not differ in age, disease duration or clinical scores at the beginning of treatment protocol. TNS significantly decreased after mUV/VIS ($p = 0.004$) and slightly increased after l-VIS treatment ($p > 0.05$) (Fig.13.a.). In the mUV/VIS group the individual scores decreased compared to baseline for sneezing ($p = 0.016$), rhinorrhea ($p = 0.007$) and nasal itching ($p = 0.014$). The scores for nasal obstruction improved slightly during phototherapy but changes did not reach statistical significance ($p > 0.05$) (Fig.13.b.). In the control group, none of the scores improved significantly at the end of treatment. In fact, a significant increase was observed in the score for nasal obstruction ($p = 0.017$) (Fig.13.b.). No improvement of nasal obstruction was recorded using acustic rhinometry (data not shown).

13. a **13. b**

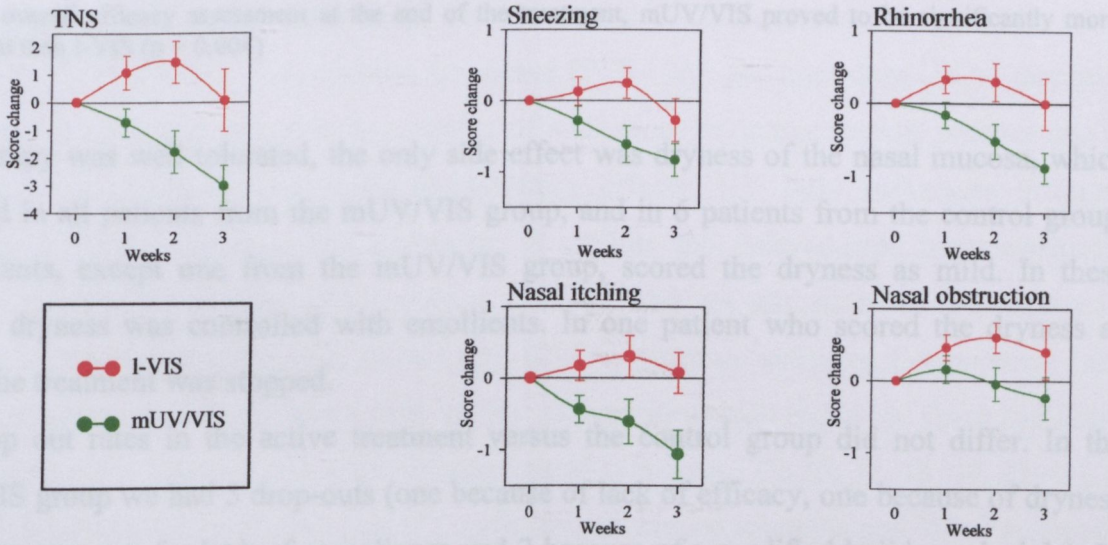


Fig. 13.: Effects of the mUV/VIS and l-VIS treatment on the TNS and individual clinical symptoms of allergic rhinitis.

TNS significantly decreased after mUV/VIS ($p = 0.004$) and slightly increased after l-VIS treatment ($p > 0.05$) (Fig.13.a.). In the mUV/VIS group the individual scores decreased compared to baseline for sneezing ($p = 0.016$), rhinorrhoea ($p = 0.007$) and nasal itching ($p = 0.014$). The scores for nasal obstruction improved slightly during phototherapy but changes did not reach statistical significance ($p > 0.05$) (Fig.13.b.). In the control group, none of the scores improved significantly at the end of treatment. In fact, a significant increase was observed in the score for nasal obstruction ($p = 0.017$) (Fig.13.b.).

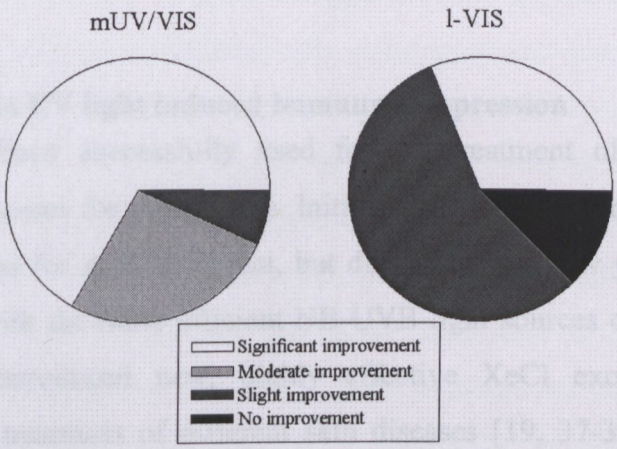


Fig. 14.: Scoring of the global outcome of treatment in mUV/VIS irradiated and control groups.

In the overall efficacy assessment at the end of the treatment, mUV/VIS proved to be significantly more efficient than l-VIS ($p = 0.004$)

The therapy was well tolerated, the only side effect was dryness of the nasal mucosa, which occurred in all patients from the mUV/VIS group, and in 6 patients from the control group. All patients, except one from the mUV/VIS group, scored the dryness as mild. In these patients dryness was controlled with emollients. In one patient who scored the dryness as severe the treatment was stopped.

The drop out rates in the active treatment versus the control group did not differ. In the mUV/VIS group we had 5 drop-outs (one because of lack of efficacy, one because of dryness of the mucosa, one for lack of compliance and 2 because of a modified holiday schedule). In the control group we had 4 drop-outs (2 in consequence of lack of efficacy, one for lack of compliance and one because of an upper respiratory infection). In the control group a significantly higher consumption of rescue medication was recorded compared to the mUV/VIS group (93 tablets in the control group versus 57 tablets in the mUV/VIS group).

5. Discussion

5.1. Mechanism of the UV light induced immunnosuppression

UV irradiation has been successfully used for the treatment of hyperproliferative and inflammatory skin diseases for many years. Initially, BB-UV light sources in the UVB range 290-320 nm were used for such treatment, but during the last few years these light sources have been replaced with the more efficient NB-UVB light sources operating at 310-313 nm [15]. We recently introduced new, highly effective XeCl excimer laser-based UVB phototherapy for the treatment of different skin diseases [19, 37-39]. The XeCl laser was found to be more effective than conventional UVB light sources in the phototherapy of skin disorders [22, 37]. These results have been confirmed by other groups and the 308 nm excimer laser is currently widely used for the treatment of skin diseases [25, 26]. UVA irradiation, 8-MOP plus UVA radiation, combined UVB-UVA and high-dose UVA1 therapies also have been used for an expanding number of indications in dermatological practice due to their immunosuppressive and immunomodulatory effects. UV light influences different inflammatory and immunological processes: it inhibits the delayed-type hypersensitivity reaction; reduces the number of Langerhans cells and induces alterations in the activity of epidermal antigen-presenting cells (APC) [28, 40] UV exposure activates T-suppressor cells and keratinocytes, which produce a wide variety of cytokines, such as IL-10. These cytokines modulate the APC function and other immunological processes [27, 30, 31, 33, 34, 41-43]. It has been demonstrated previously that UV radiation induces keratinocyte and T-cell apoptosis [22, 32] and UV radiation is known to modulate the expression of adhesion molecules on Langerhans cells and keratinocytes [14, 33, 41, 44, 45]. UV irradiation suppresses histamine release from mast cells [46] and decreases the number of infiltrating eosinophils in the skin.

5.2. Pathomechanism of allergic rhinitis

All of these inflammatory cells and processes are important in the pathogenesis of allergic rhinitis, which is an allergen-induced, IgE-mediated inflammatory disease of the nasal mucosa. During sensitization in the nasal mucosa allergen is taken and presented by antigen presenting cells (dendritic cells) to the T-helper cells. This process activates Th2 cells in atopic patients, these cells produce a variety of cytokines (IL-4, IL-13). By the effect of these mediators B lymphocytes become activated, and produce IgE antibodies, which have a central

role in the allergic reaction. The produced allergen specific IgE binds to IgE receptors (FceRI and FceRII) present on the surface of mast cells, eosinophils and dendritic cells.

In the elicity phase allergen binds to the allergen specific IgE molecules fixed to the FceRI and FceRII receptors on the surface of these cells, by the effect of this process effector cells will be activated. In the course of the mast cell activation histamine and other preformed inflammatory mediators are released. Histamine binds to H1 receptors and produces the specific allergic symptoms. This is the early or histamine dependent phase. Due to the mediators (IL-4, IL-5) produced and released by mast cells, other cells (eosinophils, macrophages, T-cells and basophils) migrate to the nasal mucosa and the inflammatory process is amplified. This is the late phase of the allergic reaction, which begins 6-12 hours after the allergen exposition. The eosinophils are very active effector cells during the cellular infiltration. Eosinophils-from the circulation- bind to vascular endothelium by the effect of IL-5, as a result of this process their migration and function is amplified. Some of the mediators released from mast cells have leukocyte chemotactic activity, so these mediators promote leukocyte migration to the site of the inflammation. Arriving to the site of inflammation the activated eosinophils release a variety of inflammatory mediators to the extracellular compartment. IL-3, IL-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF) promote the differentiation of the eosinophils, and via inhibition of their apoptosis elongate the eosinophil survival. The longer eosinophil survival is essential in the chronic allergic inflammation. In the course of the basophil degranulation histamin is released, too, so basophils take part in the early and in the late phase allergic responses, too. The cytokines of the T-helper cells promote eosinophil differentiation and elongate eosinophil survival [11, 47].

5.3. Mechanism of the UV light induced suppression of the immediate type hypersensitivity reaction in the skin

UV therapy has long been used in the treatment of different inflammatory skin diseases, and as there are many common pathogenetic factors in these skin disorders and allergic rhinitis, we have now addressed the question of whether phototherapy might also be effective for the treatment of hay fever.

The SPT is a method that is widely used to investigate the immediate hypersensitivity reaction to a specific allergen in the skin, and it has been shown that there is a good correlation between the SPT reaction and the nasal symptoms in patients with allergic rhinitis. So in the first series of our study we investigated the effects of the different kind of irradiations on the

immediate-type hypersensitivity reaction in the skin in order to identify the wavelengths, which are able to suppress most effectively the clinical symptoms of allergic rhinitis.

There was a tendency for decreasing of the wheal size in case of VIS irradiation, but VIS irradiation didn't influence significantly the wheal formation. By the effect of the UVA irradiation a tendency for decreasing of urtica formation was also observed, the suppression was statistically significant only 72 hours after the treatment. The UVB irradiation with the 308 nm XeCl excimer laser and the PUVA treatment suppressed the allergen-induced wheal development in a dose-dependent manner, the inhibition was statistically significant at erythematous doses. The fact that the XeCl laser and PUVA treatment had no effect on histamine-induced wheal formation suggests that the XeCl laser and PUVA treatment inhibit histamine release from the mast cells. Our data are in good accord with other findings suggesting that UV irradiation significantly reduced the size of the allergen induced wheal in the SPT [28, 48, 49]. Vocks et al. found that a single dose of UVB irradiation significantly reduced the allergen induced wheal size in the SPT, while the flare responses decreased significantly only after three suberythematous UVB irradiations. Whole body UVB irradiation, excluding the prick test areas didn't influence significantly the wheal and flare responses to common aeroallergens, suggesting that the UVB-irradiation induced inhibition of histamine release is a local effect of the UVB light [49]. Fjellner et al. demonstrated that moderate doses of UVB irradiation inhibited the histamine-release effect of compound 48/80 from rat peritoneal mast cells in vitro, while higher doses of UVB light caused cytotoxic histamine leakage from the mast cells [50]. UVB irradiation was also capable of inhibiting compound 48/80-induced mast cell degranulation in both mice and humans[51, 52].

In another study Fjellner et al. found that repeated PUVA treatment of human skin was followed by decreases in the itch and flare responses induced by intradermal injection of the histamine-liberating agent compound 48/80 [50]. They presumed that the inhibition of histamine release from mast cells by PUVA might be explained by a membrane-stabilizing effect in the mast cells. Danno et al. also investigated the effect of the PUVA treatment on the immediate type hypersensitivity skin reaction. An animal model was used for this study. Mouse ears were treated with 8-MOP solution plus UVA radiation. After PUVA treatment mast cell liberators, such as concanavalin-A, compound 48/80, and a vasodilator mixture including 5-hydroxytryptamine and histamine were injected intradermally. After this ear swelling response, the rate of the mast cell degranulation, and mast cell numbers were

measured. They found that PUVA treatment significantly suppressed the compound 48/80 and concanavalin-A induced ear swelling response and the mast cell degranulation [51]. In another experiment they found that subedematous doses of UVB radiation significantly suppressed the mouse ear swelling response and the mast cell degranulation evoked by intradermal injection of compound 48/80 [51]. All these results suggest that the inhibition of histamine release from mast cells by UVB irradiation and PUVA treatment is probably mediated by a direct membrane stabilization effect.

The combined UVA-UVB irradiation, the Geroxalen plus combined UVA-UVB treatment and the mUV/VIS irradiation caused a strong, statistically significant, dose-dependent inhibition of the SPT in suberythematosus doses, too. These results suggest that these combined irradiations have a more profound and rapid inhibitory effect on immediate type skin reaction than UVB or 8-MOP plus UVA alone. The underlying mechanism might be the synergistic effect of different wavelengths on histamine release.

In the SPT, the antigen induces a rapid release of histamine from the sensitized cells and results in the development of a wheal in 10-20 minutes. It has been shown that UVA light significantly inhibited histamine release from human basophils and a human mast cell line and that UVB light had an inhibitory effect only on mast cells [46]. The effect of in vitro UVA irradiation of basophils is characterized by a biphase dose dependent action on histamine release: low doses are followed by a significant inhibitory effect, in contrast high doses are followed by histamine eliberation [53] The strong inhibition of SPT reaction by combined UVA-UVB, Geroxalen plus UVA-UVB and mUV/VIS irradiation might therefore be explained by the combined actions of the different wavelengths on the skin mast cells.

5.4. Possible mechanisms of rhinophototherapy

According to the SPT results in the second series of our study we investigated the effects of different light sources in the treatment of allergic rhinitis. At first we used the biologically most effective UVB light source, the 308 nm XeCl excimer laser to assess the efficacy of phototherapy in hay fever, and we found that intranasal UVB phototherapy with medium-dose 308 nm XeCl excimer laser significantly suppressed the nasal symptoms of patients with severe hay fever, whereas in low dosages had no effect on the symptoms. For the treatment of skin diseases with NB-UVB irradiation, the therapy is started usually with a UVB dose of 0.8 x MED dose, which is gradually increased, depending on the patient's tolerance, and irradiation is performed 3-4 times weekly [15]. Since the XeCl excimer laser is more

effective than NB-UVB, and since there were no data on the tolerability of the nasal mucosa to the XeCl excimer laser, we started the treatment of the nasal cavity with a lower UVB dosage than that we usually apply in the therapy of skin diseases. It emerged that the intranasal XeCl excimer laser illumination had no effect on the clinical symptoms of patients with hay fever when the treatment was started at the low dosage of 0.25 x MED twice weekly, but a significant improvement was seen when the treatment was started with 0.4 x MED and was continued with increasing dosages four times weekly. Mild dryness of the nasal mucosa was the only side effect observed, but it disappeared completely within a few days after the last XeCl laser treatment.

Then we investigated the efficacy of intranasal PUVA treatment in hay fever and we showed that PUVA treatment of the nasal cavity also significantly reduced the nasal symptoms of patients with allergic rhinitis. The treatment of the nasal mucosa was started with a low dose of UVA and the dosage was then gradually increased. We applied four treatments weekly. Mild dryness of the nasal mucosa was the only side-effect observed during the treatment, and this was easily countered with vitamin A oil.

Based on these previous results then we applied a combination of low dose UVB, low dose UVA and visible light (mUV/VIS) for the treatment of the nasal cavity of patients suffering from allergic rhinitis. We found that mUV/VIS light significantly suppressed the clinical symptoms of allergic rhinitis. Rhinophototherapy was tolerated well and significantly reduced the clinical scores for sneezing, rhinorrhoea, nasal itching and the TNS. A reduction of almost 50 % in TNS was reported after phototherapy. Reduction of individual scores, and the overall efficacy of mUV/VIS treatment was comparable with that obtained after treatment with the new antihistamine, fexofenadine hydrochloride [54].

The mechanism of action of XeCl, PUVA and mUV/VIS intranasal phototherapy might involve many different factors that play a role in the pathogenesis of the disease. Mainly the mechanisms of the effector phase could be influenced by phototherapy, because patients were already sensitized. There are numerous in vitro data on the effects of phototherapy on immune cells, and much has also been learned as regards how UV phototherapy affects the inflammatory cells in the skin. UV irradiation results in a reduction in the number of epidermal Langerhans cell [14], increases the production of immunosuppressive cytokines in macrophages [33, 42, 43, 55] , induces apoptosis in the T cells [22], in activated mast cells [55] and eosinophils, inhibits histamine release from the mast cells in vitro [46, 56].

Our group also investigated the effect of the mUV/VIS and l-VIS light on eosinophils and inflammatory mediators in the nasal lavage, on T-cell, eosinophil and RBL-2H3 cell apoptosis and on the mediator release from RBL-2H3 cells. Koreck et al [57] found that in the mUV/VIS group the percentage of eosinophils and the ECP level in the nasal lavage decreased significantly during therapy, whereas, both the eosinophil cell count and the ECP level increased slightly in the control group. The nasal fluid IL-5 levels decreased after treatment in the mUV/VIS group and increased in the control group; as concerns the changes from the mean baseline values the difference between the two groups was statistically significant. A slight decrease of IL-4 levels was observed in nasal lavages from patients treated with mUV/VIS light and a slight increased in the samples from the control group, but changes did not reach statistical significance. mUV/VIS irradiation induced a dose-dependent increase in both, apoptotic T cells and eosinophils. No pro-apoptotic effect of l-VIS irradiation was observed in either T cells or eosinophils. Moreover, a dose dependent increase of both $CD3^+CD45RO^+$ and $CD3^+CD45RA^+$ was observed after mUV/VIS irradiation. RBL-2H3 cells were resistant to mUV/VIS induced apoptosis.

We found that following mUV/VIS irradiation the β-hexosaminidase release was inhibited. Even low doses of mUV/VIS (15 – 60 mJ/cm^2) induced a significant decrease of β-hexosaminidase release and higher doses (240 mJ/cm^2) had a complete blocking effect. In contrast, no inhibitory effect of l-VIS irradiation was observed.

Allergic inflammation is associated with a shift in the cytokine balance towards a Th2 predominance [7]. Several data indicate that Th2 cytokines (IL-5 and IL-4) are present in increased amounts in the nasal mucosa of allergic rhinitis patients [6, 7]. IL-5 is a cytokine, which promotes the maturation, activation and prolonged survival of eosinophils, the main effector cells in hay fever [58]. The suppression of prolonged eosinophil survival induced by IL-5 is a potential therapeutic strategy for the resolution of allergic rhinitis. In our study irradiation of the nasal mucosa resulted in a significant decrease in local IL-5. T lymphocytes are major sources of IL-5 [59]. Thus, apoptosis of these cells following phototherapy might be the basis of the underlying mechanism of decreased IL-5 production. Memory T cells have an important role in the perpetuation and maintenance of allergic process. Apoptosis of these cells following phototherapy might have a long-term beneficial effect. Phototherapy also resulted in a decreased number of eosinophils and a decreased level of ECP in the nasal lavage fluid. This might be attributed to the direct pro-apoptotic effect of mUV/VIS on

eosinophils and to the decreased local IL-5 level. Similar results concerning eosinophil, ECP and IL-5 levels and T lymphocytes are observed after other well-established therapies of allergic rhinitis, such as topical glucocorticoids or immunotherapy [6, 36, 60, 61]. Allergic rhinitis is also accompanied by an elevated level of IL-4 in the nasal mucosa. IL-4 is essential in promoting the commitment of T cell precursors to produce Th2 cytokines and it activates the IgE isotype switching of B cells [5]. However, the role of IL-4 in modulating eosinophil survival and function is not yet clear. IL-4 could regulate the production of CCL11/eotaxin, a potent eosinophil chemoattractant promoting tissue eosinophilia, but it is also an inducer of apoptosis of peripheral blood eosinophils [62, 63]. The pro-apoptotic effect of IL-4 is more dramatic in eosinophils separated from atopic individuals as compared with those from nonatopic subjects. Wedi et al. have suggested that IL-4 mediated eosinophil apoptosis may be of physiological relevance if the eosinophil is not primed by the survivor cytokines (IL-5, IL-3 or GM-CSF) [63]. These data suggest that the quantitative relation of IL-4 and IL-5 produced during inflammation may determine the apoptosis rate of eosinophils at the site of allergic inflammation. Our study did not reveal significant changes in IL-4 levels in the nasal lavage samples. Similar results were reported after topical glucocorticoid therapy of allergic rhinitis [64]. Thus, the reduction of IL-5 in nasal mucosa after phototherapy together with the persistence of IL-4 might further promote phototherapy induced eosinophil apoptosis.

Not only T cells and eosinophils, but also mast cells and basophils have important roles in the effector phase of the allergic reaction [9]. They are the principal source of different mediators and especially of histamine. The role of histamine in allergic rhinitis has been well studied and is mirrored by the wide use of antihistamines in the treatment of allergic rhinitis [65]. In our study, we demonstrated that mUV/VIS irradiation is able to inhibit mediator release from RBL-2H3 cells. It has been shown that β-hexosaminidase release following allergen challenge of RBL-2H3 cells passively sensitized to murine IgE correlates with histamine release and SPT [66]. Several other therapeutic agents used for the therapy of allergic rhinitis and asthma have been already tested in this in vitro model of histamine release, and have been shown to be potent in inhibiting IgE-mediated histamine release [67, 68]. Our findings are in concordance with previous studies in which the inhibitory effect of UVA and UVB light on histamine release was assessed [53]. The use of mUV/VIS, which is characterized by low dose UVA and low dose UVB is followed by a very strong inhibitory effect, and in fact a complete blocking effect could be achieved at certain doses.

The data reported here demonstrate that phototherapy was able to inhibit the effector phase of the allergic reaction at multiple checkpoints. In contrast with antihistamines, which influence predominantly histamine-mediated features of the allergic process, phototherapy has a different, more complex action spectrum. This suggests that intranasal phototherapy might be an alternative for patients not controlled by antihistamines. Our data support this indication considering that all enrolled patients were non-responders to conventional therapies, including the latest generation of antihistamines.

5.5. Possible side effects

It is well known that repeated high-dose ultraviolet light irradiation has carcinogenic potential. The ultraviolet light-induced carcinogen effect is linked to the cumulative doses of the ultraviolet light (usually requiring many years). For PUVA treatment there is a higher risk for cancer development among patients that have received more than 260 PUVA treatments with a cumulative dose of between 100 – 500 J/cm^2 [69]. If long wave-UVA (340 – 400 mJ/cm^2) is used alone - without previous photosensitization - for the treatment of skin disorders, usually much higher UV dosages are used. For example, for the treatment of atopic dermatitis wavelengths between 340-400 nm are used with an effective dose between 50-100 J/cm^2 per treatment. This results in a cumulative dose of 750 J/cm^2 over a three-week treatment period [70]. Since the irradiation dosages for the phototherapy or photo-chemotherapy of the nasal mucosa uses much lower cumulative dose than the threshold for increased cancer risks, the probability of carcinogenesis in the present therapeutically schemes is extremely low.

Similarly as for topical corticosteroids used for the treatment of the disease, UV light by its immunosuppressive effect might facilitate the appearance of viral and bacterial infections on the treated areas. However, the likelihood for this is lower than that of the presently used local immunosuppressants since ultraviolet light has a direct anti-microbial effect. We did not observe any infections during this study.

Phototherapy of the nasal cavity was tolerated well, in fact the only side effect observed- similarly as for locally applied corticostreroids- was mild dryness of the mucosa which occurred at 50% of patients during the duration of the treatment. This symptom was easily overcome with vitamin A oil, except one patient from the mUV/VIS group, who scored the dryness of the nasal mucosa as severe and the treatment was stopped.

6. Summary

Hay fever is a very common allergic disorder, its prevalence is about 8,1% among the children, 21% among the adolescents and 11% among the adults in Hungary [71]. The treatment of allergic rhinitis is a complex problem, including the elimination of the inhalative allergens from the patient's environment, the specific pharmacotherapy and immunotherapy. However in a lot of cases patients don't respond well to the conventional antiallergic treatment or the drug therapy is contraindicated, and the combined pharmacotherapy has a numerous side-effects, so every new therapeutic tool is therefore of great importance.

Since UV irradiation has been shown to exert both local and systemic immunosuppression and is effectively used in the treatment of several immune mediated skin diseases, we were interested in whether phototherapy might also be effective for the treatment of allergic rhinitis. Since there is a good correlation between the suppression of the reaction in SPT and inhibition of the clinical symptoms, firstly we investigated the effect of different wavelengths UV irradiations to inhibit the wheal formation in the SPT reaction. We found that irradiation with the 308 nm XeCl excimer laser, PUVA, the combined UVA-UVB, Geroxalen plus UVA-UVB treatment and irradiation with mUV/VIS light significantly inhibited the allergen induced immediate type hypersensitivity reaction in the skin, while UVA and VIS irradiation didn't influence significantly the SPT reaction. Based on these results we tested the effect of these different light sources in the treatment of allergic rhinitis, and we found that phototherapy of the nasal cavity with medium dose 308 nm XeCl excimer laser, 8-MOP plus UVA, or mUV/VIS light resulted in a significant improvement of the clinical symptoms of allegic rhinitis. Phototherapy was tolerated well; the only side effect was the slight dryness of the nasal mucosa. In conclusion, our findings indicate that phototherapy represents an efficient therapeutic modality for the treatment of patients suffering from allergic rhinitis.

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