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Photodynamic Therapy With Red Laser And Vixipin Accelerates Epithelial Regeneration After Chemical Ocular Burns

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Abstract: Relevance: To determine whether red-spectrum photodynamic therapy (PDT) with methylene blue, alone or combined with the antioxidant drop Vixipin, accelerates epithelial regeneration after chemical eye burns, quantified by cytomorphometry. Methods: In a prospective clinical study (2019–2021), 110 patients (155 eyes) with grade I–III chemical burns were assigned to: conventional therapy (comparison), conventional therapy + PDT (630 nm), or conventional therapy + PDT + Vixipin. Impression cytology of the cornea/conjunctiva was analyzed with ImageJ to measure nuclear and cytoplasmic areas; the nuclear-to-cytoplasmic ratio (NCR = nucleus area / cytoplasm area) was the primary endpoint. Outcomes were assessed at baseline and day 7. Group differences and within-group changes were tested at $\alpha=0.05$. Results: Baseline cytomorphometry was comparable across groups. By day 7, epithelial cytoplasm area decreased markedly, indicating maturation: ≈2.2× in the comparison group, ≈4.3× with PDT, and ≈5.0× with PDT + Vixipin. Nuclear area remained relatively stable across groups. Consequently, NCR rose significantly: from 0.069±0.016 to 0.146±0.039 (↑2.12×) in the comparison group, from 0.077±0.018 to 0.296±0.067 (↑3.84×) with PDT, and from 0.066±0.016 to 0.319±0.063 (↑4.83×) with PDT + Vixipin (all $p<0.05$), with both active arms superior to comparison and the combination superior to PDT alone. Conclusions: Red-laser PDT enhances early epithelial recovery after chemical ocular burns, and adjunctive Vixipin further augments this effect. The significant rise in NCR with concurrent cytoplasmic shrinkage supports faster epithelial renewal under the combined regimen, suggesting a practical, reproducible adjunct to standard care.

Keywords: Chemical eye burn, laser radiation, photodynamic therapy, Vixipin, nuclear-cytoplasmic ratio, morphometry, epithelial regeneration.

Introduction: Chemical burns of the ocular surface are among the most complex and dangerous pathologies in ophthalmology, leading to significant visual impairment and patient disability. In recent years, there has been an increase in cases of chemical burns, particularly in industrial and domestic settings, due to the growing use of aggressive chemical substances [1,5,9,12].

One of the key challenges in treating chemical burns of the eye is the high risk of complications, such as persistent erosions, secondary infections, corneal ulceration, conjunctival necrosis, which can ultimately result in irreversible vision loss. Existing therapeutic methods do not always provide sufficient efficacy, especially in cases of deep ocular surface damage [2,8,14].

Therefore, the search for new treatment approaches aimed at accelerating reparative processes, minimizing inflammatory reactions, and improving the biomechanical properties of ocular surface tissues is a pressing issue in modern ophthalmology [3,4,6,7].

Photodynamic therapy (PDT) using red-spectrum laser radiation and photosensitizers, along with the application of antioxidant agents such as Vixipin, represents a promising direction in the treatment of chemical eye burns. These methods facilitate epithelial regeneration, reduce inflammation, and prevent fibrotic changes, thereby increasing treatment effectiveness and improving patient prognosis [10,11].

This study aims to evaluate the effectiveness of a comprehensive treatment approach for chemical burns of the ocular surface using PDT and antioxidant therapy, as well as to develop predictive criteria and standardize diagnostic and therapeutic methods for this pathology [5,9,13].

OBJECTIVE OF THE STUDY

To assess whether adding red-spectrum PDT to standard care accelerates ocular surface epithelial healing following chemical burns, using cytomorphometric endpoints over the first week of treatment.

METHODS

The study was conducted at the Multidisciplinary Clinic of Tashkent Medical Academy and the Emergency Ophthalmic Microsurgery Department of the Clinical Hospital of Emergency Medical Care between 2019 and 2021. A total of 110 patients (155 eyes) with chemical burns of grades I–III (ICD-10: T26.5–T26.9) of various etiologies were included. Patients were divided into three groups: the comparison group (n=37, 52 eyes) received traditional treatment, the first main group (n=38, 51 eyes) received traditional treatment combined with photodynamic therapy (PDT), and the second main group (n=35, 52 eyes) received traditional treatment, PDT, and Vixipin eye drops. Exclusion criteria included grade IV burns, the presence of concomitant ophthalmic and systemic diseases affecting tissue regeneration.

Research methods included standard ophthalmologic diagnostic techniques: visual acuity testing with ametropia correction, anterior segment biomicroscopy (Carl Zeiss M-211), intraocular pressure measurement using the Maklakov method, and assessment of ocular surface damage area. Special methods included impression cytology to evaluate corneal and conjunctival epithelial status (cellulose-acetate filters PALL, USA), biochemical analysis of tear fluid to determine markers of inflammation and oxidative stress, and cell morphometry using PrimoStar (Carl Zeiss) and Leica DM 2500 microscopes with digital image analysis (NanoZoomer-SQ, Hamamatsu). The area and length of cells and nuclei were analyzed using ImageJ software (NIH, USA), and the nuclear-cytoplasmic ratio (NCR) was calculated using the formula: NCR = nucleus area / cytoplasm area, where an increased NCR indicated active regeneration processes.

The treatment protocol included PDT using a pulsed red laser (630 nm, 300 mJ/cm², exposure time 180 s) with a 1% methylene blue solution as a photosensitizer, as well as Vixipin administration three times a day for 10 days.

Statistical data analysis was performed using SPSS 25.0. Normality of distribution was assessed using the Shapiro–Wilk test. Group comparisons were conducted using Student's t-test and the Mann–Whitney U test, with a statistical significance level of $p<0.05$.

The study was conducted in accordance with the Helsinki Declaration (2013), approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan (Protocol No. 2 of 01.05.2014 and No. 11 of 20.12.2016), and all patients provided informed consent.

RESULTS AND DISCUSSION

The results of the study showed that the total volume of epithelial cells in all three study groups varied from 729 µm² to 4022 µm². In some cases, the increase in cell volume was associated with prolonged rinsing of the anterior surface of the eye with running water, which contributed to a change in the morphological characteristics of the cells, as well as with the severity of the inflammatory process due to the late referral of patients to the hospital. The average area of epithelial cells before treatment was 1954.1 ± 511.4 µm², the average cell length was 61.37 ± 16.33 µm², and the length of the nucleus was 15.06 ± 3.57 µm². Analysis of morphological changes showed that during treatment, the length of the cells remained virtually unchanged, but an increase in their diameter led to a change in the shape and circumference of the cells. On the 7th day of treatment, no such changes were observed in all three groups, indicating stabilization of the regeneration processes. One of the key indicators of delayed regeneration of damaged tissues is the change in the ratio of the areas of the nucleus and cytoplasm of the cell. Before treatment, the area of the nuclei of epithelial cells in the comparison group was 117.94±35.08 µm² (from 60 to 203 µm²), in the 1st main group - 131.52±23.84 µm² (from 85 to 186 µm²), in the 2nd main group - 123.71±24.11 µm² (from 82 to 175 µm²). There were no statistically significant differences between the groups before treatment ($p> 0.05$). However, on the 7th day of treatment, a statistically significant increase in the area of the nuclei of the cells was observed in the two main groups compared to the comparison group (p<0.05).

Table 1

Indicators of the nuclear-cytoplasmic ratio in cytograms

Parameters	Comparison group (n=21)		I main group (n=21)		II main group (n=20)	
	Before treatment	Day 7	Before treatment	Day 7	Before treatment	Day 7
Cell length	57,45±22,61	42,41±11,26*	65,55±12,12	35,12±7,19*^	61,11±10,53	30,43±8,69*^#
Nucleus length	12,73±3,57	14,64±5,19*	17,38±5,11	14,95±4,17*	15,06±4,81	13,43±5,21*^#
Cell area	1873,89±546,57	906,16±374,62*	1927,91±480,19	534,85±173,39*^	2072,03±483,85	512,47±219,78*^
Cytoplasm area	1755,94±525,58	797,70±339,23*	1796,39±471,49	417,35±145,81*^	1948,31±474,60	392,35±175,19*^#
Nucleus area	117,94±35,08	108,46±38,43*	131,52±23,84	117,51±34,84*^	123,71±24,11	120,12±47,45^
NCR	0,069±0,016	0,146±0,0395*	0,077±0,0183	0,296±0,0672*^	0,066±0,0155	0,319±0,0629*^#

Note: * - before treatment; ^ - compared to the comparison group; # - compared to the first main group; *^# - significantly different (p<0.05)

By analyzing other cellular parameters and inclusions on the 7th day of treatment, significant differences were identified among the studied groups. The most notable changes were observed in the nuclear-cytoplasmic ratio (NCR). The transition of epithelial cell shape from oval to elliptical during treatment indicated an acceleration of regenerative processes. Throughout therapy, the size of cells and their nuclei decreased by 2–3 times, while the nuclear-cytoplasmic ratio increased at varying intensities. In the comparison group, NCR increased by 2.12 times, in the first main group by 3.84 times, and in the second main group by 4.83 times, indicating a more pronounced cellular regeneration effect when photodynamic therapy (PDT) and Vixipin were used in combination compared to traditional treatment.

The change in the nucleus-to-cytoplasm ratio reflects an increase in the number of active functional cells, enhanced proliferation of organelles and cellular structures. In the main groups, the acceleration of regeneration processes was evident through a significant increase in NCR values. On the 7th day of treatment, NCR values were: in the comparison group – 0.146±0.039 (increase by 2.12 times, p<0.05), in the first main group – 0.296±0.067 (increase by 3.84 times, p<0.05), and in the second main group – 0.319±0.063 (increase by 4.83 times, p<0.05). Thus, NCR in the comparison group was significantly lower (p<0.05) than in the main groups, confirming the greater effectiveness of photodynamic therapy and Vixipin in stimulating regenerative processes.

Comparative analysis of epithelial cell regeneration showed that cell size changes varied in intensity across the studied groups. By the 7th day of treatment, the reduction in epithelial cell area was as follows: in the comparison group – 2.1 times, in the first main group – 3.6 times, and in the second main group – 4.1 times. This was attributed to a more intense normalization of the nucleus-to-cytoplasm ratio in the main groups, accompanied by a reduction in cell area while maintaining nuclear size. In the comparison group, the intensity of regenerative processes was 2.18 times lower than in the second main group, confirming the efficacy of comprehensive treatment with PDT and Vixipin. In patients with grade I–II burns from the main groups, cytograms on the 7th day of treatment revealed only layers of epithelial cells with signs of hypertrophy and hyperchromasia, uniform cytoplasmic staining without dystrophic changes, indicating complete resolution of the inflammatory process. The effect of laser radiation on cell morphogenesis was evident in the alteration of morphometric parameters of ocular surface epithelial cells. The application of red-spectrum laser radiation (630 nm, 300 mJ/cm²) in combination with PDT and Vixipin significantly influenced cell regeneration. In the comparison group, which received only traditional treatment, a moderate reduction in cell size and an increase in NCR were observed; however, the rate of these changes was significantly lower than in the main groups receiving laser therapy.

In the first and second main groups, where PDT was used, additional changes were observed, including a decrease in cytoplasmic area and an increase in nuclear area, indicating heightened proliferative activity of cells, a transformation of cell shape from oval to elliptical, which signified maturation and differentiation of epithelial cells, and a statistically significant increase in NCR ($p<0.05$) compared to the comparison group, demonstrating accelerated cell renewal.

Morphometric analysis showed that by the 7th day of treatment, the cytoplasmic area had significantly decreased compared to baseline values in all study groups, though the extent of these changes varied. In the comparison group, the cytoplasmic area decreased from 1755.94±525.58 μm² to 797.70±339.23 μm² (↓1.2 times, $p>0.05$), in the first main group (PDT) from 1796.39±471.49 μm² to 417.35±145.81 μm² (↓2.3 times, $p<0.05$), and in the second main group (PDT + Vixipin) from 1948.31±474.60 μm² to 392.35±175.19 μm² (↓2.5 times, $p<0.05$). These findings indicate that laser radiation accelerates cytoplasmic reduction, which serves as a morphological marker of active cell proliferation and maturation. Analysis of nuclear area dynamics showed a slight decrease in the comparison group from 117.94±35.08 μm² to 108.46±38.43 μm² (↓1.08 times, $p>0.05$), in the first main group from 131.52±23.84 μm² to 117.51±34.84 μm² (↓1.12 times, $p>0.05$), while in the second main group, nuclear area remained nearly unchanged at 120.12±47.45 μm² ($p>0.05$).

Thus, despite the reduction in cell size, nuclear area remained relatively stable, indicating active protein synthesis and enhanced proliferative processes, particularly under the influence of PDT and Vixipin. The NCR is a key parameter reflecting cell activity and the intensity of regenerative processes. Before treatment, NCR values showed no statistically significant differences among the groups: in the comparison group – 0.069±0.016, in the first main group – 0.077±0.0183, and in the second main group – 0.066±0.0155. By the 7th day, a significant increase in NCR was observed in the main groups: in the comparison group, NCR increased to 0.146±0.0395 (↑2.12 times, $p<0.05$), in the first main group to 0.296±0.0672 (↑3.84 times, $p<0.05$ compared to the comparison group), and in the second main group to 0.319±0.0629 (↑4.83 times, $p<0.05$ compared to both the comparison group and the first main group). Comparative analysis between groups showed that NCR differences between the comparison group and the main groups were statistically significant ($p<0.05$).

The difference between the first and second main groups was also significant ($p<0.05$), confirming the additional effectiveness of Vixipin in the second main group. Furthermore, the rate of NCR change in the comparison group was 2.18 times slower than in the second main group, highlighting the substantial acceleration of regeneration under the influence of laser radiation and Vixipin. Photodynamic therapy (PDT) in combination with laser radiation significantly influenced cell morphogenesis, leading to a reduction in cytoplasmic area and a relative increase in nuclear area, which is a morphological marker of active proliferation. NCR in the first and second main groups increased 3.84 and 4.83 times, respectively ($p<0.05$), indicating significantly accelerated regeneration. The differences in NCR between groups were statistically significant ($p<0.05$), confirming the efficacy of laser radiation and the additional benefit of Vixipin.

The second main group (PDT + Vixipin) demonstrated the highest rate of cell regeneration compared to the first main group, supporting the effectiveness of comprehensive treatment. In the comparison group, regeneration occurred 2.18 times slower than in the second main group, indicating the insufficient efficacy of traditional treatment compared to laser therapy. Thus, the use of laser radiation in combination with PDT and Vixipin is an effective strategy for accelerating epithelial cell regeneration and can be recommended as an adjunctive method for treating chemical eye burns.

CONCLUSION

Red-spectrum photodynamic therapy (PDT) with methylene blue, particularly when combined with the antioxidant drop Vixipin, favorably modulates epithelial cell morphogenesis after chemical ocular burns. Across seven days, cytomorphometry demonstrated marked contraction of the cytoplasmic compartment with relative preservation of nuclear area, yielding a robust rise in the nuclear-to-cytoplasmic ratio (NCR)—a pattern consistent with metabolically active epithelial renewal. NCR increased significantly in all groups, with the largest gains in the PDT arms (≈3.8-fold with PDT alone and ≈4.8-fold with PDT+Vixipin), indicating an accelerated trajectory of early epithelial recovery compared with conventional therapy.

These findings support PDT as a practical adjunct to standard care for chemical eye injuries, and suggest that Vixipin further augments the reparative response. Clinically, the combination regimen is associated with faster normalization of epithelial cytomorphology within the first treatment week, a window that is critical for preventing secondary complications and scarring.

Future studies should extend follow-up beyond seven days, incorporate stratification by burn etiology and severity, and employ analytic models that account for inter-eye correlation. Inclusion of proliferation and fibrosis biomarkers (e.g., Ki-67, α-SMA) and standardized PDT dosimetry will help refine mechanisms and optimize protocols. Pending such confirmation, red-light PDT—especially with Vixipin—can be considered a promising, reproducible strategy to enhance epithelial regeneration after chemical ocular burns.

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