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Ultrastructural changes of ciliary epithelial nucleus in experimental diabetes – an animal model

Diabetes, the fourth leading cause of death in most countries, is a chronic condition affecting 154 million people worldwide, according to the World Health Report 2000 (14). It is the disease spreading mostly in developed countries. Every second person over 50 or even 40 suffers from diabetes mellitus. In Europe there are 35 million diabetic patients. In Poland about one million people suffer from diabetes, and another one million have non-diagnosed diabetes (14). Independently of the type of diabetes – it is general metabolic disorder. Diabetic vascular abnormalities in adults often result in blindness and visual disability (9,14).

Ciliary body is a middle part of the uvea, between the iris and the choroid. It is a ring surrounding and supporting eye lens. Ciliary body divides anterior and posterior part of the eyeball. Morphologically we can differentiate pars plana and pars plicata – with many ciliary processes of the ciliary body. Histologically it can be divided into the muscle, stroma and epithelium. Ciliary epithelium is bilayered. Cuboidal epithelial cells situated near the stroma, contain melanin and play metabolic functions. They are called pigmented ciliary epithelium – PE. The second layer is non-pigmented ciliary epithelium – NPE. It consists of columnar cells connected with aqueous humour of the eye. They play an important role in the production and regulation of this ocular fluid. NPE cells make the blood-aqueous barrier (1–5, 8, 10–13). The blood–aqueous eye barrier (BAB) consists of two components: one is in the ciliary epithelium and protects the posterior chamber from circulating macromolecules, the second is an endothelial barrier which prevents movement of macromolecules from the blood vessels into the anterior chamber (10–13).

The aim of this study was to observe, in electron microscope, the New Zealand rabbits ciliary epithelial nucleus structure changes, depending on the duration of experimental diabetes mellitus.

MATERIAL AND METHODS

During the experiment we used an animal (New Zealand rabbit) model. The rabbit eye is histologically very similar to the human one. Metabolic processes are the same or approximate (7–9). Fifteen eyes of fifteen adult New Zealand (males) rabbits (body mass 2.880 g ± 400 g) were studied. Three eyes of healthy animals were enucleated as a control group. The animals were administered alloxan (Sigma, St. Louis, USA) in the form of 10% solution in 0.9% NaCl at the dose of 100 mg/kg b.m. Alloxan was injected into the ear marginal vein. Glycemia was measured after 7 days. If the level was 11 mmol/l or higher the animal was included in the experimental group. There were four groups of alloxan diabetes rabbits. Duration of diabetes varied: 3 weeks, 6 weeks, 3 months, 6 months. The anterior segment of each eye was cut meridionally into sectors of about 1mm thickness. The ciliary bodies were carefully dissected from the anterior segment. After buffering and staining in 1% osmium tetroxide solution, ultrathin 4μ sections were ultramicrotome cut, and after the aqueous solution of uranyl acetate contrasting, were observed in the electron microscope TESLA BS–500. The conventional electron microscopy was used to show changes of NPE and PE cells ultrastructure depending on experimental diabetes.

RESULTS

There were four groups of rabbit diabetic eyes. The control group included three eyes of healthy rabbits – without any ultrastructural changes in ciliary epithelium nucleus: smooth nuclear membrane, regularity in nuclear chromatin location. In group I (3 weeks of experimental diabetes) we noted deep indentations of nuclear membrane, a small amount of heterochromatin in NPE cells (Fig. 1). In group II (6 weeks of experimental diabetes) we observed very small nucleus, irregularity of nuclear heterochromatin distribution (Fig. 2). In group III (3 months of experimental diabetes) there were evident ultrastructural changes – especially in ciliary epithelium: flattened PE nucleus with open nuclear membrane pores. In group IV (6 months of experimental diabetes) changes of the ciliary epithelial nucleus were found. Nuclei were small, with open pores and indentations of the nuclear membrane observed (Fig. 3).

Fig. 1. TEM x 10000. Group I (3 weeks of diabetes). NPE cell – deep indentations of nuclear membrane (arrowhead), small amount of heterochromatin

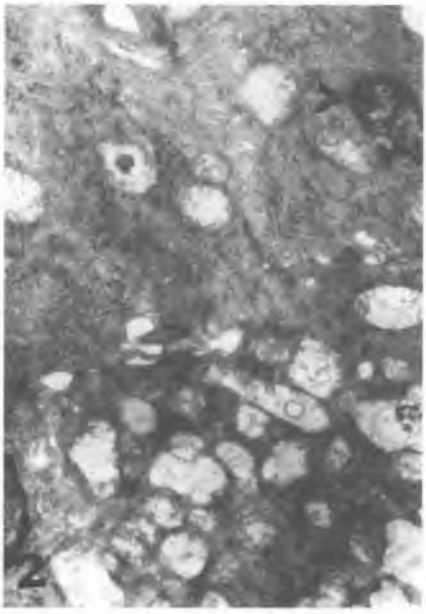

Fig. 2. TEM x 10000. Group II (6 weeks of diabetes). PE cells – small nuclei, irregularity in heterochromatin location

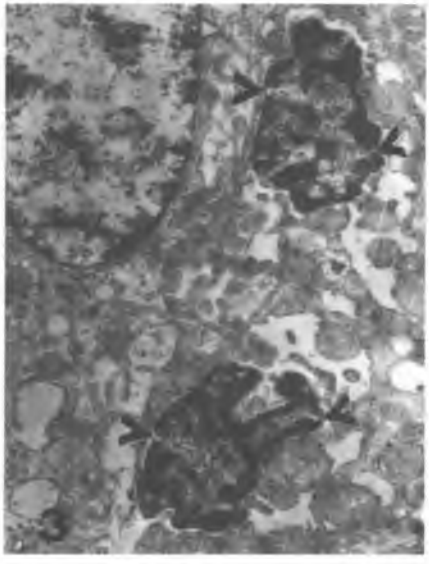

Fig. 3. TEM x 10000. Group IV (6 months of diabetes). Ciliary epithelium – small nuclear sizes, indentations of nuclear membrane, open pores (arrowheads)

DISCUSSION

In the living organism barriers are localised in the epithelium or the vascular endothelium – in a layer of cells that separates two different compartments of the body. Blood - aqueous barrier (BAB) in the eye consists of two compartments (1, 3, 10, 11, 13). BAB is a complex of epithelial and endothelial parts. Ciliary and iridial epithelia protect the posterior chamber and endothelial part prevents the movement of macromolecules to the anterior chamber. The main structures of the BAB are iris and ciliary body. Ciliary processes actively secrete aqueous humour – a clear, colourless fluid. The ciliary processes are covered by a bilaminar neuroepithelium. Ciliary body epithelium is thought to be responsible for the secretion of aqueous humour in the eye. The ciliary epithelium is composed of two cell layers, an outer pigmented layer – PE (pigmented epithelium), whose basal surface lies adjacent to the stroma of the ciliary processes and an inner non-pigmented layer – NPE (non-pigmented epithelium) whose basolateral membrane faces the posterior and vitreal spaces of the eye (1–5, 8, 10, 11, 13).

The bases of NPE cells line the posterior chamber and the bases of PE cells rest on the stroma of the ciliary body. The PE layer is continuous posteriorly with the pigmented epithelium of the retina and anteriorly with the anterior epithelium of the iris. The NPE layer is continuous with the neural retina at the ora serrata and anteriorly with the posterior epithelium of the iris. The most important component of BAB is the junctions between adjacent ciliary epithelial cells. The special type of intracellular junctions is zonulae occludentes. The zonula occludens between NPE cells represents the permeability barrier that prevents diffusion of macromolecules into the aqueous humour and is the main part of the blood – the aqueous barrier (10, 12, 13).

The diabetes mellitus is a very common disease. We have extensive information about metabolic disturbances and histological changes e.g. in connective tissue, blood vessels of kidneys or retina. There are many ultrastructural changes we can observe in blood during electron microscope study – tissue barriers, intercellular junctions, cytoplasm, endoplasmic reticulum, nucleus.

CONCLUSIONS

In the experimental diabetes severe disturbances of ciliary epithelium nucleus ultrastructure were observed. Depending on diabetes mellitus duration we found: deep indentations of nuclear membrane, open nuclear pores, irregularity in heterochromatin location and small sizes of the epithelial nucleus.

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SUMMARY

The aim of this study was to observe in electron microscope the New Zealand rabbits ciliary epithelial nucleus structure changes, depending on the duration of experimental diabetes mellitus. The animals were administered alloxan in the form of 10% solution in 0.9% NaCl, at the dose of 100 mg/kg b.m. If the glycemia level was 11 mmol/l or higher the animal was included in the experimental group. 15 eyes of 15 adult New Zealand rabbits were studied. Conventional electron microscopy was used to show ultrastructural changes of ciliary epithelium nucleus. During the study we noticed severe changes in the structure of ciliary epithelial nucleus depending on diabetes mellitus duration. We found: deep indentations of nuclear membrane, open nuclear pores, irregularity in heterochromatin location and small sizes of the epithelial nucleus.

Zmiany ultrastruktury jądra komórkowego epithelium ciała rzęskowego w przebiegu cukrzycy eksperymentalnej u zwierząt

Przedmiotem pracy są oceniane w mikroskopie elektronowym badania zmian w ultrastrukturze ciała rzęskowego gałki ocznej w przebiegu cukrzycy doświadczalnej, w różnych okresach trwania choroby u 15 królików rasy New Zealand. Zwierzętom podawano Alloxan w dawce 100 mg/kg m.c. w postaci 10-procentowego roztworu w fizjologicznym NaCl. Grupę badaną stanowiły zwierzęta, u których glikemia wyniosła 11 mmol/l lub więcej. Do oceny zależnych od czasu trwania cukrzycy zmian w jądrach nabłonka ciała rzęskowego zastosowano konwencjonalną mikroskopię elektronową. W badaniu zaobserwowano znaczne zmiany w ultrastrukturze jąder komórkowych. W zależności od czasu trwania cukrzycy występowały: głębokie wpuklenia błony jądrowej, rozszerzenia porów jądrowych, nieregularności w kondensacji chromatyny jądrowej, zmniejszenie wielkości i zmiany piknotyczne jąder komórkowych.