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MULTILAYER PLASTIC RECONSTRUCTION IN THE THREE-DIMENSIONAL STUDY OF THE HUMAN LACRIMAL GLAND

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Lacrimal glands are in the limelight for both clinicians, and morphologists, who understand that the clinical interpretation of morphological factology is to be relying on modern anatomical data. 10 specimens of the human lacrimal glands (palpebral part) have been studied. The use of the method of multilayer plastic reconstruction enables to get the megascopic reconstruction of the lacrimal gland, which can be studied from all sides, getting a visualization of the shape and dimensions, as well as allows to investigate the inner topography of the organ, the geometry of the lumen of the epithelial excretory ducts of the glands, to determine the changes in the thickness of the wall, to get a visual representation of microtopographic correlation between the different sections of the blood microcirculatory flow with the epithelial excretory ducts in the human lacrimal glands.

Key words: lacrimal gland, excretory ducts, plastic reconstruction.

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Lacrimal glands are in the limelight for both clinicians, and morphologists, who understand that the clinical interpretation of morphological factology is to be relying on modern anatomical data [9, 12, 14]. Therefore, the development of the scientific aspect which contributes to the accumulation of the specific facts about the exocrine gland, identifying the individual and general patterns, differences in their structure is the urgent task of the contemporary morphology [3, 8, 11, 15]. Noteworthy, no scientific data, obtained after 3D analysis of the macrostructure of the human lacrimal gland, have been found in the domestic scientific publications. Many related issues have not been fully studied to date due to complexity of the techniques and big labor costs. It is first related to the study of the structural hierarchy and spatial organization of the system of the excretory ducts of the human lacrimal glands. A tear plays an important role in the functioning of the tunicae of the eye (especially the conjunctiva and the fibrous tunic of the eyeball), preventing their drying out, development of keratosis and "dry eye" syndrome.

Recently, this problem has been of the significant importance for ophthalmologists, especially the issue on the tear formation, its flow through the excretory ducts, changes in its osmolarity, qualitative and compositional structure of the lacrimal fluid. Qualitative changes in the tear composition along with hyposecretion, breach of the mechanism, which ensures secretion through the excretory ducts and organic damages of the glands are the triggers of developing "dry eye" syndrome [4].

Current publications confirm that the process of secreta formation is well studied, which can not be said about the mechanisms that contribute to discharge of secreta from the acini through the excretory ducts towards the orifice [1, 13]. The mechanism of tear excretion from the human lacrimal glands is of particular interest since the structure of its excretory ducts is not fully studied. Paradoxically, paths of the tear drainage from the orifices of the common excretory ducts of the lacrimal gland to the surface of the conjunctiva into the lacrimal sac, rivrus lacrimalis, lacrimal lake, nasolacrimal duct and the mechanisms that contribute to tear flow in a specific direction, are described in details and comprehensively [15, 16]. At the same time, no data on the tubular excretory structures of the lacrimal gland, specifically, their stereomorphology have been found, as well as data on their morphometric parameters, microanatomical relationship with vessels of microcirculatory blood flow.

The purpose of the paper is to determine the regularities and specific features of the spatial organization of the system of the excretory ducts and its syntopy with the capacitive sections of the microcirculatory blood flow of human lacrimal glands.

Material and methods. 10 specimens of the human lacrimal glands (palpebral part) have been studied. To obtain 2-3 mm semi-thin epoxy sections the biological material was fixed in the buffered 4% glutaraldehyde solution (pH 7,4). Once the slices were washed and dehydrated they were embedded into the Epon-812. The series of semi-thin histological sections were obtained from the tissues, embedded into epoxy resin, and served as the base for the multilayer plastic reconstruction [2, 5, 6, 7, 10].

Results and its discussion. The current view of the morphologists on the spatial organization of the biological objects (structural units of the organs, complexes of extracellular structures and intracellular components) is based on two fundamentally different approaches [11, 12]. The first (a classical one) is based

on the creation of the three-dimensional reconstruction of the microobject using the series of slices. For this purpose we used semi-thin epoxy series of sections. This method permits to get the reconstructions, which are the "artificial product", but is unsatisfactory for a quick and simultaneous study of a large number of the objects. It demonstrates the precise three-dimensional representation of the studied microobjects, adapted to the spatial perception by our organ of vision. The second method of reconstruction has been developed and justified using the statistical methods, permitting to study a single slice or electronic micro-imaging using the standard test-systems. This stereological principle of reconstruction is more mathematical and abstract, but permits simultaneous study of the large number of objects. However, it has its own limitations. Not minimizing its significance it should be noted that the second method failed to replace the first one, but became a precursor of the novel method, namely, the method of computer reconstruction. While considering the primary task of the 3D reconstruction, i.e., the study of spatial three-dimensional organization of the microobject, the most preferable method is the classical one. We choose it to investigate the systems of excretory ducts of the studied glands. The overall processing of the material was conducted in a way to minimize the deformation of the studied object and its dimensions. Since the fixatives, containing formalin, shrink the specimen to 20%, to make plastic reconstruction casts we used specimens fixed in glutaraldehyde solution to reduce deformation of tissue. During casting the tissue was oriented to the desired plane in the block which is relative to its surface that allowed getting the series where a cut was made perpendicular to the surface of the object. In this way each section involved the most of the details. Graphical methods of reconstruction are supplementary, though permitting to get a general idea about the studied object, in particular, enable to define the boundaries of the epithelial complexes' clusters. A preliminary study of the three-dimensional organization of the excretory ducts using the method of multilayer graphic reconstruction enabled to receive their visual representation in the depth of the blocks made of transparent film materials. This preliminary analysis subsequently facilitates the process of making the models by the method of multilayer plastic reconstruction. Hand-made wax plates of the preferred thickness and required flexibility and durability, manufactured from the 1-2 mm base plate wax, were used to make the plastic models based on the serial histological sections. They are rather transparent and permit to reproduce the object's profiled contours on their surface. First, the obtained specimens of the lacrimal gland were fixed in 4% glutaraldehyde solution and osmium tetroxide and subsequently embedded into the Epon-812. The series of the semi-thin sections were stained in the phosphate buffered 0,1% toluidine blue solution. The loss of sections greater than 3% in a set is not allowed. Then, the photomicrography of each section, keeping to the ultimate magnification for the whole set, has been made. After that the contours of the analyzed structures and extra coordinates have been selectively determined. At this stage we used graphical reconstructions. The required structures and extra coordinates were reproduced from the photoprints onto the transparent plates for the preliminary evaluation, analysis and sequence for the next arrangement of the 1-2 mm wax plates. The contours of the investigated microobjects and extra coordinates that contribute to the correct arrangement of the workpieces have been reproduced on the wax plates. The 3D skeleton of the primary model has been obtained as a result of stacking of the set of wax plates-templates. At this stage the extra coordinates were removed from the skeleton. Thereafter, the final stage of the creation of the 3D wax model of the lacrimal gland has been done. Its various structures were marked with multicolored paints.

Fig.1. Plastic reconstruction of the lobule of the lacrimal gland. 1:240 linear magnification.

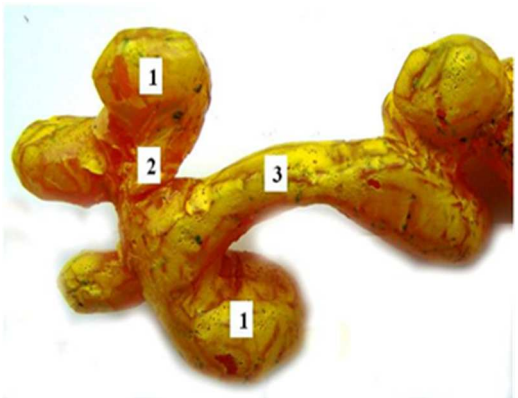

Fig. 2. Plastic reconstruction of the central intralobular duct of the lacrimal gland (coaxial elements were partially removed). 1:240 linear magnification. 1 – acinus; 2 – distal duct; 3 – intralobular axial duct.

The use of the suggested method enables to get the megascopic reconstruction of the lacrimal gland (fig. 1), which can be studied from all sides, getting a visualization of the shape and dimensions, as well as allows to investigate the inner topography of the organ, the geometry of the lumen of the epithelial excretory

ducts of the glands, to determine the changes in the thickness of the wall, to get a visual representation of microtopographic correlation between the different sections of the microcirculatory blood flow with the epithelial excretory ducts in the human lacrimal glands.

Conclusions

1. The epithelial tubular structures of the human lacrimal gland form a bifurcated system of the excretory ducts where no typical intercalated ducts have been found;
2. The lacrimal gland contains intralobular, lobular, interlobular and common excretory ducts, through which the secreta of predominantly protein nature scrects drains (fig. 2).
3. The specific features of the human lacrimal gland's structure are absence of the intercalated ducts, which are the connective link between the acini and excretory ducts, and maximum strict order in the volume of the lobule and its structural components (acini and intralobular ducts).

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Реферати

ВИВЧЕННЯ ПРОСТОРОВОЇ ОРГАНІЗАЦІЇ СЛЬОЗОВОЇ ЗАЛОЗИ ЛЮДИНИ ЗА ДОПОМОГОЮ БАГАТОШАРОВОЇ ПЛАСТИЧНОЇ РЕКОНСТРУКЦІЇ

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Сльозні залози перебувають у сфері уваги не тільки клініцистів, але і морфологів, між якими є розуміння того що, клінічне тлумачення морфологічної фактології повинно опиратися на сучасні анатомічні відомості. Матеріалом дослідження слугували 10 препаратів сльозових залоз (пальпебральна частка) людини. Використання способу багатошарової пластичної реконструкції дозволяє отримати збільшену реконструкцію сльозової залози, яку можна вивчати з різних боків, отримуючи вичерпне уявлення про форму та розміри, а також дозволяє вивчити внутрішній рельєф органа, геометрію просвіту епітеліальних вивідних протоків залоз, визначити зміни товщини стінки, одержати наочне уявлення про мікротопографічні взаємовідносини

ИЗУЧЕНИЯ ПРОСТРАНСТВЕННОЙ ОРГАНИЗАЦИИ СЛЕЗНОЙ ЖЕЛЕЗЫ ЧЕЛОВЕКА С ПОМОЩЬЮ МНОГОСЛОЙНОЙ ПЛАСТИЧЕСКОЙ РЕКОНСТРУКЦИИ

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Слезные железы находятся в сфере внимания не только клиницистов, но и морфологов, между которыми есть понимание того, что, клиническое толкование морфологической фактологии должно опираться на современные анатомические сведения. Материалом исследования послужили 10 препаратов слезных желез (пальбебральная часточка) человека. Использование способа многослойной пластической реконструкции позволяет получить увеличенную реконструкцию слезной железы, которую можно изучать с разных сторон, получая исчерпывающее представление о форме и размерах, а также позволяет изучить внутренний рельеф органа, геометрию просвета эпителиальных взводных протоков желез, определить изменения толщины стенки, получить наглядное представление

різноманітних ланок мікроциркуляторного русла з епітеліальними екскреторними протоками в сльозових залозах людини.

Ключові слова: сльозова залоза, екскреторні протоки, пластична реконструкція.

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о микротопографии взаимо-отношения различных звеньев микроциркуляторного русла с эпителиальными экскре-торными протоками в слезных железах человека.

Ключевые слова: слезная железа, экскреторные протоки, пластическая реконструкция.

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INDICATORS CELL CYCLE AND DNA FRAGMENTATION OF SPLEEN CELLS IN EARLY TERMS AFTER THERMAL BURNS OF SKIN AT THE BACKGROUND OF INTRODUCTION 0.9% NaCl SOLUTION

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In the experiment, during 7 days, changes in cell cycle and DNA fragmentation of spleen cells in rats after burn injuries were studied in the background of the introduction of 0.9% NaCl solution. After skin burn in the background of the introduction of 0.9% NaCl solution, after 1 day, greater values of the interval SUB-G0G1 and the G0G1 phase and, at the same time, lower values of the phases S, G2+M and the index of proliferation were determined, indicating the pathological induction of apoptosis and violations of the synthetic processes of splenocytes. After 3 days after burning the skin, large average values of the G0G1, proliferation and proliferation indexes were determined and, at the same time, the highest possible apoptosis rate compared to similar animal numbers in the 1 day after burn, which could be considered as the activation of mechanisms for compensating for pathological effects thermal damage at the given time. 7 days after skin burn, the average values of the G0G1 and the proliferation index are close to the similar figures for a group of animals without burn injuries of the skin, with the introduction of 0.9% NaCl solution, while the values of the S-phase (at almost 2.5 times) and the SUB-G0G1 interval (2.7 times), indicating insufficient compensation for the proliferative activity of the spleen cells against the background of increased apoptosis

Key words: cell cycle indexes, DNA fragmentation, spleen, rats, burn skin, 0.9% NaCl solution.

A prerequisite for the development of infectious complications in burning skin is the complex of immunity lesions that occurs due to the toxic effects of metabolism and toxins, which can lead to sepsis and death of the patient [3]. One of the most important components of the development of immune deficiency against the background of burns is the lesion of the spleen, as the main organ of humoral immunity and reticuloendothelial system [4, 13]. The study of the characteristics of the response of the spleen cells against the background of burn injury has been carried out quite long ago, but the data obtained are quite contradictory [1,10] and do not allow to form unambiguous views on the damage of this organ at the cellular level, which inhibits the development of effective methods for correction of immunosuppression with burn injury to the body.

The purpose of the study is to establish the characteristics of the cell cycle and DNA fragmentation of the cells of the spleen 1, 3 and 7 days after burn injury at the background of the introduction of a 0.9% solution of NaCl.

Material and methods. Within the framework of scientific cooperation between National Pirogov Memorial Medical University, Vinnytsya and SI "Institute of blood pathology and transfusion medicine of NAMS of Ukraine" (Lviv) and National Pirogov Memorial Medical University, Vinnytsya and the National Medical University named after O.O. Bogomolets an experimental study of the effect of the control infusion drug - 0.9% solution of NaCl on the structure of the spleen of the intact rats, as well as in the early stages (1, 3 and 7 days) after a burn injury to the skin. The research was carried out on laboratory white rats, males weighing 155-160 g, obtained from the vivarium of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. During the experiment all animals were kept under vivarium of National Pirogov Memorial Medical University, Vinnytsya (indoor temperature - within 24-25 ° C, humidity - within 40-60%) on a standard water and food ration, with free access to water and food. All experiments were carried out taking into account the recommendations of the European Commission on conducting medical-biological research on the use of animals and medical recommendations of the State Pharmacological Center of the Ministry of Health of Ukraine and "Rules for the clinical evaluation of safety of pharmacological agents (GLP)" [8, 14] and the rules of humane treatment of experimental animals (approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsya - Minutes № 1 by 14.01.2010). The 0.9% solution of NaCl were injected into the lower vena cava after its catheterization in aseptic conditions through the femoral vein at a dose of 10 ml/kg body weight of the animal. After each administration of 0.9% solution NaCl, the lumen of the catheter under the skin was filled with titrated heparin solution (0.1 ml of