Ph.D. Thesis

MODERN MINIMALLY INVASIVE DIAGNOSTIC AND THERAPEUTIC OPTIONS FOR THE CHRONIC INFLAMMATION MEDIATED DISEASES OF THE AIRWAYS

Zsolt Bella M.D.

Supervisor:

László Rovó M.D., Ph.D.

Department of Oto-Rhino-Laryngology & Head and Neck Surgery

University of Szeged, Faculty of Medicine

2010

LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. Zs. Bella, A. Torkos, L. Tiszlavicz, L. Iván, J. Jóri: Cholesterol granuloma of the maxillary sinus resembling an invasive, destructive tumor. *Europian Archives of Oto-Rhino-Laryngology*, 262(7), 531-533, 2005. **IF: 0,895**

II. L. Rovó, M. Széll, **Zs. Bella**, A. Korsós, L. Kemény, J. Jóri: The -509 C/T genotype of TGFβ1 might contribute to the pathogenesis of benign airway stenosis. *Otolaryngology-Head and Neck Surgery* 142(3), 441-3, 2010. **IF:1,463(2009)**

III. A. Koreck, A. Szechenyi, M. Morocz, A. Cimpean, **Zs. Bella**, E. Garaczi, M. Raica, T.R. Olariu, I. Rasko, L. Kemeny: Effects of intranasal phototherapy on nasal mucosa in patients with allergic rhinitis. *Journal of Photochemistry and Photobiology B: Biology*, 89(2-3), 163-169, 2007. **IF:1,919**

IV. Koreck A., Szechenyi A., Morocz M., A. Cimpean A., **Bella.Zs**., Garaczi E.:Az intranazális fototerápia effektivitása allergiás rhinitisben: Az első kettős vak placebo kontrollált vizsgálat. *Allergológia és Klinikai Immunológia*, 7(2), 83-84, 2004.

V. Kadocsa E., Koreck A., **Bella Zs**., Csoma Zs., Ignácz F., Alexa M., Dobozy A., Jóri J., Kemény L.: Intranazális fototerápia: új terápiás eljárás allergiás rhinitisben *Fül-Orr-Gégegyógyászat* 52(2), 108-114, 2006.

VI. Bella Zs., Kiricsi Á., Viharosné D-R. É, Dallos A., Kiss M., Kemény L., Jóri J., Kadocsa E. Rhinofototerápia perzisztáló allergiás rhinitisben-Humán, randomizált, kettős vak, placebo kontrollált, prospektív, előzetes vizsgálat. *Fül-Orr-Gégegyógyászat* 56(2), 107-115, 2010.

VII. Zs. Bella, E. Kadocsa, L. Kemeny, A. Koreck: Narrow-band UVB phototherapy of Nasal Polyps: Results of a Pilot Study. *Journal of Photochemistry and Photobiology B: Biology* 100(3), 123-127, 2010. Available online 2 June 2010. **F:1,871**(2009)

VIII. A. Koreck, **Zs. Bella**, E. Kadocsa, A. Perenyi, T.R. Olariu, L. Tiszlavicz, I. Nemeth, M. Kiss, J. Jóri, L. Kemény: Intranasal PUVA phototherapy in nasal polyposis – a pilot study. *Romanian Archives of Microbiology and Immunology*, 1/2010, accepted

Publications published related to the topic, but not used in the PhD procedure:

D. Mitchell, L. Paniker, G. Sanchez, **Zs. Bella,** E. Garaczi, M. Szell, Q. Hamid, L. Kemeny, A. Koreck.: Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cellular and Molecular Med*, **14**, 313-322, 2008 **IF: 5,228**

Abstracts in reviewed papers:

Zs. Bella, L. Rovó, L. Tiszlavicz, M. Marek, J. Czigner: A combination of endoscopic laser surgery and segment resection for the treatment of cricotracheal stenosis. *European Surgical Research*, 34, Suppl. , 2002

A Koreck, A Szechenyi, A Cimpean, **Z Bella**, E Garaczi, M Raica, I Rasko, L Kemény**:** DNA Damage and Repair in Nasal Mucosa After Rhinophototherapy. *Allergy* 62 (Suppl.83): 167-551, Nr. 636, 2007.

Z. Bella, E. Kadocsa, J. Jóri: Rhinophototerapy in allergic rhinitis-clinical experiences. *Allergy* 62 (Suppl.83): 167-551, Nr. 635, 2007.

Zs. Bella, E. Kadocsa, J. Jóri: Rhinophototerapy in allergic rhinitis. Eur Arch Otorhinolaryngology 264 (Suppl 1), S265-S354, 2007.

E. Garaczi, **Zs. Bella**, M. Boros-Gyevi, E. Tóth, Zs. Csoma, L. Kemény and A. Koreck: Intranasal phototherapy is more effective than fexofenadine hydrochloride in the treatment of seasonal allergic rhinitis. *World Allergy Organization Journal*, p S76, 2007.

Zs. Bella, E Kadocsa, L Székely, J Jóri: Rhinophototherapy in grass pollen induced allergic rhinitis *World Allergy Organization Journal*: p S77, 2007.

Z. Bella, E. Kadocsa, A. Koreck, L. Kemeny**:** UV Phototherapy of Nasal Polyps: Results of a Pilot Study. *Otolaryngology - Head and Neck Surgery*, 139 (2) P170, 2008.

M. Desrosiers, **Z. Bella**, A. Javer, S. al-Mot, L. Kemeny, A. Koreck, Q. Hamid: Ultraviolet Light: A Potential New Therapy for Nasal Polyps *Journal of Allergy and Clinical Immunology* 121(2), Supplement 1, S264, 2008.

E.Kadocsa, **Zs. Bella**, G.Jakab: Evaluation of specific nasal allergen provocation test in patients with ragweed pollenosis. *Allergy* 64 (Supplement90), 179-538, No. 511, 2009.

Zs. Bella, A.Kiricsi, E.Viharosne Dosa Racz, E. Kadocsa**.:** Rhinophototerapy in persistant allergic rhinitis: Randomised double-blind placebo-controled study. *Allergy* 64(Supplement90:), 179-538, No. 1186, 2009.

Rethy L., Kadocsa E., **Bella Z.,** Kiss M., Garaczi E., Kemeny L., Benson M.,: Old molecules as new markers for poor response to local steroid treatment in ragweed-induced allrgic rhinitis. Allergy 65 (Supplement 92), 683, No1863, 2010.

Zsiros O., Kadocsa E., **Bella Z.,** Tiszlavicz L., Nemeth I., Jóri J., Kemeny L.,: The antiproliferativ effect of the UV phototherapy on nasal polyps- in vitrohystopathological examination. Allergy 65 (Supplement 92), 631, No1716, 2010.

CONTENTS

INTRODUCTION AND AIMS:

Inflammatory diseases of the airways are illnesses of high incidence affecting large populations, bearing great social and economical importance by having an impact on workplace and at-home activities as well as the quality of life. Anatomical structures such as the nasal cavity, the larynx, the trachea and the lower airways taking part in breathing contribute to ensure physiological breathing as a whole unit, therefore their diseases develop more and more obviously in clinical practice, in correlation with each other. The *concept of 'one airway, one disease'* has also been declared by the WHO ARIA document (Bousquet 2008).

Being the gateway in the air flow for breathing, the nose plays an indispensable part in the homeostasis of the s. It warms, humidifies and filtrates air, thus protecting the lower airways. The nose and the bronchial systems are anatomically connected to each other. Their surface and histological structures are similar as both areas are covered with multilayered ciliary epithelium almost everywhere, and they are in contact by means of numerous indirect neural and systemic mechanisms, too. The nature of the nasobronchial link and the nasobronchial reflex has yet not been fully cleared, however, it is a fact that nasal diseases (allergic rhinitis, nasal polyposis, common cold) resulting the release of inflammatory mediators may have a consequence on the lower airway, too. The link studied the most is the one between allergic rhinitis and asthma.

We have launched several projects for the study of diseases of the airways, in cooperation with joint work groups of the Department of Oto-Rhino-Laryngology and Head-Neck Surgery at the University of Szeged, the Department of Dermatology and Allergology, and the Institute of Pathology. We have researched pathophysiologically similar molecular biological processes which nevertheless presented differrent symptoms based on their anatomical localization. As a practicing clinician, during my work my primary aim and task was to study and introduce the application opportunities in practice of already acquired knowledge in the field of clinical diagnostics and therapy.

1. In the past 20 years, our clinic has become of the the leading centers in Hungary in the treatment of scarry airway stenosis in adults and children. In the case of large airway stenosis, some external agent causes the mechanical damage of the endotracheal mucosa. The often iatrogenic cause (mispositioned tracheotomy, long term intubation), initiates an inflammatory cascade mechanism, which then results scarring in the pursuit of restitution. In reference to

cricotracheal resection introduced in patients with laryngeal and/or tracheal stenosis, we had the opportunity to conduct a histological study of the removed surgical specimens. By means of the increase of „minimally invasive" endotracheal interventions preceding resection aimed at the elimination of acute asphyxia, the inflammatory cell infiltration and scar tissue formation affects deeper and deeper tissue layers. Particularly apparent is the large fibrosis extending to all tissue layers of the trachea observable during the CO_2 laser vaporisation(s) with increased number and extension, which manifests in an increased clinically stenosis predisposition. Histological examinations even in patients classified in the same group showed large individual differences. Individually patient-dependant, genetically determined molecular biological mechanisms affecting the inflammatory base process stand behind the scar formation, which has thus far been explained with exogeneous causes. Recognition of these processes result the creation of new diagnostic and therapeutic modalities. Diseases with airway scarring can be related to the expression and polymorphism of certain determined genes, just as it was suspected earlier in connection with fibrotic diseases of airways or other organs (Lawson 2006)

2. Ultraviolet (UV) light is one of the major environmental hazards, and its role is well-known in the triggering of skin tumors and skin aging. However, phototherapy (UV and visible light (VIS) has a significant local and systemic immunosuppressive effect, therefore it has been broadly used as a remedy for the treatment of various inflammatory, immune-mediated skin diseases (Bónis 1997). Phototherapy exerts its immunosuppressive effect in the skin by means of inducing T-cells apoptosis, inhibiting the number and function of Langerhans cells and increasing the quantity of immunomodulatory cytokines (IL-10) (kang 1998). Our research results suggest that irradiating the nasal mucosa with light of different wavelengths (UV and visible, mUV/VIS) is efficient in reducing the symptoms of allergic rhinitis (Koreck 2005). Based on the above results, the Hungarian Medical Research Council (ETT TUKEB) declared the mUV/VIS-based therapy of allergic rhinitis to be a medical procedure (file no.: 351/KO/02, certificate no.: 60008/20/ETT/2002). The Rhinolight® phototherapeutic device used for the treatment is a CE-marked medical device. The efficacy of the Rhinolight® intranasal phototherapy in seasonal allergic rhinitis was justified by our research work group using a randomized, double-blind, placebo-controlled clinical study (Koreck 2005). Positive therapeutic experience gained in the treatment of chronic immune-mediated diseases of the nasal mucosa inspired the creation of new phototherapeutic protocols and the designation of new indication areas and target groups.

Allergic rhinitis (AR) has become by now the most common chronic disease worldwide, with an estimated number of patients to be over 500 million. According to WHO figures, half of Europe's population can become hay fever sufferer by 2015 due to the global and explosive growth of incidence. Studies for prevalence in Hungary also reported on a significant increase (Balogh 2007, Bittera 2008). In addition, its social and economic importance is further intensified as severe and/or persistent cases increase asthma prevalence, remarkably constrain nighttime resting and daytime activities, which cause additional decline in the quality of life. The success of all initiatives for prevention, primarily aimed at the change of lifestyle and environment is doubtful, due to the lack of widespread social inclusion. Currently, the most successful way of alleviating the harmful consequences can be the application of effective treatment modalities.

The group of CRS disorders annually accounts as many as 22 million office visits and more than 500,000 emergency department visits in the U.S., according to some estimates. Annual CRS-related healthcare expenditures may reach as much as $3.5 billion (Benninger 2004). Inflammation of viral or bacterial origin are the most frequent of the above. On some occasions special and rare forms may develop, which primarily present differentialdiagnostic difficulties (Bella 2005). Nevertheless, despite having established a proper diagnosis and following the therapeutic principles, we often experience treatment failure.

Nasal polyposis appear in some 20% of chronic rhinosinusitis patients. In developed countries, also in Hungary, nasal polyposis is a disease affecting large population, characterized by a high recurrence rate, bad recovery inclination, and significantly reduced quality of life. Depending on different sociocultural and environmental impacts, these days its prevalence varies between 1,3-5,6%. 20-25% occurence was documented after block dissection in cadavers, therefore the pathological deformation is much more frequent than that of the diagnozed and treated cases. Treatment of nasal polyposis has still not been solved until now. Recurrence of polyps is still high even under the generally accepted combined, surgical-steroid treatment strategy, so neither surgical treatment, nor steroid therapy administered in the long run, but not even their combination can result total recovery of the patient (Fokkens 2007). Even the application of further conservative treatment modalities (nasal lavage, long-term low-dose systemic macrolid antibiotics and local antimycotics, aspirin desensitization, anti-IgE, anti-IL-5, etc.) cannot offer a final solution. Also, it poses a problem that possible complications and side-effects of both surgery and the steroid treatment are known.

Histological characteristics of nasal polyposis very much resembles that of certain other immunological skin diseases of proliferative nature (eg. psoriasis), which have already been successfully treated with PUVA for some 30 years now (per os combined use of UVA-light and 8-methoxypsoralen photosensitizer). The intranasal PUVA treatment significantly reduced nasal symptoms of allergic rhinitis patients (Csoma 2006). During our preliminary, open- label, prospective clinical study, we however did not manage to achieve macroscopic change after a 6-week PUVA treatment, yet we experienced a significant reduction of eosinophil cationic protein (ECP) and IL-5 levels in the nasal lavage, also a significant decrease of eosinophils in the polyp tissue. (Koreck-Bella 2010). Similar etiological factors of allergic diseases and the favorable experience in the application of intranasal PUVA treatment hold out promising results and inspire further development of UV phototherapy and the examination of its use in nasal polyposis.

MY DUTIES AND AIMS IN THIS COMPLEX STUDY WERE:

1.1. ***Histopathological examinations of stenotic and scarry trachea specimens removed using cricotracheal resection.***

1.2. ***Examination of TGFβ superfamily playing a decisive role in the regulation of inflammatory processes, and the predisposition role of its various poyimorphisms in the formation of laryngotracheal scarring.***

2.1. The basic condition for the application of the new, promising phototherapeutic treatment was the exclusion of the nasal mucosa damaging effect of UV beams used in the therapeutic range and dose (Justification of safe usability). ***Evaluation the in vivo effect of intranasal phototherapy, by assessing DNA damage and repair mechanism in nasal mucosa***.

2.2. So far, little experience has been available regarding its applicability in persistent allergic rhinitis (PAR). The aim of our research executed with the support of the Jedlik Ányos Programme (NKFP1-00004/2005) during *a human, randomized, double-blind, placebo-controlled, prospective clinical study was* ***the development of a new RL-therapeutic protocol, and the establishment of its efficacy and safe usability in the treatment of PAR.*** (Ethical licence: SZTE, Regional Human Medical Biological Research Ethics Committee, file no.: 110/2007, permit no.: 2288.).

2.3**.** Our experimental histological studies have justified that UVB penetrates well in vivo into the polyp tissue, it reaches in the stroma the lymphocytes responsible for the inflammation, and induces apopotosis in them. On the basis of these results, the objective of our clinical studies: ***To examine the clinical efficacy and tolerability of NB-UVB phototherapy in bilateral nasal polyps***

1. HISTOPATHOLOGICAL AND MOLECULAR BIOLOGICAL STUDIES OF DISEASES WITH SCAR FORMATION IN THE AIRWAY

1.1. Histopathological and molecular biological studies of processes with laryngotracheal scarring

1.1.1. Introduction

Air enters the lung through the larynx and the trachea, thus, any obstruction of this area leads to either asphyxia, or in severe cases, to the drop in the quality of life or to a state incompatible with life. The most frequent type of the stenosis is the intraluminar scar formation due to the injuries of airways tissue or anatomical structures. During the last decades this symptom has been the complication of the prolonged intubation and mechanical ventillation due to traffic accidents, complicated surgical and intensive care interventions in several hundred thousand cases.. The cuff of the ventilatory tube may cause an ulceration on the airway.

Wound healing involves three temporally overlapping stages: an inflammatory stage, a proliferative stage, and a phase of contraction and remodeling–. Growth factors (TGF-β) PDGF, FGF) have a decisive role in the formation of fibrosis, which facilitate proliferation of fibroblasts, the production of matrix and inhibit the functioning of enzymes decomposing matrices. This factor bears a central role in the formation of fibrosis by means of rearrangement of extracellular matrix components,

1.1.2. Materials and methods

Thus, the process of scarring is determined by a complex inflammatory process. In the mid-90s a significant trend became widespread for the endoscopic solution of scarry stenoses (Shapsay1987). But international experience and our own results point out that the restenosis predisposition is significant, therefore the ultimate definitive solution can often be only external surgery and the removal of the stenotic part. The endotracheal endoscopic technique, and, if required, open resection have been used as a routine application for the resolution of tracheal stenoses at our clinic since 1996. These interventions enabled us to carry out the retrospective histological examitation of the removed scarry tracheal segments (n=27). Patients were selected into three groups based on the number and extension of CO_2 laser

interventions (recanalization) conducted prior to the resection for the elimination of acute asphyxia. Group 1. is the control group (6 patients), who did not receive laser treatment. Specimens of patients with max. one described laser treatments were selected into group 2 (12 patients), while specimens of patients with 2-4 extended laser treatments affecting cartilage were selected into group 3 (9 patients).. For the evaluation of the degree of change we set up a semiquantitative score (0-3).

1.1.3. Results:

Erosion of the epithelium was observed in all three groups. In the third group erosion and metaplasia appeared in all cases. A significant difference was observed between the control group and group 3. Irrespectively of the number and extension of laser treatments, inflammation and fibrosis were expressed in all three groups. The volume of inflammation among patients having received extended laser treatment was significantly higher than in the control group. The subepithelial layer is characterized by the tissue image of granulation tissue and intense fibrosis in all study groups, during which fibroblasts appear and produce connective tissue matrices. The perichondrial fibrosis in the third group was of larger degree as compared to the control group. As regards the degree of fibrosis, significant difference could be demonstrated between group 2 and 3. Cartilage necrosis, inflammation destructing cartilage was caused by the multiple, extended laser treatment. Single laser treatment caused either no or only small-degree cartilage necrosis.

1.1.4. Discussion:

Minimally invasive interventions gaining ground lately involve less burden on patients. However, in many cases they only result temporary solution to the problem, and as histopathological studies proved, in the course of time they further aggravate stenosis. Contrary to the short-term advantages of laser treatments, their histological analysis showed a number of negative long-run consequences, many times the mechaism of increased development of restenosis. By the increasing of the number and extension if laser interventions, the occurence and degree of inflammatory, necrotic and fibrotic processes affecting deeper tissue layers (such as cartilages) rises. Our results histologically confirm clinical observations stating that there is a connection between the severity of the scarring and the size of the trauma triggered.

The deviations of high volume observed in each therapy groups also indicate other possible factors affecting the grade of inflammation and fibrosis. Of these, the role of regulatory factors showing indivudal deviations and determining the scar formation process on a molecular biological level seems obvious.

1.2. Molecular biological studies to assess the genetic predisposition of diseases with airway scarring

1.2.1. Introduction:

Acquired benign laryngotracheal stenosis associated with abnormal fibrotic processes is one of the most severe complications of endotracheal intubation. We aimed to conduct an initial study to identify genetic susceptibility factors for this disease. Prospective genetic study (January 2003 – December 2006).

1.2.2. Methods:

We have compared the frequency of four polymorphisms of the transforming growth factor-β1 (TGFβ1) gene in patients with acquired laryngotracheal stenosis due to endotracheal intubation (n=36) and in intensive care patients who had also undergone endotracheal intubation, but had never presented tracheal stenosis (n=30). These polymorphisms of TGFβ1 have been reported to be either susceptibility or protective factors in various fibrotic abnormalities in the airways.

1.2.3. Results:

Genotype distribution of the -509 C/T polymorphism showed significant difference between the benign airway stenosis patients and the control group. Statistical analysis revealed that the ratio of heterozygous mutants was significantly ($p=0.0053$) higher among the control patients. These data suggest a protective function for the heterozygous C/T genotype against acquired tracheal stenosis; alternatively, the C/C genotype might be a susceptibility factor for tracheal stenosis in those undergoing endotracheal intubation (OR=4.5; 95% CI= 1.5123–13.3902).

1.2.4. Conclusions:

The results of our preliminary study suggests, that in addition to other factors, genetic

predisposition may contribute to the pathogenesis of acquired benign laryngotracheal stenosis: the presence of the wild type allele of the TGFβ1 -509 C/T polymorphism that has a high rate of heterozygosity in the general population may play a role in the pathogenesis of this disease.

2. SAFETY AND THERAPEUTIC ASPECTS OF INFLAMMATION-MEDIATING UV LIGHT

2.1. Effects of intranasal phototherapy on nasal mucosa-safety study

2.1.1. Introduction

Rhinophototherapy has been shown to be effective in the treatment of allergic rhinitis. Considering that phototherapy with ultraviolet light (UV) induces DNA damage, it is of outstanding importance to evaluate the damage and repair process in human nasal mucosa.

2.1.2. Methods

We have investigated eight patients undergoing intranasal phototherapy using a modified Comet assay technique and by staining nasal cytology samples for cyclobutane pyrimidine dimers (CPDs), which are UV specific photoproducts.

2.1.3. Results

Immediately after last treatment Comet assay of nasal cytology samples showed a significant increase in DNA damage compared to baseline. Ten days after the last irradiation a significant decrease in DNA damage was observed compared to data obtained immediately after finishing the treatment protocol. Difference between baseline and 10 days after last treatment was not statistically significant. Two months after ending therapy, DNA damage detected by Comet assay in patients treated with intranasal phototherapy was similar with that of healthy individuals. None of the samples collected before starting intranasal phototherapy stained positive for CPDs. In all samples collected immediately after last treatment strong positive staining for CPDs was detected. The number of positive cells significantly decreased 10 days after last treatment, but residual positive staining was present in all the examined samples. This finding is consistent with data reported in skin samples after UV irradiation. Cytology samples examined two months after ending therapy contained no CPD positive cells.

2.1.4. Conclusion

Our results suggest that UV damage induced by intranasal phototherapy is efficiently repaired in nasal mucosa.

2.2. Intranasal mixedUV/VIS phototherapy for the treatment of intermittant allergic rhinitis

2.2.1. Introduction According to the previously published results, the Rhinolight® (RL) intranasal phototherapy can be applied efficiently and safely in intermittent allergic rhinitis. The objective of this present clinical study has been to establish and prove the phototherapy's efficacy and safe applicability in persistent allergic rhinitis (PAR).

2.2.2. Methods:

Based on a randomization, 34 PAR patients were assigned into two groups, 25 people finished the study after all. The RL-group received a mixed Rhinolight® therapy containing UVB-UVA high-intensity visible light (dose:1.6-2.7 J/cm2/nasal cavity/treatment), while the placebo group was administered a high-intensity visible white intranasal phototherapy, altogether 13 times, for 6 weeks. The evaluation was conducted based on the results of the diary of symptoms (daily, in the morning and in the evening), nasal inspiratory peak flow (NIPF), quantitative smell threshold (Pennsylvania Smell Treshold Test) and mucociliar transport function (saccharin test). The ICAM-1 adhesion molecule expression of the epithelial cells was determined from the nasal mucosa sample, which objectively indicates the level of allergen-induced inflammation.

2.2.3. Results:

Baseline nasal symptom scores showed significant improvement ($p<0,05$), in both patient groups by the end of the treatment, which improvement still lasted until the end of the one-month follow-up. By the end of the treatment, morning sneezing ($p=0,034$), rhinorrhea ($p=0,0019$), nasal obstruction ($p=0,021$), calculated total nasal symptom score (TNS, $p=0,019$) NIPF ($p=0,0019$), the evening sneezing ($p=0,017$) and NIPF ($p=0,0077$) scores showed significant improvement in the RL group compared to the placebo group. By the end of the 4-week follow-up period, the scores of morning and evening nasal itching ($p=0,004$, $p=0.0003$), the evening rhinorrhea ($p=0,0034$) and the TNS ($p=0,0017$) in the RL group were

significantly better than in the placebo group. No significant change was observed in either the smelling or mucociliar functions in any of the groups compared with the baseline values ($p>0,05$). The number of ICAM-1 positive cells in the RL group stopped, yet this change was not significant ($p>0,05$). No severe side-effects were observed during the treatment. 9 patients had to be excluded due to upper-airway infection.

2.2.4. Conclusion:

The significant symptomatic differences observed in the improvement of both the nasal symptoms and the NIPF between the RL and the placebo group proved the efficacy of the RL treatment in persistent allergic rhinitis. The 6-week RL treatment did not cause damage of either the smelling or mucociliar functions, neither did it trigger increased nasal mucosa irritation.

2.3. UV phototherapy for the treatment of nasal polyposis

2.3.1. Introduction Nasal polyposis (NP) is characterized by high recurrence rate despite medical and\or surgical treatment. The major mechanism of action of ultraviolet B light (UVB) is induction of apoptosis in inflammatory cells. Therefore phototherapy may represent a new therapeutic approach in NP. A pilot feasibility study was performed to assess the clinical efficacy and tolerability of UVB phototherapy in NP.

2.3.2. Methods:

Thirteen subjects with bilateral grade 1-3 NP were enrolled in an open-labeled prospective pilot study. Patients were exposed to gradually increasing doses of UVB light over a 12 week period (3 exposures/week). Subjects rated their nasal obstruction symptom scores weekly on a visual analogue scale from 0 to 6. The NOSE quality of life questionnaire was used at baseline and end of treatment period. Adverse events were monitored by endoscopy.

2.3.3. Results and Conclusion:

Ten subjects completed the study. Nasal obstruction symptom scores and quality of life (NOSE) improved at end of treatment compared to baseline. Treatments were well tolerated and no device related adverse events were reported. The results suggest that phototherapy may represent a potential new treatment option in nasal polyps.

3. CONCLUSION AND NEW RESULTS OF THE THESIS

The appearance and course of diseases are determined by genetic and environmental factors together. 99% of the genome is the same in every human. The genetic variability (heterogenity) accounting for less than 1% determines the individual responses and the versatility of biological and physiological processes generated to the same noxa formed in the human body, which are traceable with molecular biological methods. Identification of etiological factors and knowledge of genome-level predilection factors in modern medicine prepares the potential to introduce a custom-made therapy tailored to the individual.

The better understanding of chronic inflammatory processes taking place in the background of airway diseases may contribute to the reassessment of thus far less successful treatment methods. Our accurate knowledge allow the creation of new prevention programmes and the introduction of modern conservative and minimally invasive therapeutic treatment modalities.

Reviewing the objectives and new results of our studies, let us summarize our conclusions:

Hystopathological examination of stenotic, scarry trachea specimens removed with cricotracheal resection:

We have introduced a new semiquantitative method for the classification of the hystopathological severity of benign laryngotracheal stenoses, which is capable of evaluating the effect of different treatment types.

The occurence and degree of inflammatory, necrotic and fibrotic processes affecting deep tissue layers such as the cartilage increases with the growth of the number and extension of endotracheal laser interventions. **We have histologically justified the** clinical observation stating that **there is a connection between the severity of the scarring and the size of the trauma triggered.**

The deviations of high volume observed in each therapy groups also indicate other possible factors affecting the grade of inflammation and fibrosis. Of these, **the role of regulatory factors showing indivudal deviations and determining the scar formation process** on a molecular biological level seems obvious.

Examination of TGF-β superfamily playing a decisive role in the regulation of inflammatory processes, and the predisposition role of its various polymorphisms in the formation of laryngotracheal scarring.

Acquired benign laryngotracheal stenosis associated with abnormal fibrotic processes is one of the most severe complications of endotracheal intubation. We aimed to conduct an initial study to identify genetic susceptibility factors for this disease. The results of our preliminary study suggests that in addition to other factors, **genetic predisposition may contribute to the pathogenesis of acquired benign laryngotracheal stenosis**: the presence of the wild type allele of the TGFβ1 -509 C/T polymorphism that has a high rate of heterozygosity in the general population may play a role in the pathogenesis of this disease. Confirmation our findings by further studies on larger groups of patients may help optimizing the intensive patient care to avoid or diminish the risk of this hardly manageable complication.

The evaluation the in vivo effect of intranasal phototherapy, by assessing DNA damage and repair mechanism in nasal mucosa.

The DNA-damaging effect of high-energy ultraviolet light irradiation is well known, and this effect may indicate the first step of carciogenesis. The majority of literature data refer to the damage formed after UV irradiation of the skin, this is why the parallel study of the impact exerted by UV light on keratinocytes and nasal epithelial cells is important. We gained the epidermal keratinocytes from the skin of donors who had undergone plastic surgery in our institute. Nasal epithelial cells were separated and bred from samples of mucosa resection surgeries. We detected the measure of UV-induced DNA damage using an alkalic Comet assay. During the application of the method, linearly with *the raising of UV doses, the extent of DNA damage also increased in both cell types, therefore we justified that the* ***Comet assay chosen is capable of demonstrating the DNA damage caused by UV light.***

We detected the effect of the intranasal mUV/VIS phototherapy on DNA damage and repair mechanisms in vivo. In our studies, we processed tha nasal mucosa samples of 26 allergic rhinitis patients in a double-blind experimental system using a Comet assay. Our results have shown that in the experimental study design we chose **the *UV/VIS phototherapy did not cause significant DNA damage.***

These results suggest a limited contribution of UV specific DNA damage to the overall cell damage of nasal mucosa in symptomatic allergic rhinitis patients detected at this time-

point with the Comet assay technique. Therefore, ***direct detection of UV specific photoproducts*** *by techniques such as immunostaining of tissue specimens* ***is of outstanding importance for evaluating DNA damage and repair of nasal mucosa*** *(CPD staining).* Our results showed that CPDs can be detected in nasal mucosa samples immediately after irradiation and residual staining was present 10 days after last irradiation. We found that skin and airway mucosa exhibit similar kinetics in repairing UV induced DNA damage.

In this pilot study we have shown for the first time that ***nasal mucosa exposed to UV light possess the capacity to repair DNA damage*** *which suggests that* ***the multistep process of carcinogenesis has not been triggered.***

Preparation of a new therapeutic protocol, establishment of its efficacy and safe usability in the treatment of PAR.

During our current studies we find out whether the ***RL procedure can be used in a safe and effective way for moderate and severe PAR, either***. Apart from the improvement of *subjective nasal symptoms*, the NIPF presents the efficacy of the RL phototherapy. Abatement of inflammatory processes in the nasal mucosa are also indicated by the decreasing tendency of *ICAM-1 adhesion molecule expression*. *Despite using the* **new, 6-week** (13 treatments) **format of the RL therapy** *and doubling the total dose (2 weeks/6 treatments), we still did not observe any side-effects which were different from that of the placebo.* Neither did we observe a decrease in either the *smelling or the mucociliar functions,* which is another proof for the safety of the RL therapy. However, it must be noted that due to the heterogenity of the allergic patient group, the resumption of studies with large patients numbers involved are necessary for the elevation of our results' value.

The examination of the clinical efficacy and tolerability of NB-UVB phototherapy in bilateral nasal polyps

Our experimental histological studies have justified that UVB penetrates well in vivo into the polyp tissue, it reaches in the stroma the lymphocytes responsible for the inflammation, and induces apopotosis in them. Intranasal phototherapy showed that the UV-induced DNA damage response of respiratory epithelium is very similar to that of the human epidermis and that nasal mucosa is able to efficiently repair UVB induced DNA damage (57,87). NB-UVB phototherapy may represent an alternative, steroid-sparring treatment for patients with nasal

polyps. The severity of NP has generally been correlated to the degree of nasal obstruction and therefore any new treatment has to have a significant effect on this symptom. We have shown that both ***nasal obstruction and quality of life (NOSE) of patients improved significantly after NB-UVB phototherapy***. The therapeutic outcome was stable for at least 3 months after end of treatment despite the fact that most of the patients had not received any specific therapy. Our preliminary data suggest that targeted NB-UVB phototherapy may represent a promising new therapeutic modality in nasal polyposis especially in patients with previous sinus surgeries, but a larger, double-blind study is warranted in the future to assess the therapeutic power of a shorter regimen and to further assess the safety profile of this new treatment

ACKNOWLEDGEMENTS

I would like to express my special thanks to **Edit Kadocsa** and **László Rovó** for providing me the opportunity to accomplish my scientific work and I would like to express my deepest grade of gratitude for their pieces of advice both regarding my scientific, clinical and personal life.

I would like to express my sincere gratitude to **Prof. Jenő Czigner** and **Prof. József Jóri**, the former and present heads of Department Oto-Rhino-Laryngology Head & Neck Surgery for their continous help and encouragement during my clinical and research activities.

I am deeply obliged to my collegue and friend, **Andrea (Ildi) Koreck** for the special cooporation and great help during our common work and projects.

I greatly acknowledge the continous support, encouragement and help of **Prof. Lajos Kemény**, who provided me the opportunity to begin my scientific work and continous collaboration to his Department.

My deepest thanks to my great Team of Rhinology to **Ágnes Kiricsi, Enikő Madaras, Gabriella Jakab, Márta Dobó, Judit Nagy.**

Very thanks for the histological and laboratory works to **Mária Kiss, Márta Széll, Edina Garaczi, László Tiszlavicz, Enikő Nagy, Ádám Perényi and István Németh.**

I am very thankful to all **colleagues** and friends of the Departments of Oto-Rhino-Laryngology Head & Neck Surgery, Allergology & Dermatology and Institute of Pathology - their support is greatly appreciated.

My thoughts and graduate also go towards my **family, wife and friends** whose continous support, encouragement, belief and patience filled me with energy and making everything worthwile.

This work was supported by the Jedlik Ányos Program (NKFP1-00004/2005), Rhinolight Ltd., Hungary and Allux Medical Inc. California, USA.