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Influence of the new medicine – 2-CDA (Cladribine) on the ultrastructure of the extrabulbar segment of the optic nerve in rabbits of New Zealand breed

2-CDA (Cladribine) acts on resting cells. It disturbs metabolism in cells with low metabolic activity leading to an exhaustion of the reserves of high energetic compounds (12). Cladribine has a particular affinity for lymphoid cells. It is a new drug from the group of purine analogs. It is incorporated in the place of regular nucleotides to the chain of DNA, thus disturbing its synthesis in rapidly proliferating cells. Cladribine is the drug widely applied in treatment of leukaemias (9, 10). Its immunosuppressive properties contributed to the application of this medicine in the experimental treatment of multiple sclerosis (4, 8, 11).

The purpose of the research was the estimation of the influence of Cladribine on morphology of extrabulbar segment of the optic nerve after the administration of this medicine in recommended doses to experimental animals.

MATERIAL AND METHODS

The experiment was carried out on the rabbits of New Zealand breed weighting about 3 kg. Rabbits received water and standard granulated fodder *ad libitum*. Animals were divided into two groups: one control and one experimental. The control group included animals receiving subcutaneously 0.9% NaCl. The experimental group included animals receiving subcutaneously Cladribine in the dose 0.07 mg/kg/24h each morning for 6 days, three cycles with 5-week intervals, which corresponds to the schema of the experimental

treatment in multiple sclerosis (4). After 24 hrs from the last 0.9% NaCl dose in the control group and Cladribine dose in the experimental group the rabbits were killed and specimens of the optic nerve were collected for ultrastructural examinations. The obtained tissue material was fixed in glutaraldehyde and OsO_4 and embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate according to the Reynold's method. Observations and pictures were taken in Tesla BS-500 transmission electron microscope.

RESULTS AND DISCUSSION

The observations of slides of extrabulbar segment of the optic nerve in electron microscope revealed nerve fibers arranged in bundles of different thickness. Regular layers of myelin sheaths surrounding axons were visible. Axons and myelin sheaths were regular in shape. Normal mitochondria were observed within nerve fibers. Longitudinal rows of oligodendrocytes were located within bundles of nerve fibers between axons. Ultrastructural examinations of extrabulbar segment of the optic nerve in the experimental group indicate the similarity in comparison with the morphological picture observed in the control group. Evident morphological changes of nerve fibers in the experimental group were not observed. Their structure (Fig. 1) was similar to the control group. The cell nuclei of oligodendrocytes in slides from the experimental group possessed similar struc-

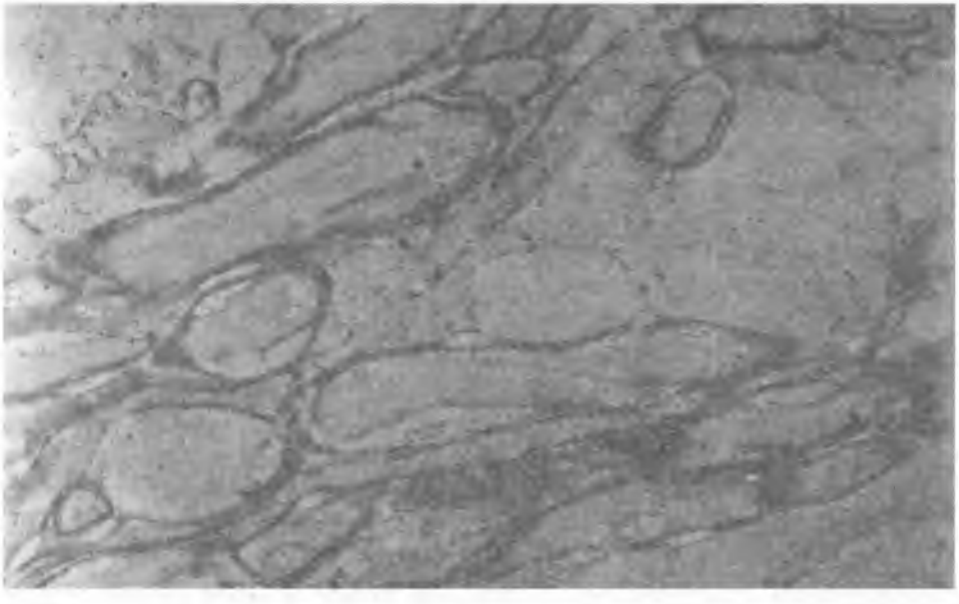

Fig. 1. Experimental group. Extrabulbar segment of the optic nerve – the structure of nerve fibers. Magn. about 4000x

ture as the control group. The nuclei of oligodendrocytes and microglia possessed equally dispersed chromatin forming electron dense aggregations located within the whole nucleus (Fig. 2).

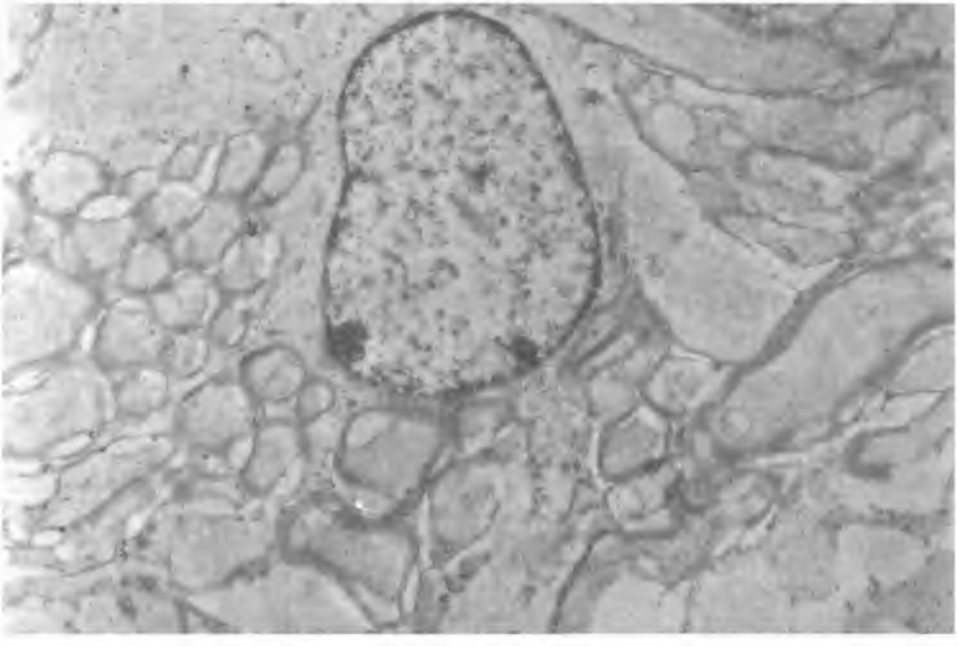

Fig. 2. Experimental group. The nucleus of oligodendrocyte with equally dispersed chromatin. Magn. about 4000x

The purpose of performed ultrastructural examinations was to show the possible negative influence of Cladribine on extrabulbar segment of the optic nerve after the administration of the medicine in experimental animals. Only Matyja et al. research work that regarded the organotypic cultures of hippocampal and cerebellar cells was published (6). These cultures were obtained from rats 1–3 days after birth. Their cells in different stages of development were exposed to 2-CDA and 2-BDA in the concentrations up to 10 μM for 10 days. The normal pattern and dynamics of differentiation of nerve and glial cells were observed in the light and electron microscopes. This research revealed that the examined substances did not cause disturbances of the cell ultrastructure and that 2-CDA and its daughter substance do not show the cytotoxic effects on normal rat tissue of central nervous system (6). Observations of Cheson et al. indicate that 2-CDA administered in recommended doses shows moderate and reversible neurotoxicity in 15% of patients (1). Djaldetti et al. led the electron microscope examinations on the effect of the action of high doses of 2-CDA on the mouse peripheral nervous system (2). Schwann cells of myelinated and nonmyelinated nerve fibers were investigated. Two experimental

groups receiving Cladribine intravenously for 7 days were formed: the first group received 1 mg/kg/24h, the second group – 0.5 mg/kg/24h. Schwann cells of both myelinated and nonmyelinated nerve fibers of animals receiving higher doses of 2-CDA showed the damage of nucleus and nucleolus, the decrease of the amount of heterochromatin, vacuolization and desorganization of myelin sheaths. Schwann cells of animals treated with the smaller doses were not damaged. Authors concluded that Cladribine can cause peripheral neuropathy damaging Schwann cells only in doses significantly higher than therapeutic (2). This is consistent with our observations in which experimental group did not show morphological changes.

Scientists reported the optic nerve toxicity of many antineoplastic and immunosuppressive drugs. Green observed the swelling of mitochondria and axons, the presence of irregular and wrinkled myelin sheaths and complete loss of axons in the optic nerve after the administration of 0.1 ug Vincristine (3). Kaiser-Kupfer et al. present the secondary axonal degeneration of retinal axons caused by the therapy with high-doses of Tamoxifen (5). Mayer et al. investigated the influence of dexamethasone and corticosterone on the morphology of the rat optic nerve. Using autoradiographic methods they suggested that glial cells are target cells for glucocorticosteroids. The interactions on the cell surface may strongly influence the expression of regulative functions of glucocorticosteroids through the changes of the amount and functions of cytoplasmic receptors for glucocorticosteroids (7).

CONCLUSIONS

1. Ultrastructural examinations of the extrabulbar segment of the optic nerve show that Cladribine administered according to the schedule of the experimental treatment in sclerosis multiplex does not cause the damage of the optic nerve in experimental animals

2. The achieved results suggest the necessity of new research works regarding the eye structures of mesenchymal origin.

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SUMMARY

Experiments were carried out on the rabbits of New Zealand breed weighting about 3 kg. Rabbits from the experimental group received Cladribine in the dose of 0.07 mg/kg/24h each morning subcutaneously for 6 days, three cycles with 5-week intervals. Specimens of the optic nerve were stained according to the Reynold's method and observed in Tesla BS-500 transmission electron microscope. Results achieved from examinations of slides in experimental group indicate that Cladribine administered in the dose corresponding to therapeutic dose used in humans for experimental treatment of sclerosis multiplex does not cause the damage of extrabulbar segment of optic nerve in experimental animals. The achieved results suggest necessity of new research works regarding the eye structures of mesenchymal origin.

Wpływ nowego leku immunosupresyjnego - 2-CDA (Kladrybina) na ultrastrukturę odcinka pozagałkowego nerwu wzrokowego królików rasy nowozelandzkiej

Badanie wykonano na królikach samicach rasy nowozelandzkiej o masie ciała ok. 3 kg. Zwierzętom grupy doświadczalnej podawano Kladrybinę w dawce 0,07 mg/kg m. c. /dobę przez 6 dni, co 5 tygodni w 3 cyklach. Do badań pobierano fragmenty nerwu wzrokowego. Ultracienkie skrawki barwiono według metody Reynoldsa i badano w mikroskopie elektronowym Tesla BS-500. Wyniki uzyskane z badań preparatów grupy doświadczalnej wykazują, że podanie Kladrybiny w ilości odpowiadającej dawce leczniczej stosowanej u człowieka w eksperymentalnej terapii stwardnienia rozsianego nie powoduje uszkodzenia struktur odcinka pozagałkowego nerwu wzrokowego u zwierząt doświadczalnych. Uzyskane wyniki skłaniać mogą jednak do podjęcia dalszych badań w obrębie struktur oka pochodzenia mezenchymatycznego.