In Vitro Study of the Role of Lenticular Epithelial Calcium Channels and Aquaporins in the Development of Cataract

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The role of calcium and water channels (aquaporins) in the pathogenesis of cataract was studied *in vitro*. Aquaporin blockade caused opacity of the lens sooner than changes in calcium ion concentration in culture medium.

Key Words: *lens; cataractogenesis; aquaporins; calcium channels*

Numerous methods for *in vitro* studies of cataract were described. The lens remains transparent only if its structure is preserved, which depends on various factors (physicochemical status of lenticular proteins, membrane lipids, balanced functioning of enzymate systems, water electrolyte balance of the lens). If one of these parameters is impaired, lens opacity develops. Classical models of cataract simulation are traumatic injury, exposure to ionizing radiation, lens culturing in medium with high content of carbohydrates (glucose, galactose, xylose), the so-called "sugar" or diabetic cataract, exposure of the lens to chemical agents toxic for lenticular cells and fibers (naphthalene, dinitrophenol, antimitotic agents, enzyme inhibitors, calcium chloride, hydrogen peroxide, mercuric salts).

Cataract development can be studied *in vitro* in an organ culture. We cultured rat lenses in normal saline. This method for the study of cataractogenesis attracts attention due to easy evaluation of changes in the lens and possibility of evaluating the cataractogenic activity of different substances after their addition to the culture medium.

Disorders in the content of calcium ions in lenticular cells play an important role in the pathogenesis of cataract. It is known that in 75% patients with cataract the content of Ca ions in lenses is 4-fold higher than in normal subjects [3]. Calcium enters the lens through cationic channels and its elimination is mediated by Ca-ATPase of the sarco- and endoplasmic reticulum and plasma membrane. The increase in calcium entry into the lens with age is compensated for by hyperactivity of Ca-ATPase pumps. However, membrane permeability for calcium drastically increases during the development of senile cataract, while activity of Ca-ATPase decreases by about 50%, which leads to a significant increase in Ca content in the lens. Reduction of Ca-ATPase activity in diabetic cataract is presumably caused by disorders in lipid membrane structure or hyperoxidation of this enzyme or both these mechanisms. Elevation of Ca content leads to a series of pathological processes: protease (calpains 1 and 2, calpastatin) activation, Na,K-ATPase inhibition, cell growth, synthesis of pathological proteins, impairment of normal spatial protein structure, increase in Ca entry into the cell, apoptosis, and membrane permeability. High concentration of Ca ions impairs the work of sodium pumps and exhibits a direct cytotoxic effect on this structure [1]. All these factors lead to impairment of the molecular structure of the lens and result in its opacity [4,8,10-13]. According to recent data, reduction of Ca content in the lens also causes cataract, because it is linked with increase of water channel permeability, which inevitably leads to opacity [7].

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Water balance between the cytoplasm and extracellular space of fiber cells is essential for the maintenance of lens transparency. The system of humor circulation between the capsular epithelium and crystallines is important for normal functioning of lenticular structures. At the molecular level the regulation of epithelial humor transport is realized by aquaporins (plasma membrane proteins acting as water channels) [5]. AQP0 Aquaporin (MIP26 is the main membrane integral protein) is detected on fiber cells of the lens and AQP1 on epithelial cells of the anterior pole of the lens [5,6]. It is proven that impairment of aquaporin structure leads to lens opacity. Congenital cataract develops in mutation of genes encoding AQP0 synthesis [3]. Development of senile cataract is also linked with AQP0 dysfunction [9].

We studied cataractogenesis *in vitro* by blocking calcium and water channels (aquaporins) and by increasing the content of calcium ions in culture medium.

MATERIALS AND METHODS

The study was carried out on 155 lenses of adult outbred rats of both sexes. The animals were narcotized with ether. The lenses were isolated under sterile conditions via the posterior access under a binocular magnifying glass. The eyeball was open, the vitreous body was removed, Zinn's ligaments were crossed, and the lens was accurately mobilized without damaging the capsule. The lenses with mechanical injuries or capsular defects were not taken into the study.

Culturing was carried out in sealed tubes in 5 ml 0.9% sterile NaCl with 80 mg/liter gentamicin sulfate at 37°C. The content of $CaCl_2$ in the medium was 0.7 mM. Cataractogenesis inductors were added into nutrient medium at the start of culturing. The lenses were divided into 5 experimental groups with different culturing conditions: group 1 (n=31; control): 5 ml 0.9% sterile NaCl with 80 mg/liter gentamicin sulfate; group 2 (n=31): 5 ml 0.9% sterile

TABLE 1. Comparative Evaluation of the Period of Lens Opacity under Different Conditions of *In Vitro* Culturing (n=31)

Group, parameter	Day of opacity manifestation		
	2nd degree	3rd degree	4th degree
Control (saline)			
Me	2	5	6
Q	(2; 4)	(4; 6)	(5; 8)
X_{min}	1	2	4
X_{max}	6	8	12
Saline and $CaCl_2$ (15 mM)			
Me	1	3	4
Q	(1; 2)	(2; 3)	(3; 5)
X_{min}	1	2	3
X_{max}	2	4	4
Saline and verapamil (1.5×10^{-2} mM)			
Me	2	4	4
Q	(2; 2)	(3; 5)	(3; 6)
X_{min}	1	3	3
X_{max}	5	8	8
Saline, $CaCl_2$ (15 mM), and verapamil (1.5×10^{-2} mM)			
Me	1	2	3
Q	(1; 1)	(2; 2)	(2; 4)
X_{min}	1	1	1
X_{max}	2	3	7
Saline and $HgCl_2$ (0.3 mM)			
Me	1	2	2
Q	(1; 1)	(1; 2)	(2; 2)
X_{min}	1	1	1
X_{max}	2	2	3

Note. Me: median; Q: interquartile range (25%; 75%); X_{min} and X_{max}: minimum and maximum ranges of the sample.

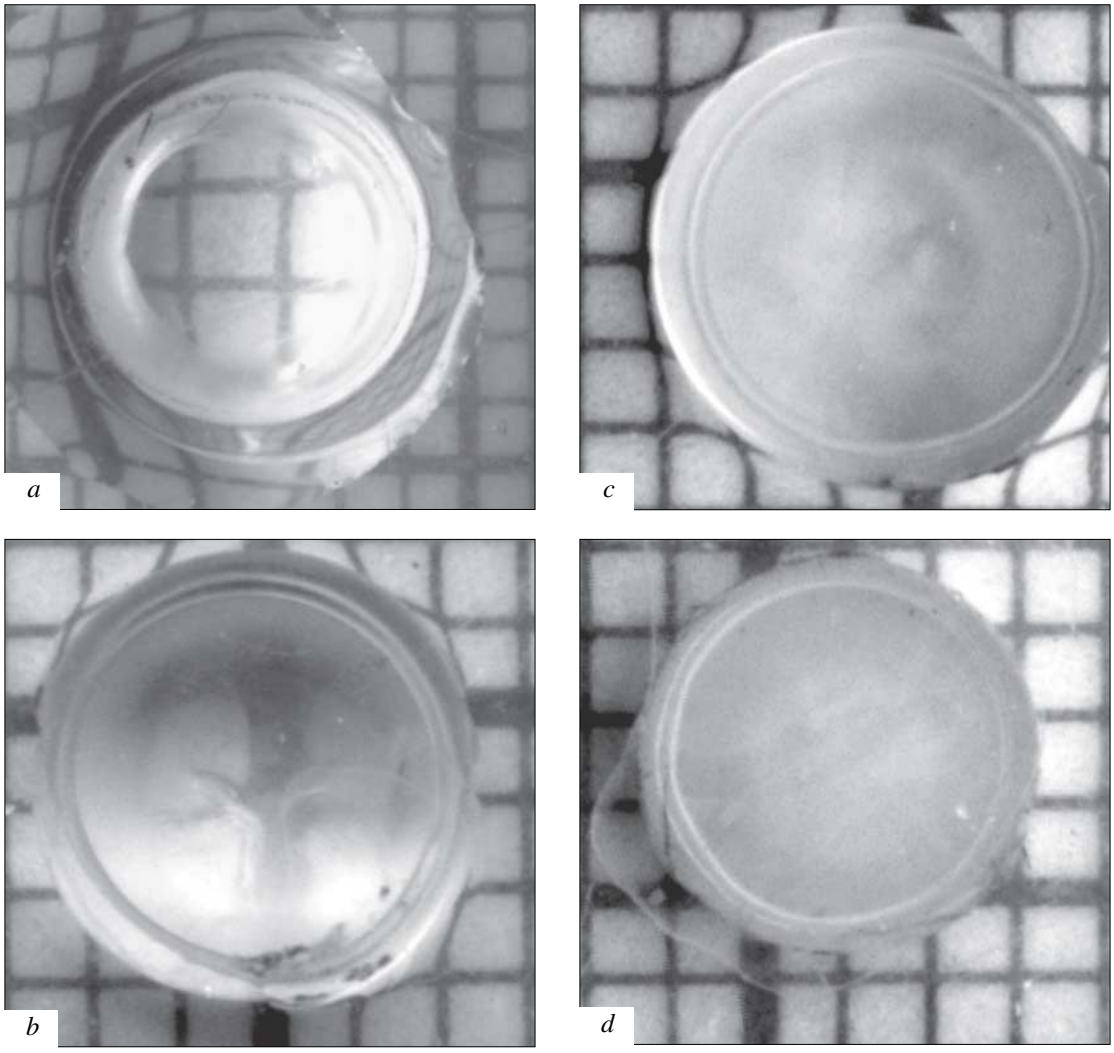

Fig. 1. Degree of rat lens opacity. *a*) grade I: intact (transparent) lens; *b*) grade II: initial opacity; *c*) grade III: intermediate opacity; *d*) grade IV: complete opacity.

NaCl with 80 mg/liter gentamicin sulfate and 15 mM $CaCl_2$; group 3 (n=31): 5 ml 0.9% sterile NaCl with 80 mg/liter gentamicin sulfate and 1.5×10^{-2} mM verapamil (calcium channel blocker); group 4 (n=31): 5 ml 0.9% sterile NaCl with 80 mg/liter gentamicin sulfate, 15 mM $CaCl_2$, and 1.5×10^{-2} mM verapamil; and group 5 (n=31): 5 ml 0.9% sterile NaCl with 80 mg/liter gentamicin sulfate and 0.3 mM $HgCl_2$ (water channel blocker).

Lens opacity was evaluated visually using a lined paper sublayer. Complete opacity of the lens was diagnosed by when the lines on the sublayer, placed under the lens, were not seen (Fig. 1).

The samplings obtained in the experiment were verified using Kolmogorov—Smirnov, Shapiro—Wilk, and Epps—Pally tests. Statistical data processing revealed that the distribution of resultant values was not normal. The differences between the independent samples were detected using Kolmogorov—Smirnov, Mann—Whitney, and Kramer—Welch nonparametric tests at 95% confidence probability.

RESULTS

Comparison of the results in the samples at 95% confidence probability showed that addition of cataractogenesis inductors into the culture medium caused rapid development (in comparison with the control) of lens opacity (Table 1). Blockade of Ca channels with verapamil in the presence of normal concentration of Ca ions in the medium (0.7 mM) also accelerated lens opacity compared to the control (complete opacity was observed on day 4). This effect of verapamil can be attributed to deficit of Ca ions created in the lens, which modulates the intensity of the metabolic processes, because calcium acts as activator of enzyme systems. As a

result, lens opacity develops. Rapid development of lens opacity after simultaneous addition of 15 mM $CaCl_2$ and verapamil is due to high gradient of calcium ions at the interface of the incubation medium—lens capsule epithelium—crystallines of lens nucleus surface layers rather than to calcium channel blocking. Passive transport of Ca^{2+} into liquid medium between the lens capsule epithelium and crystallines is stimulated under these conditions.

Presumably, blockade of calcium channels leads to disorders in water exchange [7]. However, water channels play a more important role in the development of lens opacity. Mercuric chloride (aquaporin blocker) in a concentration of 0.3 mM sharply accelerated the development of lens opacity: initial opacity was noted during day 1 of culturing and complete on day 2 (Table 1). This effect of 0.3 mM mercuric chloride is regarded as the effect of a functional blocker, but not as a toxic effect on the tissue realized at a concentration of 0.7 mM. In addition, we showed that the blocking effect of 0.3 mM mercuric chloride on aqueous channels is reversible and could be arrested with mercaptoethanol [2].

Hence, these data indicate that dysfunction of the channels realizing water and calcium exchange through the lenticular epithelial layer plays the key role in cataract development. These data are particularly important for the genesis of senile cataract, because activity of water-electrolyte metabolism in tissues, including the lens, decreases significantly with age.

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