TESIS OF Ph.D. DISSERTATION

THE EFFECT OF SURFACE EXCIMER LASER TREATMENT OF THE CORNEA AND ITS ENVIRONMENT - EXPERIMENTAL AND CLINICAL STUDY

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INTRODUCTION

As the cornea has surface position, it is easy to access for surgical interventions. The so-called 'refractive surgery' represents a special field, its aim is the correction of refractive errors through the change of the surface curvature of the central cornea. For changing the refraction of the eye, it is a basic need that we should be able to change the shape of the central corneal surface in tiny steps and we should be able to calibrate accurately how much refractive change is caused by a certain intervention. The considerable technical development of the last decades made possible to construct such microsurgical tools and laser instruments, with which it is practicable to satisfy these requirements. Nowadays the refractive surgery can be considered to one of the most dynamically developing field of the ophthalmology. The excimer laser photorefractive keratectomy (PRK), one of the main branch of the refractive surgery these days, has roughly a 15-year past. The therapeutic application (phototherapeutic keratectomy, PTK) was started in the second half of the 80's, too.

The research on the excimer laser is interested not only with the technical developing. In the achievement and maintenance of the aimed effect the biological reaction of the corneal tissues has great significance, too. After the abrasion performed as a part of the laser treatment and the remove of the anterior layers of the stroma the organism 'try to substitute' the lost tissues. During the wound healing the cornea surface formed by the excimer laser can change, and this may influence the accuracy of the treatment, the stability of the achieved refractive change, and, through the smoothness or roughness of the surface, the best corrected visual acuity. The transparency and the mechanical strength of the cornea may decrease as a consequence of the laser treatment and the wound healing, the innervation of the treated area may sustain a loss temporarily, and status of the endothelium should be controlled. The changes in the cornea have more distant effects: they influence the composition and the rate of the production of the tear. The first experiment on cornea with the excimer laser equipment of the Optical and Quantumelectonical Department of the József Attila University in Szeged were happened in 1991. The refractive and therapeutic excimer laser treatments on patients started in Hungary in Budapest, in the 1st Department of the Semmelweis University in 1992. I have had the possibility to work in both groups. In my dissertation I give an
OBJECTS

1. The observation of physical phenomena in the cornea and its environment at the hit of the excimer laser pulses
   a. The aim was the detection of the shock wave formation with the help of optical equipment, and to follow its propagation in the function of time. The ablation plume was documented in a similar way. The change of the surface contour of the cornea, the propagation of surface wave was examined.
   b. The shape, the quality and the roughness of the wound surface were studied with scanning electron microscope after treatments through grids of different shapes.

2. The study of wound healing in animal experiment model
   a. The changes of different layers of rabbit cornea were followed on slides prepared in different time points after excimer laser keratectomy by transmission electron microscope.
   b. An animal model of PRK treatment of residual myopia after radial keratectomy (RK) was planned for examination the wound healing process.

3. The size up of possibilities and the limits of the photorefractive keratectomy in the treatment of hyperopia
   The criteria of suitability for hyperopic refractive surgery were examined. The clinical results of PRK treatment of spherical hyperopia were analysed, the results with two applied masks were compared.

4. The search of possibilities and limits of phototherapeutic keratectomy
   a. The results of excimer laser treatment of severe astigmatism after perforating keratoplasty were analysed.
   b. The possibility of treating recurrent erosion, which originated from alkali burn, with excimer laser was examined.
   c. The experiences of PTK treatment of two members of a family, who are suffering from Groenouw I dystrophy were estimated.
5. The examination of the protein composition of the tear following excimer laser treatment

The change of tear protein composition and concentration of the mean tear proteins after PRK was examined.

METHODS

1. The physical effects of excimer laser on enucleated pig eyes
The experiments were carried out in the Optical and Quantumelectonical Department of the József Attila University in Szeged. The photoablation on the pig eyes was performed with 193-nm Lambda Phiscs EMG 102 MSC excimer laser equipment.

a. Observation of shock wave, ablation plume and surface wave
For documentation of shock wave, ablation plume and surface wave, the frozen images were recorded by an ultrafast photographic arrangement. The records were obtained by Brinnel microscope equipped with a video camera (ITT Nokia VMC 3680 AF). The eyes were illuminated by 1-ns-long pulses from a medium pressure nitrogen-laser-pumped short pulse dye laser (Rhodamine 6G, \( \lambda = 590 \text{ nm} \)). The photographs were taken from the screen of the color monitor. The records were made for different delay times.

b. Scanning electron microscopic examination of rabbit cornea
The structure of the photoablated wound surface was examined by Phillips scanning electron microscope. The treatment was carried out through round, oblong or square-grid bronze masks of different size. Through the holes of the grid it was possible to perform a 150 \( \mu \text{m} \) x 150 \( \mu \text{m} \) ablation. 0,15 \( \mu \text{m} \) per pulse tissue defect was caused, the maximal ablation depth was 170 \( \mu \text{m} \).

2. The study of the wound healing through animal experience model

a. The follow up of wound healing after excimer laser treatment
The experience was carried out on one eye of 8 rabbits with the help of Lambda Phisics EMG 102 MSC excimer laser equipment. A 5-mm diameter filter-paper disc, soaked with alcohol was placed on the surface of the cornea for 2 seconds. During the excimer laser treatment the energy density on the cornea surface was 320 \( \text{mJ/cm}^2 \), which created 15 \( \mu \text{m} \) ablation by shoot. After the removal of the epithelium the photoablation was
performed in the centre of the cornea, through a 3-mm diameter bronze mask, in 150 µm planned depth. Both corneas of 2-2 rabbits were removed 1 day, 3 weeks, 4 weeks and 1,5 month after the treatment, and transmission electron microscopical slides were prepared. The slides were examined by JEM 7A electron microscope.

b. The examination of wound healing after photorefractive keratectomy with preceding radial keratotomy

The experiments were carried out in the 1st Department of Ophthalmology of Semmelweis University. The experiments were performed on both eyes of 9 one-year-old grey rabbits (18 eyes). During the radial keratotomy 8 regularly arranged incisions were made by diamond knife, which extended into the deepest stroma, leaving a 3-mm-diameter central optical zone unattached. 2 weeks later excimer laser treatment was performed with 193 nm Aesculap-Meditec MEL 60 equipment (energy density 250 mJ/cm², repetition frequency 20 Hz). The epithelium of the 6-mm-diameter central zone was removed by hockey-knife, then PRK treatment was performed in 54 µm depth (which corresponds to -6,0 diopters), through a home made paper mask of 6-mm-diameter. One eye of three rabbits did not went through a laser treatment, these served as control. 3-3 animals were sacrificed 3, 4 and 6 weeks after radial keratotomy (and so 1, 2 and 4 weeks after PRK), 1-1 control eyes belonged to each groups. Light microscopical slides were produced.

3, 4: Refractive and therapeutic treatments

The Refractive and therapeutic treatments were carried out in the 1st Department of Ophthalmology of Semmelweis University. During the preoperative examination uncorrected and best corrected near and distance visual acuity were tested, the refractive error was estimated. Slit-lamp and fundus examination were performed, tonometry, corneal topography and pachymetry were done.

The laser treatment was performed in drop anaesthesia (Oxibucain HCl or Tetracain). Aesculap-Meditec MEL 60 equipment was used. The energy density was 250 mJ/cm², the repetition frequency was 20 Hz in scan mode. In spot mode the laser beam was circular, with 1,5-mm diameter, 800-1200 mJ/cm² energy density, the ablation rate was 2 µm per pulse, the repetition frequency was set to 3 Hz.
Postoperative treatment and follow up: After surgery the eye was patched for one day, then antibiotic eyedrops were prescribed until complete re-epithelisation of the cornea; after that steroid eyedrops were given in tapered doses for 3-6 months. The postoperative examinations included UCVA, BCVA, and subjective refraction for both far and for near vision; pachymetry, slitlamp biomicroscopy for evaluation of the haze. Applanation tonometry was performed on every occasion during the period of the steroid therapy.

3. The possibilities and limits of photorefractive keratectomy in the treatment of hyperopia

20 eyes of 14 patients (age: 21-51 years, mean preoperative spherical equivalent: +4,23±1,18D) went through PRK treatment between January and December of 1994. After removing the epithelium mechanically, the laser ablation was performed through a butterfly-shaped rotation mask. First a smaller, then from September of 1994 a new, larger mask were used, which worked with a bigger ablation zone. The results gained with the use of the two masks were statistically compared.

1st group: With the first mask (outer diameter 7,0 mm, ablation zone diameter 4,0 mm) 6 eyes of 4 patients were treated (age: 37-49 years, mean preoperative spherical equivalent: +3,50±0,57D).

2nd group: With the second mask (outer diameter 7,0 mm, ablation zone diameter 5,0 mm) 10 eyes of 14 patients were treated (age: 21-51 years, mean preoperative spherical equivalent: +4,54±1,25D).

4. Search for possibilities and limits of phototherapeutic keratectomy

a. Refractive excimer laser treatment of cornea transplantatum

Excimer laser photorefractive keratectomy was performed on seven eyes of six patients, which went through cornea transplantation previously. The interval between the keratoplasty and the PRK extended from 1 year to 18 years. The mean preoperative spherical refractive error was -5,5±2,9D, the mean astigma was -6,8±2,7D. After mechanical removal of the epithelium, the excimer laser treatment was performed in scan mode using a rotation mask, or a contraction mask for correction of simple myopic astigmatism.
b. Treatment of complication of alkali burn with phototherapeutic keratectomy with reference to 2 cases
One eye of 2 patients suffered severe alkali burn. In spite of the applied conservative treatment recurrent erosion developed on both eyes, so excimer laser treatment was decided. The edges of the epithelium were scarped by hockey knife, then the basis of the erosion, and the edges of the epithelium were treated in spot mode.

c. Excimer laser treatment of Groenouw I granular dystrophy
Two members of a family, mother and son, suffering from granular dystrophy, were treated with phototherapeutic keratectomy. After the mechanical removal of epithelium and dropping of methylcellulose, myopic mask was used for the treatment, the aperture was restricted for 7,0 mm to 6,0 mm. The ablation was carried out in two steps, slit-lamp examination was performed between them.

5. The change of tear protein composition caused by photorefractive keratectomy
A. 5-10 µl tear sample was collected in 50 µl glass capillaries from one eye of 23 patients (32,9±11,0 years) before treatment and on the 4th postoperative day. The laser treatment was performed with 193 nm Aesculap-Meditec MEL70G-scan 'flying spot’ excimer laser.
B. 'Normal tear' was collected without stimulation from one eye of 9 volunteers (30.7±4.0 years) as control. After this 'reflex tear' was collected, gained with the help of nasal stimulation.

The tear proteins were separated by sodium-dodecyl-sulfate (SDS-PAGE) poliacrylamid gel-electrophoresis. Curves, characterise the concentrations of the individual proteins were gained from the gels with the help of digital image analysis.

Statistical analysis:
1. With the help of discriminance analysis the hypothesis was tested that there is a difference in the protein pattern, which characterises all of the proteins together, between the preoperative and postoperative samples.
2. The densitometric peaks characterising of five more tear proteins, the lactoferrin, the albumin, the IgA (heavy chain was measured), the lipocalin and the lysisym, were compared separately by paired t-test.
RESULTS AND CONCLUSIONS

1. Physical effect of excimer laser on pig eyes

a. Observation of shock wave, ablation plume and surface wave

With the help of ultrafast photo-system ablation plume was observed. It turned out from the follow up, that, because of the fast explosion of the molecules, the produced depression of the cornea is not filled by debris. In this way the next pulse affects the underlying material with its whole energy, and it deepen the ablation in a predicted way. The shock wave was found to spread with a very high speed - 4000 m/s - in the air above the cornea. We demonstrated the surface wave, which spreads on the surface of the cornea towards the periphery after the hit of the excimer laser pulse, for the first time. The clinical significance of the surface wave is that its amplitude is relatively big, compared to the thickness of the cornea, and so it may cause the damage of the cornea.

b. Scanning electron microscopic examination of rabbit cornea

The wound edges of the 193 nm excimer laser treatment were found to be sharp, parallel, only minimal cell damage could be observed in the epithelium surrounding the wound, the treatment did not cause the thickening of the stroma. It follows from the latter, that the inter-lamellar gap formation and the thickening of the lamellas is not the direct, physical effect of the excimer laser, rather cased by a tissue reaction, oedema forming.

The fine, membrane-like formation found on the surface of the lamellas in the deeper layers of the cornea is probably the same as the pseudomembrane observed during light and electron microscopical investigations of other authors.

2. Study of wound healing through animal model

a. The follow up of wound healing after excimer laser treatment

The process of the wound healing was followed-up with the help of electron microscope on rabbit cornea in the first 6 weeks. According to our experiences the reepithelisation after excimer laser treatment leads to development of excessive number of epithel layers, and multiple layer of basal cell in it. The regeneration of basement membrane has not been completed in six weeks. The activity of the scarring process on the surface
of the stroma is the highest on the 3rd-4th weeks. The keratocytes have still contained expressed endoplasmatic reticule after 3-4 weeks, although after 6 weeks the number of active cells is low. The collagen fibres in the scar tissue are arranged irregularly. Our results show that the active phase of the wound healing of the cornea after the excimer laser treatment ends to the 6th week. In this time the activity of the cells returns to the normal level. However, the disintegration of the collages fibre structure persists for a longer time.

b. The examination of wound healing after photorefractive keratectomy with preceding radial keratotomy

When the excimer laser treatment was performed on eyes, which previously went through radial keratotomy, the most interesting finding was the multilayered endothelium, above which stroma oedema was found. This shows that the altered endothelium is not able to fulfil its dehydrating task totally. It is likely that corneal damage inflicted by the primary intervention (RK) is severe enough to aggravate the otherwise negligible effects of the secondly procedure (superficial PRK) on the endothelium. Some observed extension of the epithelium refers to the development of abnormal epithelial-stroma connection. According to the above, monitoring of the status of the endothelium is recommended in patients undergoing radial keratotomy followed by photorefractive keratotomy.

3. The possibilities and limits of photorefractive keratectomy in the treatment of hyperopia

The excimer laser treatment of the hyperopic patients reduced the refractive error. Regression was found in more cases between the 3rd and the 6th months. According to our observation, the predictability and the stability falls behind the results experienced at the myopic eyes. Our methodical experience is that the near uncorrected visual acuity is a more reliable parameter as a marker for the refractive results of surgical hyperopic treatments, than the far uncorrected visual acuity. No significant difference was found between the two masks.

4. Search for possibilities and limits of phototherapeutic keratectomy

a. Refractive excimer laser treatment of cornea transplantatum
The Refractive excimer laser treatment of cornea transplantatum falls behind the results of similar interventions on intact cornea. The laser treatment reduces the astigmatism effectively at our patients. However, after 3 months regression and decrease of best corrected visual acuity followed in a considerable part of the cases, with strong haze and irregular astigmatism. The decompensation of the cornea button also occurred. Because of these, we concluded that the excimer laser treatment of high astigmatism in corneal transplantatum is not recommended. It is possible that prescribing more intensive steroid drop therapy after photorefractive keratectomy can prevent the development of strong scar tissue. Smaller ablation depth, endothel cell counting before the treatment may also help to prevent the development of strong surface haze.

b. Treatment of complication of alkali burn with phototherapeutic keratectomy

One of our patient, who was treated with phototherapeutic keratectomy because of recurrent erosion developed after alkali burn, healed without any complication. In the case of our second patient, although the reepithelisation of the cornea completed, one month later the perforation of the cornea occurred. According to the histological examination, we think that the connection between the new epithelium and the stroma was not enough tight, and erosion developed once more. This led to bacterial infection, inflammation, and than to the disintegration of the structure of the cornea. The alkali burn starts a complex process in the eye, of which the non-healing and recurring erosion is a part. In the case of erosion developed as a consequence of a mechanical injury or corneal dystrophy, the reepithelisation means the healing of the disease in the same time. However, in the case of alkali burn we have to take into account that the connection of the new epithelium with the underlying tissue is not enough regular and strong because of the absence of the basement membrane and the Bowman membrane, which may lead to further recurrence. Because of this, in case we decide beside the treatment, tight postoperative follow up is needed, and antibiotic and anti-collagenase may prevent the development of such complications.

c. Excimer laser treatment of Groenouw I granular dystrophy

Our experiences show that the granular dystrophy can be treated effectively by excimer laser. Because of the danger of the hyperopic shift, it is advisable to performed as minimal intervention as possible, clearing of only the area of pupil set as an aim, and
not to make effort to remove all of the deposits at the same session. It is wiser to repeat the photoablation in a later time point.

5. The change of tear protein composition caused by photorefractive keratectomy

The protein composition of tear samples collected before photorefractive kerectomy and on the 4th day after treatment was compared. The analysis of discriminance found a significant difference in the protein patterns. This type of analysis of the electrophoretic densitographs uses all peak information simultaneously. Significant decrease in three of the main protein peaks, lactoferrin, IgA heavy chain, and lysozym, was also found after PRK, the decrease in the level of lipocalin was borderline significant, the concentration of albumin did not change.

The excimer laser ablation of the cornea has an acute effect on the lacrimal gland protein secretion. Changes in the tear composition may lead to feeling of dryness and to a decrease of the tear film stability postoperatively.

SUMMARY

The aim of my dissertation is the examination of the effect of the 193-nm excimer laser treatment on the cornea and on the composition of the tear film, which covers the cornea, and to estimate the results of certain types of refractive and therapeutic treatments. We found that the excimer laser gives rise to such physical changes which permits to remove tissue from the surface layers of the cornea in a well controlled way, and the environment does not suffer considerable damage. On the other hand, during the wound healing scar tissue develops, which later on influences the refractive properties of the cornea, and so the success of the treatment, too. The shape of the ablation and the type of the original refractive error also influence the result of the photorefractive keratectomy. In this way, although the laser treatment of the hyperopic patients reduced the refractive error in every case, the predictability and the stability were less than in the case of myopia. Our experiences with the phototherapeutic treatments show that the granular dystrophy can be treated effectively with excimer laser, and because of the danger of hyperopic shift, it is advisable to treat the minimum in one section. When the
refractive or therapeutic treatment is carried out on a previously operated or injured cornea (radial keratotomy, corneal transplantation, alkali burn), generally there are more complications (multilayered endothelium, severe scar formation, corneal decompensation) than in cases of intact corneas. Changes in the protein secretion of the lacrimal gland were experienced as an acute side effect of the excimer laser corneal ablation.
PAPERS AND PRESENTATION IN THE THEME OF THE DISSERTATION

PAPERS


Eur J Ophthalmol (közlésre elfogadva)

LECTURES

1. J. Mohay, I. Ratkay, Á. Füst, I. Süveges, G. Szabó, B. Hopp, B. Rácz, Zs. Bor:
Scanning electron microscopical study of corneal incisions induced by an excimer laser
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2. Ratkay I., Füst Á., Mohay J., Süveges I., Szabó G., Hopp B., Rácz B., Bor Zs.:
Szaruhártya scanning elektronmikroszkópiás vizsgálata excimer laser keratectomiat követően
Első Nemzeti Orvosi Laser Kongresszus, Budapest, 1992 aug. 31- szept 1.

3. Süveges I., Mohay J., Bor Zs., Ratkay I., Füst Á.: A szaruhártya sebgyógyulása
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Magyar Szemorvos Társaság Kongresszusa, Győr, 1992 szept. 4-5.

4. I. Ratkay, Á. Füst, I. Süveges, B. Hopp: Excimer laser ablation of the cornea
XVI-th International Meeting of Ophthalmologists Alpe-Adria, Győr, 1992 szept. 3.
5. I. Ratkay, I. Süveges, Á. Füst, Zs. Bor: Corneal wound healing after photorefractive keratectomy
International Conference on Cornea, Eye Banking and External Diseases, Jerusalem, Israel, June 20-24. 1993

6. Á. Füst, I. Ratkay, I. Süveges, Zs. Bor: Elektronmicroscopical investigations after excimer laser photoablation on rabbit cornea
International Conference on Cornea, Eye Banking and External Diseases, Jerusalem, Israel, June 20-24. 1993

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15. Á.Füst, I.Süveges, Á.Kerényi, J.Németh, Z.Zs.Nagy: Photorefractive keratectomy following radial keratotomy - histological and electron microscope study on rabbit corneas

16. Á.Füst, I.Süveges: Update on PRK (referátum)


19. Füst Á, Veres A, Nagy Z.Zs., Maka E, Grus FH, Süveges I: A könny fehérje mintázatának változása fotorefraktív keratektómia hatására