THE DEVELOPMENT OF A KERATITIS CAUSED BY P. AERUGINOSA UNDER CONDITIONS OF VARIOUS TYPES OF CORNEAL SURFACE DAMAGE IN THE EXPERIMENT

Kryvetska Nelia Volodymyrivna

Assistent, Department of Ophthalmology
National Pirogov Memorial Medical University Vinnytsya, Ukraine

Bacteria dominate among the causes of infectious keratitis [1,2]. Among them, purulent corneal lesions caused by P. aeruginosa have a rapid course, high rate of severe complications are resistant to antimicrobial therapy and lead to a significant decrease in visual acuity [3,4]. Within recent years, Pseudomonas has shown an increase in the frequency of seeding at purulent keratitis both in monoculture and in associations up to 39.0%-44.6% and 20.0%, respectively [3].

A special cohort consists of P. aeruginosa keratitis associated with contact lenses wearing. It has a high frequency of occurrence, which, according to various authors, can reach 44-65% among mentioned corneal lesions [5,6].

Materials and methods: The research was conducted at the vivarium of Pirogov National Medical University, Vinnytsia during 2019-2020 in compliance with the ethical requirements stipulated by the Directive 2010/63/EU of the European Parliament and the Council of September 22, 2010 on the protection of animals used for scientific purposes, the norms of biomedical ethics, which were approved by the First National Congress of Bioethics of Ukraine (2001), as well as in accordance with the Law of Ukraine No. 3447-IV (2006).

A total of 32 rabbits (adult animals with a body weight of 3-3.5 kg) were involved in the study. For infection, a museum strain of Pseudomonas aeruginosa was used in the form of a suspension of a one-day culture of the microorganism at a concentration of $5 \times 10^8$ CFU/ml. All animals were randomly divided into 3 groups.

Animals of experimental group 1 (8 rabbits) were undergone scarification (making 5 linear scratches of 5 mm each) in the center of the cornea and instillation into the conjunctival sac of 0.2-0.4 ml of the suspension of the pathogen with its additional introduction subconjunctivally 0.1 ml.

In group 2 (12 rabbits), deepithelialization of the cornea in the central part on an area of about 1 cm$^2$ was performed by scraping its surface layer with the tip of a 21G injection needle. The microbial suspension was applied to the eye in drops.

To the animals of the group 3 (12 rabbits) after the processing as mentioned for group 2, the surface of the cornea was covered for 24 hours with a sterile soft
contact lens made from balafilcon A (water content: 36%, oxygen permeability DK/t: 110.0).

The initial assessment of bacterial eye affection was performed after 24 hours. The course of the disease was monitored daily by evaluating keratitis clinical signs, collecting material for culturing, conducting an ophthalmological examination of the cornea with fluorescein staining and photofixation.

Results. An infection with P. aeruginosa at linear scratches of the corneal surface (group 1) resulted in a bacterial conjunctivitis without persistent purulent corneal inflammation. The next day, all animals of this group developed conjunctivitis. There were cases of intense (5 eyes) or moderate (3 eyes) hyperemia of the conjunctiva and third eyelid, swelling of these structures, and lacrimation. Three out of eight rabbits had thick white purulent discharge; fibrin threads were observed in the remaining eyes. Corneal changes in all cases were present in the form of erosions, almost imperceptible during ophthalmological examination. A fluorescein test revealed a local relatively well-defined area of staining that was smaller than the scarification zone. An epithelialization of erosions occurred spontaneously within 3-4 days.

In the second experimental group, in which the animals were de-epithelialized with the subsequent application of a microbial suspension of P. aeruginosa, the appearance of keratitis was noted in all cases. A day after infection, 3 out of 12 rabbits developed profuse white viscous purulent discharge; the rest had moderate muco-purulent and fibrinous secretions. Intense redness and swelling of the conjunctiva and third eyelid was observed in all eyes. There was inflammatory infiltration of the cornea with pronounced local edema and a positive fluorescein test over the entire area of deep epithelialization in all cases. The focus of inflammation was surrounded by a transparent rim. Within 3-4 days, the affection progressed, which was expressed in the spread of the infiltrate and swelling of the cornea beyond the deep epithelialization zone with intense fluorescein staining, but not beyond the diameter of the deep epithelialized surface in any direction.

In the animals of the third group, the purulent keratitis appeared in all cases, and the inflammatory process was characterized by a significantly larger area and depth of the lesion. Intense hyperemia of the bulbar conjunctiva and third eyelids, pronounced chemosis, abundant purulent discharge, which often caused the eyelids to stick together, were invariably observed. The area of corneal opacification significantly exceeded the area of deep epithelialization up to subtotal and total damage. Due to the pronounced intensity of clouding, the deeper structures of the eye were only partially visible (in 8 cases) or were not identified at all (4 observations).

During the second day of the study, the process progressed in all 12 animals of the III group, twice with ulceration. Instillation of fluorescein caused uneven diffuse penetration of the cornea with penetration of the dye into deeper layers and decentralized distribution well beyond the deep epithelialization zone without clear demarcation. In 4 cases, there was deformation of the cornea with loss of its sphericity; corneal abscess occurred twice. In the most severe cases, there was keratomalacia and perforation of the cornea (2 observations each).

Discussion. The conducted study demonstrates the importance of complete de-epithelialization of part of the cornea in combination with a long stay of the
pathogen on the affected surface. In contrast, linear corneal scarifications, co-inoculated with P. aeruginosa did not result in persistent bacterial keratitis requiring treatment. Apparently, the eye infection was eliminated by the animal's local bactericidal and regenerative protection systems.

Complete deepithelialization of the corneal surface accompanied by P. aeruginosa inoculation resulted in the prevalence of moderate keratitis in animals within 48 hours. In the absence of treatment, the disease mostly progressed to semi-severe. The use of the same scheme together with contact lenses in almost all cases led to the early development of severe keratitis. This difference in the results in the 2nd and 3rd groups allows predicting the severity of the experimental disease depending on the goal of the researcher. The proposed experimental methods are distinguished by their ease of implementation and repeatability of results.

Conclusions The proposed models of P. aeruginosa keratitis make it possible to reproduce in an experiment a bacterial lesion of the cornea of varying degrees of severity. The conditions for modeling P. aeruginosa keratitis in rabbits are: complete de-epithelialization of part of the cornea and long-term presence of the pathogen on the affected surface. Instillation of a microbial suspension of P. aeruginosa on the deep epithelialized surface of the eye followed by covering with a soft contact lens leads to the development of severe forms of bacterial keratitis.

References: