(32)

Healing of alkali burned rabbit corneas with persistent superficial ulceration after excimer laser phototherapeutic keratectomy. Clinical and electron microscopic findings

Gojenie uporczywego powierzchownego owrzodzenia rogówki po oparzeniu zasadą u królików poddanych fototerapeutycznej keratektomii laserowej. Obrazy – kliniczny i w mikroskopii elektronowej

Lakimenko Stanislav[1], Buznyk Oleksiy[1], Shchypun Sergiy[2], Dumbrova Nataliya[3], Molchanyuk Nataliya[3]

[1] Department of Eye Burns, Ophthalmic Plastic Surgery, Keratoplasty and Keratoprosthesis, The Filatov Institute of Eye Diseases and Tissue Therapy
Head: Professor Nataliya V. Pasechnikova, MD, PhD
[2] Center of laser correction of vision "Tarus"
Head: Sergiy K. Shchypun, MD, PhD
[3] Departmnt of Pathomorphology and Electron Microscopy, The Filatov Institute of Eye Diseases and Tissue Therapy
Head: Professor Nataliya V. Pasechnikova, MD, PhD

Summary: Purpose: To evaluate epithelial and stromal healing of alkali-burned rabbit corneas with persistent superficial ulceration after phototherapeutic keratectomy (PTK).
Material and methods: 36 rabbits (36 eyes), with superficial corneal ulcers, which were formed after 10% NaOH corneal burn, were used. PTK was performed on the 18th day after the burn in 18 rabbits. The base and edges of corneal ulcers in 18 other rabbits were scraped with scalpel on the 18th day after the burn (control group). Clinical course and electron microscopic changes of post-burn corneal ulcers were investigated after PTK and in the control group.
Results: Clinical course of corneal ulcers has shown that PTK promoted corneal epithelialization and led to formation of less dense corneal opacities compared to control group. Electron microscopy investigations have shown that PTK 1) activated basal layer epithelial cells at the corneal ulcer edge; 2) accelerated epithelial basal complex renewal; 3) increased amount of macrophages with cytoplasmic inclusions of necrotic tissues in corneal stroma; 4) increased amount of fibroblasts with activated intracellular organellae in corneal stroma. It led to acceleration of corneal epithelization, cleaning of corneal stroma from necrotic tissues in the area of corneal ulcer and to more physiologic stromal architecture renewal.
Conclusions: PTK promotes healing of post-burn persistent corneal ulcers in rabbits.

Słowa kluczowe: wrzód rogówki, oparzenie oka, laser, fototerapeutyczna keratektomia.
Key words: corneal ulcer, eye burn, excimer laser, phototherapeutic keratectomy.

Introduction

Persistent corneal ulcers are usually formed during the 3rd-4th week after severe burns – grade IV, Roper-Hall classification (1). Collagenase synthesis by corneal epithelial and stromal cells at the ulcer edge and base is the main link in pathogenesis of these ulcers (2,3). The process might lead to corneal perforation in some cases. Nevertheless, clinical application of collagenase inhibitors has shown unsatisfactory results in prevention and treatment of post-burn corneal ulceration (4). Contact lens wear (5) requires further research because of deficiency in observations of patients with post-burn persistent ulcers. Lamellar corneal grafting (6), conjunctival flap (7), amniotic membrane transplantation (8) lead to corneal epithelialization in most cases, but these procedures often result in formation of dense corneal opacity and low visual outcome. Therefore, search for a precise surgical tool for treatment of persistent post-burn corneal ulcers is still of current importance.

Excimer laser phototherapeutic keratectomy (PTK) with wavelength of 193 nm has been applied in treatment of a number of corneal pathologies during past decades (9). Due to lack of studies excimer laser application at the burned cornea is still questionable (10,11).

Material and methods

We used 36 "shinshilla" rabbits (36 eyes), 2.2-3.0 kg in weight. 12 rabbits have been used for clinical examinations (6 – PTK group, 6 – control group), and 24 rabbits (12 – PTK group, 12 – control group) – for microscopic examinations.

All manipulations with animals have been carried out following "Principles of laboratory animal care" (NIH publication No. 85–23, revised 1985).

Post-Burn Corneal Ulcer Model

After topical anesthesia with proparacaine 0.5% and eye globe fixation a filter paper 10 mm in diameter, soaked in 10% NaOH, has been applied in optical zone of the cornea of all animals for 25 sec. Filter paper has been soaked in alkali solution until it became soft enough to conform rabbit cornea after application. Immediately after filter paper removal the eye has been rinsed with isotonic solution for 120 sec. Corneal defects 10 mm in diameter and dense corneal opacity were formed in all rabbits immediately after the burn (Fig. 1).

Fig. 1. Immediately after 10% NaOH burn. Dense corneal opacity (10 points in scale from Fig. 2).
Ryc. 1. Intensywne zmętnienie rogówki (10 punktów w skali z ryc. 2) bezpośrednio po oparzeniu 10% wodorotlenkiem sodu.

The defects have been decreasing in size during the first week after the burn (the mean size of the ulcers was 2.3 ± 1.0 mm on the 7th day after the burn). On the 10th day after the burn ulcers increased in size to 4-6 mm in diameter (4.7 ± 0.5 mm). The ulcers were not decreasing in size during next 7 days. Mean size of corneal ulcers on the 17th day after the burn was 4.8 ± 0.3 mm (range 4-5.5 mm).

Indication for surgery

It was non-decreasing size of the ulcer during 7 days. This period was chosen because absence of decrease of ulcer sizes after this period of conventional treatment is usual indication for surgery in patients with persistent corneal ulceration. In our burn model the ulcers were not decreasing in size during the 11th-17th days after the burn (mean sizes of ulcers on the 10th day were 4.7 ± 0.5 mm, on the 17th day – 4.8 ± 0.3 mm). It was considered formation of a persistent corneal ulcer (Fig. 3a, 4a), thus it was indication for surgery.

Phototherapeutic Keratectomy

"Schwind Keratom" (Schwind, Kleinostheim, Germany), excimer laser machine was used. PTK was performed on the 18th day after the burn in 18 rabbits (18 eyes), after intramuscular anesthesia with thiopentalum-natrium 1 mg/kg, topical anesthesia with proparacaine 0.5% and eye globe fixation with forceps at limbus area. Diameter of ablation was equal to the maximal diameter of ulcer (5-6 mm). Frequency 12 Hz. Criterion of PTK sufficiency was removal of whitish debris from ulcer edges and base. Due to similar infiltration of the ulcers, usual modes of excimer laser machine were at 27-30 μm ablation.

Surgical Keratectomy

After animals were anesthetized as in PTK group corneal ulcers in 18 control rabbits (18 eyes), underwent scraping of ulcer edges and base with a scalpel on the 18th day after the burn. Criterion of surgical keratectomy (SK) sufficiency was the same as in PTK group.

Medical treatment

Rabbits of both groups received drops of gentamycine 0.3% 4 times a day during the first week after the burn and water solution of boric acid 2% 4 times a day during the 8th-18th day after the burn. Postoperative care (after PTK and surgical scraping), in rabbits of both groups consisted of gentamycin 0.3% 4 times a day during the first week postop (18th-25th day after the burn), and water solution of boric acid 2% 4 times a day during the 2nd-3rd weeks after PTK and manual scraping (26th-40th day after the burn).

Methods of clinical examination

One observer (O.B.), performed all the examinations. Slit lamp microscopy was done every 2 days until epithelialization was complete, after that – every week during the second month and in 3 months after the procedures. Clinical outcome measures were time of corneal epithelialization (days after surgery), density of corneal opacity by scale from 1 to 10 (Fig. 2), and presence of complications (corneal perforations). Corneal epithelialization was considered to be complete when cornea was not stained after 1 drop of fluoresceinum 0,1% instillation.

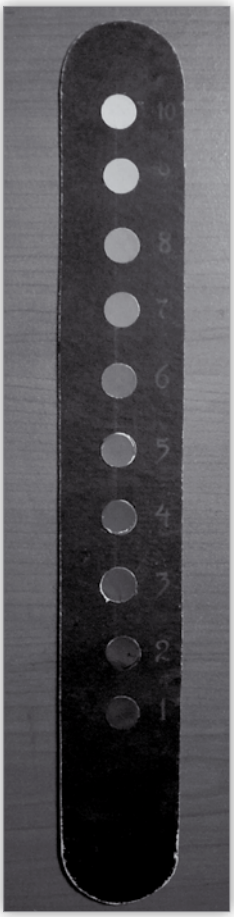

Fig. 2. V.V. Voino-Lasenetskiy scale for grading of corneal opacity density.
Ryc. 2. Skala V.V. Voino-Lasenetskiego służąca ocenie stopnia zmętnienia rogówki.

Measurement of corneal ulcer size

Ulcer size has been measured by calculation of half-sum of ulcer maximal horizontal and vertical diameters in millimeters.

Corneal pachymetry

Corneal thickness at the center of ulcer, at the ulcer edge and at the edge of corneal opacity was measured in both groups with Humphrey Instruments Inc Model 855 pachymeter (San Leandro, CA, USA), before the surgery, 1 day, 1 week, 1 month and 3 months after the surgery.

Transmission Electron Microscopy

6 animals (3 – PTK and 3 – control) were sacrificed using an overdose of Phenobarbital sodium on the 1st, 7th, 14th, 28th day after the procedures (19, 21, 25, 32, 39 days after the burn). Corneas were prepared in standard way (12) for examination by transmis-

ISSN 0023-2157 Index 362646

sion electron microscope. State of epithelium, epithelial basal complex and stroma were studied.

Statistical analysis

Mean ± standard deviation was calculated for sizes of corneal ulcers, time of corneal epithelialization and density of corneal opacity. Non-parametric Kruskal-Walis test was used to estimate intergroup differences. Bonferroni correction factor was used in case of plural comparisons.

Results

Clinical Observations

There were no corneal perforations after the procedures in both groups. Mean sizes of corneal ulcers in rabbits of both groups before the procedures (17th day after the burn) were 4.6 ± 0.2 and 4.9 ± 0.3 mm respectively (P = 0.508). However, at the end of the 1st week corneal ulcers in PTK group were smaller than in the control group – 3.9 ± 0.2 mm and 5.5 ± 0.4 mm

Groups patients/ Grupy pacjentów	18th day after burn/ 18. dzień po oparzeniu M ± SD	7th day after procedure/ 7. dzien po zabiegu M ± SD	14th day after procedure/ 14. dzień po zabiegu M ± SD	21st day after procedure/ 21. dzień po zabiegu M ± SD	Day of epithelialization/ dzień po wynabłonkowaniu M ± SD
PTK	4.6 ± 0.2 n = 6	3.9 ± 0.2 n = 6	3.5 ± 0.3 n = 6	0.2 ± 0.2 n = 6	20.3 ± 1.7 n = 6
SK	4.9 ± 0.3 n = 6	5.5 ± 0.4 n = 6	5.1 ± 0.4 n = 6	3.2 ± 0.4 n = 6	25.2 ± 3.4 n = 6
P	0.508	0.009	0.009	0.003	0.013
Bonferroni corrected P		0.036	0.036	0.012	-

Tab. I. Mean size of corneal ulcers (mm) before (18th day after burn) and in follow-up after the procedures, terms of corneal epithelialization (days after procedure).
Tab. I. Średni wymiar wrzodu rogówki (mm) przed zabiegiem (18. dzień po oparzeniu) i po zabiegu, czas nabłonkowania (dni po zabiegu).

Groups patients/ Grupy pacjentów	18th day after burn/ 18. dzień po oparzeniu M ± SD	7th day after procedure/ 7. dzien po zabiegu M ± SD	14th day after procedure/ 14. dzień po zabiegu M ± SD	1 month after procedure/ 1 miesiąc po procedurze M ± SD	3 months after procedure/ 3 miesiące po procedurze M ± SD
PTK	8.5 ± 0.5 n = 6	8.2 ± 0.4 n = 6	8.3 ± 0.5 n = 6	7.7 ± 0.5 n = 6	5.8 ± 1.2 n = 6
SK	8.3 ± 0.5 n = 6	8.3 ± 0.5 n = 6	8.3 ± 0.5 n = 6	8.3 ± 0.5 n = 6	7.7 ± 0.5 n = 6
P	0.575	0.523	1.000	0.058	0.008
Bonferroni corrected P	-	-	-	-	0.028

Tab. II. Mean value of corneal opacity density (points) before (18th day after burn) and in follow-up after the procedures.
Tab. II. Średnia wartość gęstości zmętnienia rogówki (pkt) przed zabiegiem (18. dzień po oparzeniu) i po zabiegach.

Groups patients/ Grupy pacjentów	18th day after burn/ 18. dzień po oparzeniu M ± SD	7th day after procedure/ 7. dzien po zabiegu M ± SD	14th day after procedure/ 14. dzień po zabiegu M ± SD	1 month after procedure/ 1 miesiąc po procedurze M ± SD	3 months after procedure/ 3 miesiące po procedurze M ± SD
PTK	430.3 ± 5.8 n = 6	428.6 ± 8.6 n = 6	427.9 ± 7.3 n = 6	425.7 ± 8.5 n= 6	425.5 ± 7.4 n = 6
SK	430.0 ± 7.8 n = 6	430.3 ± 8.3 n = 6	428.5 ± 9.1 n = 6	427.9 ± 7.7 n = 6	427.1 ± 8.9 n = 6
P	1.000	1.000	1.000	1.000	1.000

Tab. III. Results of corneal pachymetry (μm) before (18th day after burn) and in follow-up after the procedures.
Tab. III. Wyniki pachymetrii (μm) przed zabiegiem (18. dzien po oparzeniu) i po zabiegach.

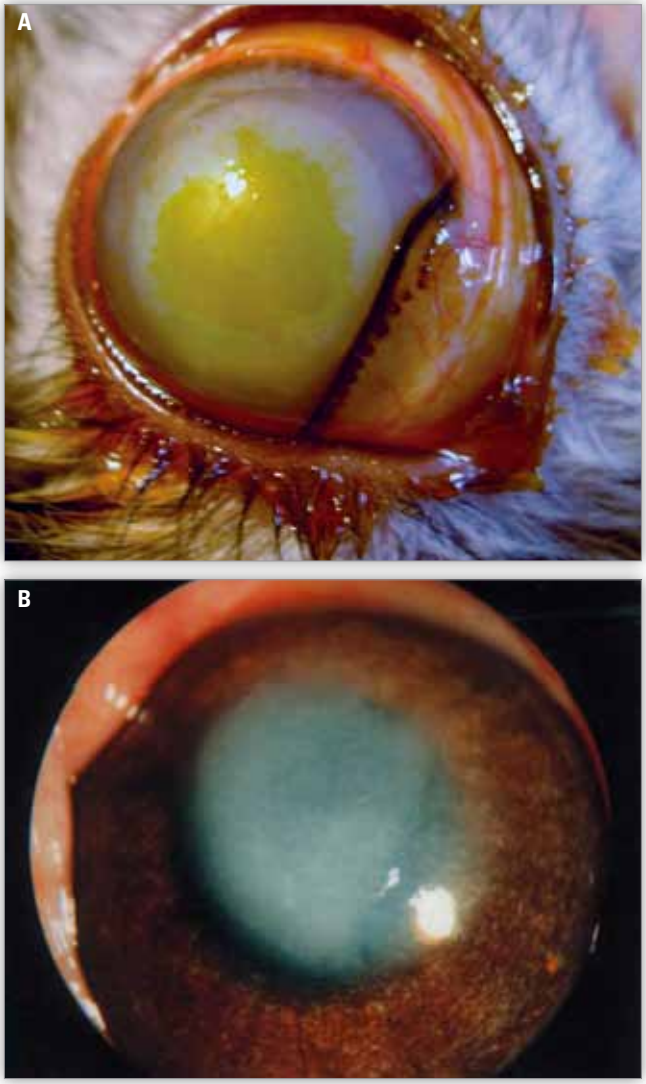

Fig. 3. Rabbit eye from PTK group: A – 18 days after 10% NaOH burn. Persistent corneal ulcer 5x5 mm, dense corneal opacity; B – 3 months after 30 μm PTK (108 days after the burn). Non-intensive (6 points in scale from Fig. 2), non-vascularised corneal opacity.

Ryc. 3. Oko królika z grupy PTK A – 18 dni po oparzeniu 10% wodorotlenkiem sodu. Znaczne owrzodzenie rogówki 5x5 mm i jej zmętnienie; B – 3 miesiące po 30 μm PTK (108 dni po oparzeniu). Zmętnienie rogówki nieunaczynione (6 punktów w skali z ryc. 2).

respectively (Bonferroni corrected P = 0.036). On the 14th and 21st day after the procedures corneal ulcers in PTK group were smaller than in SK group as well (Table I).

Corneal epithelialization occurred in both groups. Mean time of epithelialization after PTK were 20.3 ± 0.7 days, after surgical scraping were 25.2 ± 1.4 days (P = 0.013).

Difference in corneal opacity density before the procedures and 1 month after was insignificant. However, 3 months after the procedures corneal opacity density in PTK group was less intensive than in SK group – 5.8 ± 1.2 points in PTK group, 7.7 ± 0.5 points in SK group (Bonferroni corrected P = 0.028) – Table II, Fig. 3a-b, 4a-b.

Corneal pachymetry

Corneal thickness in the ulcer center and edge in rabbits of both groups could not be measured due to dense corneal opacity in all follow-up points. Corneal thickness measurements before and during follow up after the procedures at the edge of corneal opacity are presented in Table III.

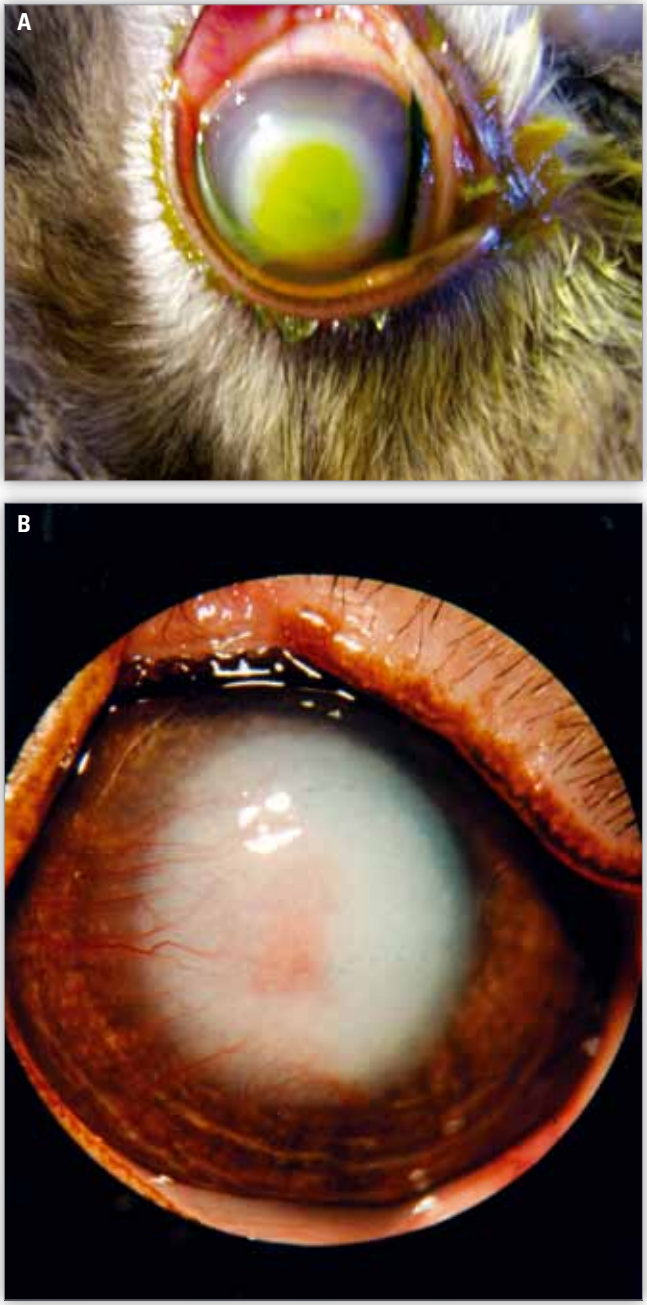

Fig. 4. Rabbit eye from the control group: A – 18 days after 10% NaOH burn. Persistent corneal ulcer 6x5 mm, dense corneal opacity; B – 3 months after surgical keratectomy (108 days after the burn). Intensive (9 points in scale from Fig. 2), vascularised corneal opacity.

Ryc. 4. Oko królika z grupy kontrolnej: A – 18 dni po oparzeniu 10% wodorotlenkiem sodu. Wrzód rogówki 6x5 mm, intensywne zmętnienie rogówki; B – 3 miesiące po PTK (180 dni po oparzeniu). Intensywne (9 punktów w skali z ryc. 2), unaczynione zmętnienie rogówki.

Transmission Electron Microscopic observations

1st postoperative day (19th day after the burn)

PTK group

Epithelium at the edge of the ulcer is polymorphic. Some epithelial cells have signs of dystrophic changes: homogenization

ISSN 0023-2157 Index 362646

of cytoplasm, intracellular structures edema, number of organellae decrease, perinuclear edema. At the same time, sections of epithelial cells with prevalence of basal cells with abundant cytoplasm, big number of granular components and small mitochondria are located nearby. These cells are lying more loosely, they have microvillae on their surface, intercellular specific contacts are infrequent between them. Basal complex: plasmolemma of basal cells, basal membrane, hemidesmosomes are found in separate areas only, basal complex is not formed. Corneal stroma is preserved partly at the ulcer area. Its pathologically changed part is characterized by structures' homogenization or destruction, presence of thin fine fibrils and connective tissue cells with packed cytoplasm and intracellular organellae edema. However, some fibroblasts have big nuclei, cytoplasm with developed granular endoplasmatic reticulum, free ribosomes and polysomes (Fig. 5). There are big macrophages with inclusion of connective tissue elements in cytoplasm as well.

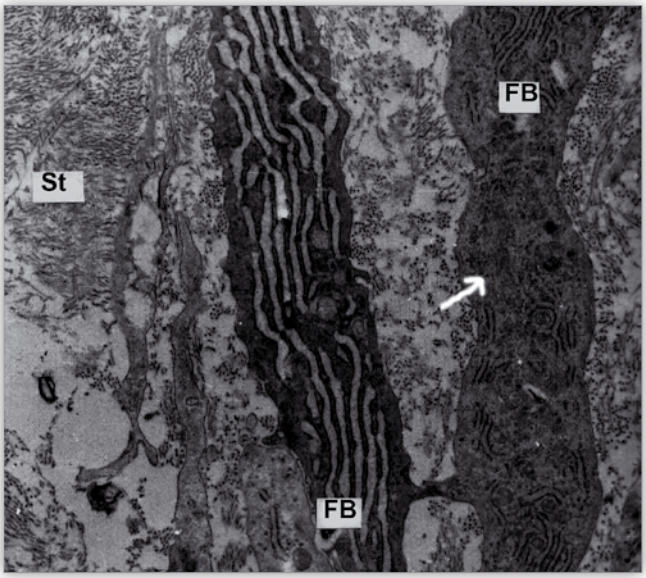

Fig. 5. 1 day after PTK and 19 days after alkali burn. Fibroblasts are in corneal stroma: one cell is with edema of intracellular structures and homogenization of cytoplasm (arrow), another cell has cytoplasm with big number of elements of endoplasmic reticulum, free ribosomes and polysomes. Magnification x 4000.
Footnotes: FB – fibroblast, St – corneal stroma.

Ryc. 5. 1 dzień po PTK i 19 dni po oparzeniu zasadą. Fibroblasty w zrębie rogówki: 1 komórka z obrzękiem wewnątrzkomórkowych struktur i homogenizacją cytoplazmy (strzałka), inna komórka ma cytoplazmę z dużą ilością elementów w endoplazmatycznym retikulum, wolne rybosomy i polisomy. Powiększenie x 400.
Odnośnik: FB – fibroblast, St – zrąb rogówki.

SK group

Corneal stroma destruction is observed in the ulcer area except for absence of epithelium. Stroma in this area is presented as unstructured mass, cellular detritus and separate accumulations of short disorientated fibrils. Remnants of wisps of more typically orientated collagen fibrils are found a little deeper. They are verged on areas of partial or full disintegration of connective tissue structures (Fig. 6).

Electronically dense or electronically transparent remnants of keratocytes are found in this area as well. Formation of epithelial cell layer is taking place at the periphery of burned area. These

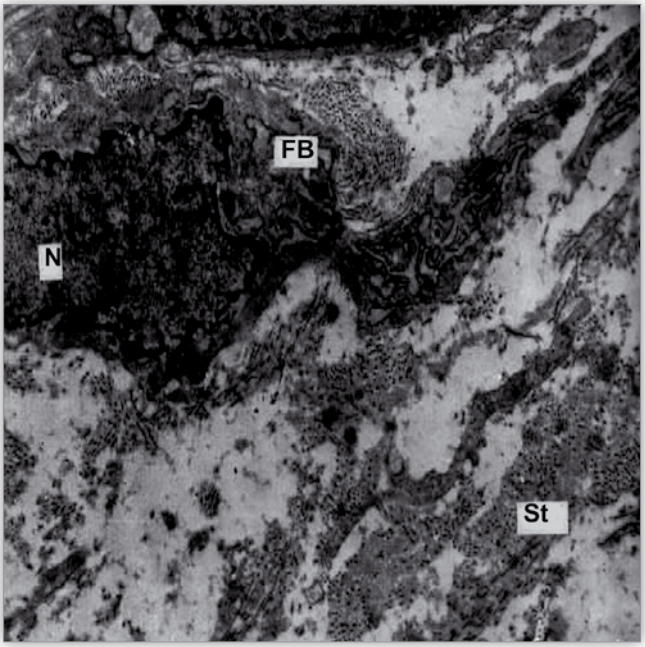

Fig. 6. 1 day after SK and 19 days after alkali burn. Marked destruction of connective tissue elements is in corneal stroma in zone of ulcer. Magnification x 4000.
Footnotes: FB – fibroblast, N – nucleus, St – corneal stroma.

Ryc. 6. 1 dzień po SK i 19 dni po oparzeniu zasadą. Zaznaczona destrukcja elementów tkanki łącznej w zrębie rogówki i strefie wrzodu. Powiększenie x 4000.
Odnośnik: FB – fibroblast, N – jądro, St – zrąb rogówki.

cells, which are situated loosely as a rule, have not well developed intercellular contacts. Condition of intracellular structures is polymorphic. Some cells contain big number of well-developed organellae, especially of that ones which are taking part in protein synthesis. At the same time, big number of cells has homogenization and intracellular structures destruction. Basal complex: basal cells plasmolemma, basal membrane, hemidesmosomes are revealed in single areas only. Subepithelial stromal tissue is loose, has small areas of structure destruction.

7th postoperative day (25th day after the burn)

PTK group

Epithelial cells at ulcer periphery are presented by basal cells mainly that differ from typical epithelial cells. They contain cytoplasm of moderate electron density, which is rich with granular components, elements of granular endoplasmatic reticulum, lisosomes and fine mitochondria. Cellular nuclei are big, oval, contain 1-2 very large nucleoli (Fig. 7). Basal complex is not formed as well.

More mature epithelial cells with activated basal cells are located more peripherally. Subepithelial stromal tissue is loose, contain disorientated short thin fibrils, areas of homogenization and electronically transparent areas of cellular detritus. Partly preserved wisps of collagen fibrils are located in middle layers and at ulcer periphery. Stromal cells in this area are destructed or have signs of intracellular edema. At the same time, there is big number of fibroblasts with abundant cytoplasm rich in ribosomes, polysomes and granular endoplasmatic reticulum components. They contain big nuclei with nucleoli. Number of such cells is relatively bigger in corneal tissue after PTK than in corneas after SK at the same period. Active macrophages with

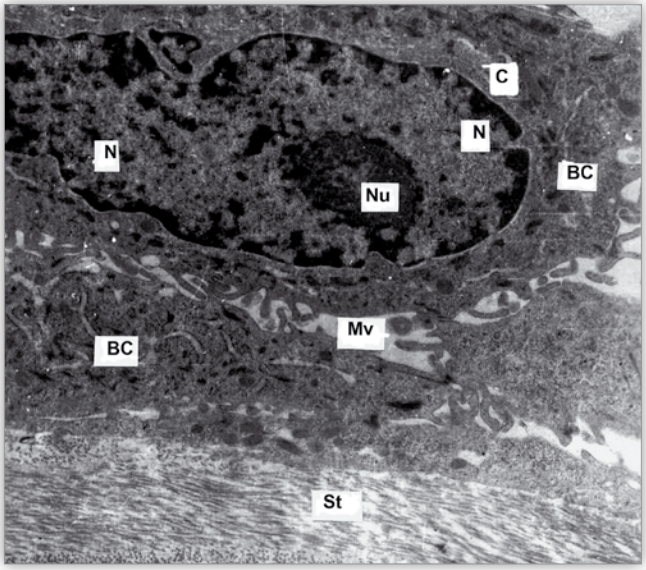

Fig. 7. 7 days after PTK and 25 days after alkali burn. Basal cells of corneal epithelium with big nucleus and nucleolus. Great number of elements of endoplasmatic reticulum, free ribosomes and polysomes are in the cytoplasm. Cellular surface has microvillas. Special contacts are not seen. Magnification x 11 000.
Footnotes: BC – basal cell, N – nucleus, Nu – nucleolus, MV – microvillas, C – cytoplasm, St – corneal stroma.

Ryc. 7. 7 dni po PTK i 25 dni po oparzeniu zasadą. Komórki podstawne nabłonka rogówki z dużymi jądrami i jąderkami. Duża ilość elementów endoplazmatycznych retikulum, wolne rybosomy i polisomy w cytoplazmie. Powierzchnia komórek ma mikrokosmki. Specjalne wiązania nie są widoczne. Powiększenie x 11000.
Odnośnik: BC – komórki podstawne, Nu – jąderka, MV – mikrokosmki, C – cytoplazma, St – zrąb rogówki.

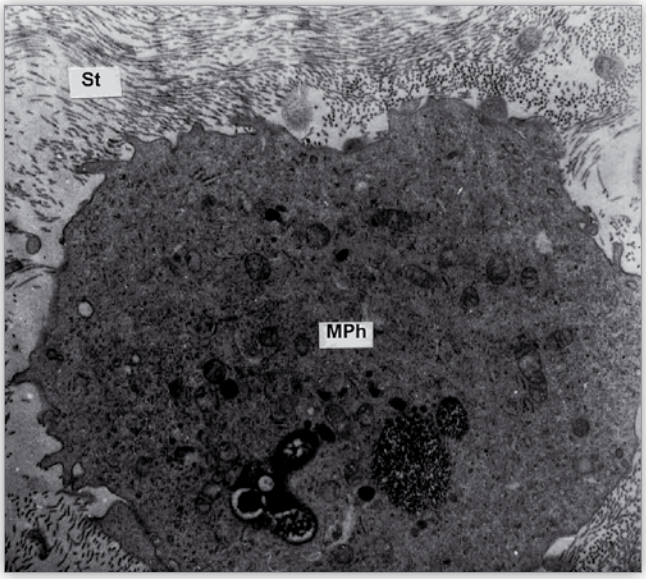

Fig. 8. 7 days after PTK and 25 days after alkali burn. Big macrophage is in corneal stroma. Magnification x 4000.
Footnotes: St – corneal stroma, Mph – macrophage.

Ryc. 8. 7 dni po PTK i 25 dni po oparzeniu zasadą. Duży makrofag w zrębie rogówki. Powiększenie x 4000.
Odnośnik: St – zrąb rogówki, Mph – makrofag.

abundant cytoplasm and inclusions of connective tissue structures, which are being destructed, are observed as well (Fig. 8).

SK group

Epithelium is being formed at the ulcer area. Basal epithelial cells are rich in organellae, their number is increased, however, they are situated in one layer, cells with elements of alteration are located between them. Basal complex in this group is seldom found.

14th postoperative day (32nd day after burn)

PTK group

Corneal epithelium is formed and close to normal. Intercellular contacts are well defined. Basal complex is formed as well (Fig. 9). However, basal cells still have activated protein synthesis apparatus. Subepithelial stromal tissue is close to normal one at periphery of corneal ulcer remnants. Relatively well-ordered cells similar to fibroblasts with increased number of organellas and flattened cells – keratocytes are located between wisps of stromal collagen fibrils. Gatherings of active fibroblasts with abundant cytoplasm and inclusions of connective tissue structures are located in areas with less organized stromal tissue.

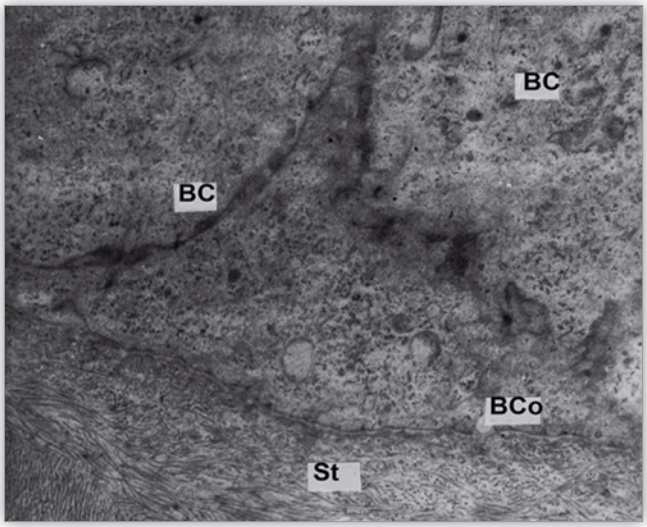

Fig. 9. 14 days after PTK and 32 days after alkali burn. Basal cells of corneal epithelium with formed intercellular contacts and basal complex. The subepithelial layer of stroma is formed too. Magnification x 4000.
Footnotes: BC – basal cell, St – corneal stroma, BCo – basal complex.

Ryc. 9. 14 dni po PTK i 32 dni po oparzeniu zasadą. Komórki podstawne nabłonka rogówki z uformowanymi połączeniami międzykomórkowymi a kompleksem podstawnym. Podnabłonkowa warstwa zrębu jest również uformowana. Powiększenie x 4000.
Odnośnik: BC komórki podstawne, St – zrąb rogówki, BCo – kompleks podstawny.

SK group

Corneal stroma architecture is being renewed as well. At the same time, unstructured areas and areas with wisps of thick short collagen fibrils with transverse crossings, which are typical for fibrous tissue formation and are not peculiar to corneal stromal tissue, are found too (Fig. 10).

Newly formed collagen fibrils, which are located near fibroblasts, and collagen fibrils wisps are more structured and less pathologically changed in PTK group than in SK group, where considerable pathologic changes are being found simultaneously with signs of connective tissue regeneration.

ISSN 0023-2157 Index 362646

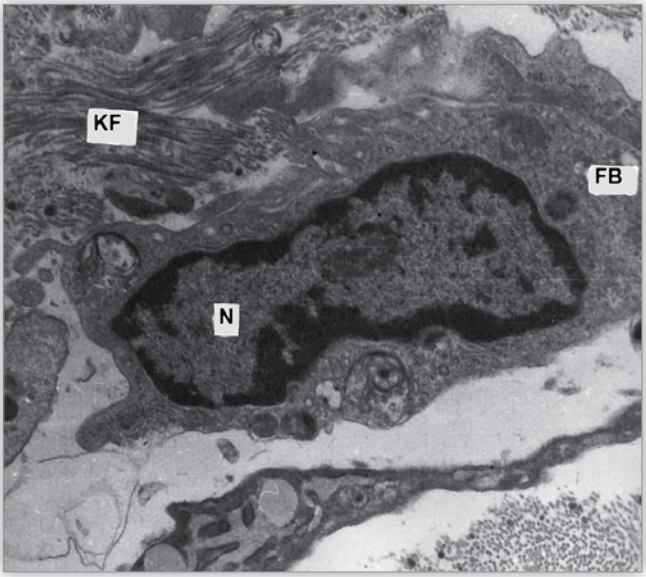

Fig. 10. 14 days after SK and 32 days after alkali burn. Corneal stromal cells and wisps of coarse collagen fibers. Magnification x 6000.
Footnotes: KF – collagen fibrils, FB – fibroblast, N – nucleus.

Ryc. 10. 14 dni po SK i 32 dni po oparzeniu zasadą. Komórki zrębu rogówki i wiązki gruboziarnistych włókien kolagenu. Powiększenie x 6000.
Odnośnik: KF – włókienka kolagenowe, FB – fibroblast, N – jądro.

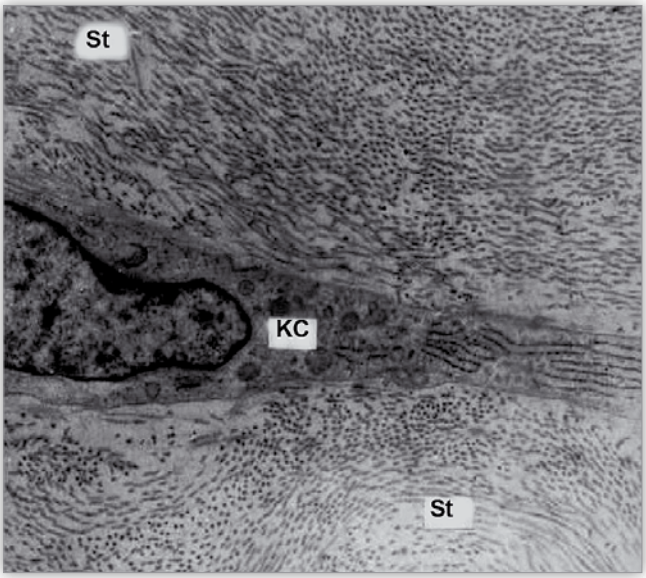

Fig. 11. 28 days after PTK and 46 days after alkali burn. Collagen fibrils wisps of corneal stroma with keratocyte fragment. Magnification x 6000.
Footnotes: KC – keratocyte, St – corneal stroma.

Ryc. 11. 28 dni po PTK i 46 dni po oparzeniu zasadą. Wiązki gruboziarnistych włókien kolagenu w zrębie rogówki z fragmentem keratocytu. Powiększenie x 6000.
Odnośnik: KC – keratocyty, St – zrąb rogówki.

28th postoperative day (46th day after burn)
PTK group

Epithelial cell layer is formed, basal cells still have activated protein synthesis apparatus at area more peripheral to pre-existed corneal ulcer. At center of former corneal ulcer, epithelium is more loose, basal complex is less organized. It differs by big number of activated basal cells and smaller number of mature cells layers that are typical for intact epithelium. Corneal stroma is relatively organized. Keratocytes are located between collagen fibrils (Fig. 11), active fibroblasts are being found as well. Number of connective tissue cells is relatively increased compared to normal.

SK group

Basal complex is still not formed at the place of ulcer, hence epithelium is separated from underlying tissues in some areas. Sites of destruction of connective tissue structures are observed in subepithelial area. Collagen fibrils peculiar to fibrous tissue are located deeper and peripherally to these areas (Fig. 12).

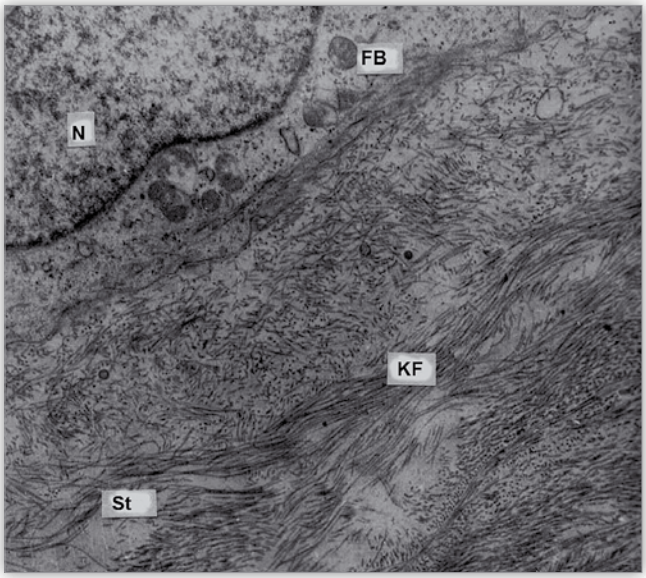

Fig. 12. 28 days after SK and 46 days after alkali burn. Corneal stroma plates are formed of loose thin collagen fibrils wisps. Fragment of fibroblast-like cell. Magnification x 4000.
Footnotes: FB – fibroblast, KF – collagen fibrils, St – corneal stroma, N – nucleus.

Ryc. 12. 28 dni po SK i 46 dni po oparzeniu zasadą. Blaszki zrębu rogówki są tworzone z luźnych, cienkich wiązek włókien kolagenu. Fragment komórki fibroblastopodobnej. Powiększenie x 4000.
Odnośnik: FB – fibroblast, KF – włókienka kolagenu, St – zrąb rogówki, N – jądro.

Discussion

Our clinical examinations have shown that PTK with machine modes at 27-30 μm ablation does not cause complications, accelerates epithelialization of post-burn superficial persistent corneal ulcers in rabbits and leads to formation of less dense corneal opacities in 3 months follow-up compared to SK. This data correlates with single Kottek's, et al. observation (11) where authors reported about acceleration of corneal epithelialization after PTK in a patient with post-burn persistent ulceration.

Pachymetry of the cornea at the centre of ulcer could not be performed due to dense opacities. Inter-group difference in corneal thickness at periphery of corneal opacity was insignificant both before the procedures and in the follow-up. In our opinion, this is an indirect sign of relatively similar grade of preoperative corneal edema in both groups. We can suppose that relatively equal amount of corneal tissue was removed in each case due to similar corneal thickness in both groups postoperatively.

Microscopic investigations were carried out to explore possible ways of excimer laser keratectomy influence on regeneration of post-burn ulcerated rabbit corneas.

Epithelium

Experimental studies (2,3) have shown that corneal ulceration is supported by collagenase synthesis by corneal epithelial and stromal cells at ulcer edges and base. In our opinion, from one side PTK removes epithelial and stromal cells from ulcer edge and base, which are the sources of collagenase. On the other side, such exposition of excimer laser radiation may inhibit collagenolytic activity of corneal epithelial and stromal cells. This hypothesis needs further investigation. Activation of protein synthesis apparatus in epithelial basal cells near the ulcer edge has been observed after PTK. Number of epithelial cells layers increased due to intensified reproduction of the basal cells. Our electron microscopy findings confirm results of other authors, who carried out histological examinations of healthy animal corneas after excimer laser ablation (12). Activation of epithelial cells leads to relatively complete renewal of epithelial layer and basal complex at the place of corneal ulcer in 28 days after PTK in our model.

Stroma

Increased number of fibroblasts and macrophages in corneal stroma, which we observed in our cases as well, was observed after excimer laser ablation of healthy animal corneas. It was supposed to be the basis of corneal "haze" by some authors (12,13). We could not assess the presence of "haze" after PTK because of intensive corneal opacities in our cases. On other hand, we have got corneas that were more transparent in PTK group than in control corneas at the end of investigation. We hypothesize that macrophage stimulation led to faster cleaning of corneal stroma from necrotic tissues in the area of corneal ulcer and fibroblast stimulation led to more physiologic stromal architecture renewal.

Phenomenon that excimer laser ablation doesn't cause additional damage of alkali-burned cornea, but vice versa promotes its more physiologic regeneration we can explain with a theory that excimer laser in yet unknown way stimulates epithelial-stromal communications in the cornea. It is known that epithelial-stromal interactions are the basis of corneal healing after its damage (14), they are shown up with a cascade of successive reactions. Starting moment is activation of plasminogen system and matrix metalloproteinases that starts different growth factors expression and in sum leads to beginning of a reparative process. In addition, we cannot exclude that excimer laser radiation stimulates corneal nerve regeneration that in the same queue influences the regeneration of corneal epithelium and stoma (15), although some researchers did not find difference in corneal nerve regeneration after excimer laser ablation and surgical keratectomy (16).

Conclusions

Superficial excimer laser ablation did not cause additional damage of burned cornea with persistent ulcer and promoted regeneration of corneal epithelium and stoma in our rabbit model.

References:

1. Roper-Hall MJ: *Thermal and chemical burns*. Trans Ophthalmol Soc UK 1965, 85, 631-653.
2. Brown SI, Weller CA: *The pathogenesis and treatment of collagenase-induced diseases of the cornea*. Trans Am Acad Ophthalmol Otolaryngol 1970, 74, 375-383.
3. Fujisawa K, Katakami C, Yamamoto M: *Keratocyte activity during wound healing of alkali-burned cornea.* Nippon Ganka Gakkai Zassshi 1991, 95, 59-66.
4. Wagoner MD: *Chemical injuries of the eye: current concepts in pathophysiology and therapy*. Surv Ophthalmol 1997, 41(4), 275-312.
5. Kanpolat A, Ucakhan OO: *Therapeutic use of Focus Night & Day contact lenses*. Cornea 2003, 22(8), 726-734.
6. Paufique L, Philips S: *Acute eye lesions treated with lamellar corneal grafting*. Br J Ophthalmol 1950, 34, 746-748.
7. Khodadoust A, Porazinski-Quinter A: *Microsurgical Approach to the Conjunctival Flap*. Arch Ophthalmol 2003, 121, 1189-1193.
8. Lee SH, Tseng SCG: *Amniotic membrane transplantation for persistent epithelial defects with ulceration*. Am J Ophthalmol 1997, 123, 303-312.
9. Fagerholm P: *Phototherapeutic Keratectomy: 12 years of experience*. Acta Ophthalmol Scand 2003, 81(1), 19-32.
10. Chuck RS, Behrens A, Wellik S et al.: *Re-epithelization in cornea organ culture after chemical burns and excimer laser treatment*. Arch Ophthalmol 2001, 119(11), 1637-1642.
11. Kottek AA, Redbrake C, Schlossmacher BW, Kuckelkorn R: *Therapie einer persistierenden Erosio nach scwerer Verätzung mit dem Excimer-Laser*. Klin Monatsbl Augenheilkd 1996, 208(4), 251-253.
12. Fantes FE, Hanna KD, Waring GO III: *Wound healing after excimer laser keratomileusis (photorefractive keratectomy) in monkeys*. Arch Ophthalmol 1989, 107(7), 895-901.
13. O'Brien TP, Li Q, Ashraf MF, Matteson DM, Stark WJ, Chan C-C: *Inflammatory response in the early stages of wound healing after excimer laser keratectomy. Arch Ophthalmol* 1998, 116, 1470-1474.
14. Wilson SE, Netto M, Ambrósio R: *Corneal cells: chatty in development, homeostasis, wound healing and disease.* Am J Ophthalmol 2003, 136, 530-536.
15. Jones MA, Marfurt CF: *Sympathetic stimulation of corneal epithelial proliferation in wounded and nonwounded rat eyes.* Invest Ophthalmol Vis Sci 1996, 37, 2535-2547.
16. Trabucchi G, Brancato R, Verdi M, Carones F, Sala C: *Corneal nerve damage and regeneration after excimer laser photokeratectomy in rabbit eyes*. Invest Ophthalmol Vis Sci 1994, 35(1), 229-235.

The study was originally received 14.06.2010 (1228)/
Praca wpłynęła do Redakcji 14.06.2010 r. (1228)
Accepted for publication 10.07.2010/
Zakwalifikowano do druku 10.07.2010 r.

Reprint requests to/ Adres do korespondecji:
Oleksiy Buznyk, MD, PhD
Frantsuzkyi bulvar 49/51, 65061, Odessa, Ukraine
e-mail: a_buznik@bk.ru

ISSN 0023-2157 Index 362646