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Administration of a low molecular fraction (below 5 kDa) from human cord blood as an eye gel component in an experimental model of cornea mechanical damage

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Cornea mechanical trauma leads to apoptosis activation, protein metabolism and glycosaminoglycans (GAGs) synthesis failure, which courses to disorganization of connective tissue, disruption of the balance between biosynthesis and catabolism of collagen in the corneal stroma, resulting in its turbidity. Therefore nowadays the promising direction in ophthalmology is searching new approaches to cure of posttraumatic pathologies of cornea that target to activate mechanisms which ensure not only a full regeneration of corneal structures, but also a qualitative restoration of its transparency as one of the main conditions for the functioning of the visual analyzer.The work is aimed to study the influence of a low molecular fraction (below 5 kDa) isolated from human cord blood (HCBF) as an eye gel component on the expression of apoptotic changes, synthesis of collagen and GAGs in the cornea with the experimental model cornea mechanical damage in rabbits.

The extraction of a fraction containing components with molecular weights below 5 kDa from human cord blood was performed by the ultrafiltration method using a membrane module "Sartorius" (Germany). Once isolated, the fraction was lyophilized and introduced into gel composition. Gel *Actovegin*® (commercial preparation, "Nycomed", Austria) was used as a comparator agent at the concentration 40 mg/ml. The rabbits (Chinchilla, n=30, 60 eyes) with experimental cornea mechanical damage got the following application on the damaged cornea area during 21 days 4 times per day: the 1st group – HCBF-containing gel; the 2nd group - gel *Actovegin*®; the 3rd group (control) – gel-placebo; the 4th group consisted of intact animals. Wound healing action of HCBF-containing gel was studied on the 3rd, 7th, 10th, 14th and 21st days by morphological data (intensity of inflammation and cornea turbidity); histochemical data (sulfated and non-sulphated GAGs were identified by staining of the cornea histological sections with alcian blue by Stidmen method and Hale method); immunohistochemical data (apoptosis index of damaged cornea cells was determined by Brosman method, and quantity of types I and III collagen was determined by monoclonal antibodies labeled with fluorescein isothiocyanate to the respective types of collagens).

Morphological examination of the cornea of rabbits with the mechanical trauma showed that HCBF-containing gel application decreased in inflammatory processes, accelerated the recovery of the corneal frontal epithelium and prevented the development of its vascularization and turbidity. Histochemical study of cornea showed the decreasing in non-sulfated and sulfated GAGs content after mechanical trauma formation in all experimental groups of animals. Normalization of non-sulphated GAGs content was observed 7 days earlier after the administration of HCBF-containing gel as compared to the reference drug *Actovegin*®. Normalization of sulphated GAGs content was determined on the 21st day after HCBF-containing gel application that was not found after *Actovegin*® administration and in the control group. Apoptotic changes studying showed that cornea cell apoptotic index on the 21st day after HCBF-containing gel administration was 2.12±0.01 (norm is 2.03±0.02) that is 43% lower as compared to the reference drug application (3.07±0.05). Immunohistochemical examination of types I and III collagen synthesis showed its normalization on the 21st day after HCBF-containing gel application and this types collagen content was 20.3% and 17.0% respectively lower as compared to *Actovegin*®. It was demonstrated that the application of the HCBF-containing gel caused a normalization of synthesis of GAGs and types I and III collagen, significant reduction in apoptotic index which was accompanied by a decrease in the cornea lesion area, normalization of stroma structure and full restoration of the cornea transparency in shorter terms than the reference drug *Actovegin*® did after mechanical damage. Thus, the low molecular fraction (below 5 kDa) from human cord blood as an eye gel component has a reparative activity and is promising for the development of the preparation based on it.

Biography

Alexander K. Gulevsky is a Professor of Cryobiology and the Lead of the Laboratory of Biochemistry of Cold Adaptation at the Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences in Kharkov, Ukraine. His main field of research is drug discovery, cryobiology and biochemistry. He has published more than 300 papers, several co-edited books, and holds several patents. He has plenty of postgraduate students. He has received several awards including Honored Worker of Science and Technology of Ukraine by the National Academy of Sciences of Ukraine.

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