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Ultrastructural changes of retinal ganglion cells in rabbits of New Zealand breed after experimental administration of 2-CDA (Cladribine)

2-chloro-2'deoxyadenosine exerts toxic influence on many malignant cell lines derived from myeloid and lymphoid cells (T and B-types) (1, 3). The medicine is the most effective in the treatment of lymphoid neoplasm: hairy-cell leukemia, chronic lymphocytic leukemia and non-Hodgkin lymphoma (3, 7, 8, 10). The suppression of the immune system during the treatment of malignant neoplasm causes serious infectious side effects, but it may be useful in the treatment of diseases caused by an excessive immune system stimulation.

The aim of the performed experiments was to show the negative influence of Cladribine on morphology of retinal ganglion cells after administration of the medicine to experimental animals.

MATERIAL AND METHODS

The experiment was carried out on rabbit females of New Zealand breed weighing about 3 kg. The rabbits received water and standard granulated fodder *ad libitum*. Animals were divided into two groups: one control and one experimental group. The control group included animals receiving 0.9% NaCl (subcutaneously) in the appropriate dose. The experimental group included rabbits receiving Cladribine in the dose of 0.1 mg/kg which corresponds to the schema of the experimental treatment in the hairy cell leukemia. The medicine was administered everyday morning for 7 days s.c. in the disinfected left lateral skin fold on the level of lumbar vertebral region (6). After 24 hrs from the last dose of 0.9% NaCl in the control group and the last dose of Cladribine in the

experimental group the rabbits were killed and specimens of the retina adjacent to optic disc 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

Electron microscope examinations of slides from the control group reveal the structure of ganglion cell perykarions. Nerve cell bodies are regular in shape. Their nuclei are large, clear and rounded. Equally dispersed chromatin fills the nuclei interior. It forms small, electron dense granules equally dispersed within the whole nucleus. The nucleolus often located eccentrically is visible. The nuclear envelope consists of two membranes separated by the perinuclear cisternae. The external membrane of the nuclear envelope possesses ribosomes. Delicate indentation of the nuclear envelope into the interior of the nucleus is observed in some ganglion cells. The nuclear pores are visible. Cell organelles such as endoplasmic reticulum, mitochondria and lysosomes are visible in the neuronal cytoplasm. Electron microscopic examinations of retinal ganglion cells reveal slight changes in comparison with the control group. The majority of ganglion cells shows a regular morphological structure of the nucleus and cell organelles: mitochondria, endoplasmic reticulum and lysosomes in comparison to the control group (Fig. 1). The inden-

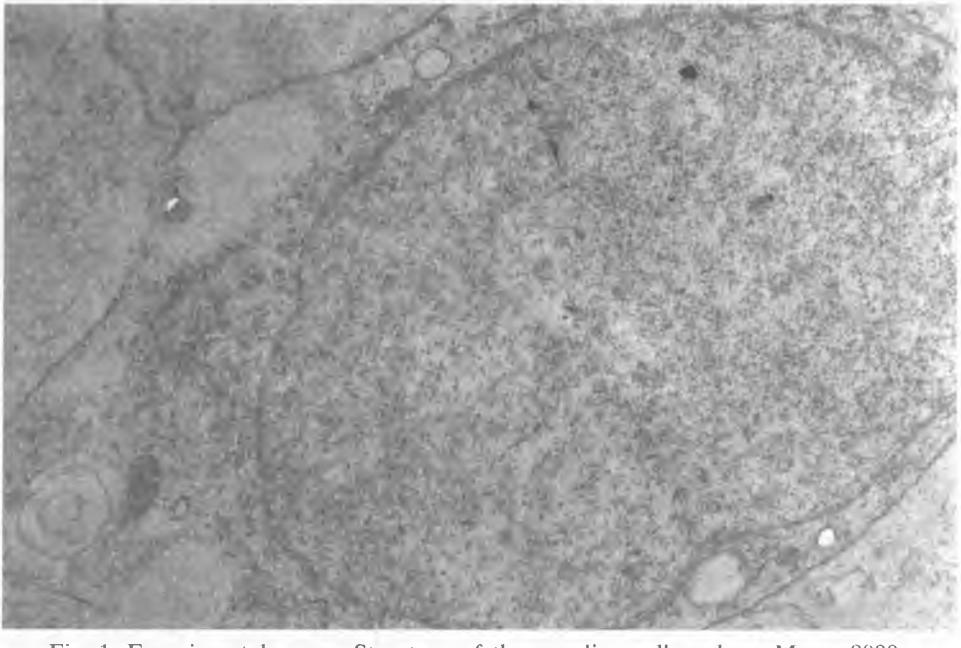

Fig. 1. Experimental group. Structure of the ganglion cell nucleus. Magn. 8000x

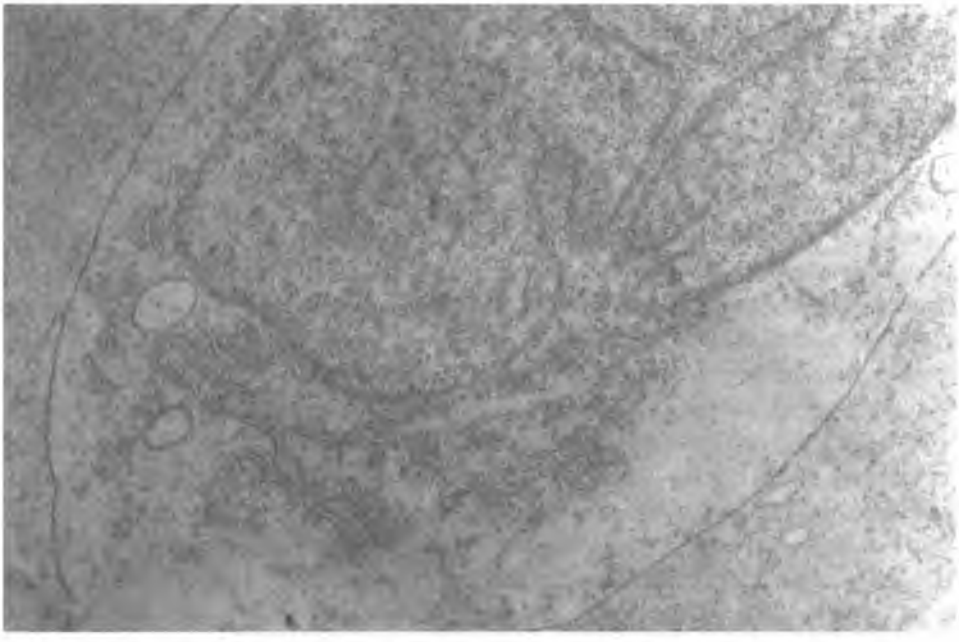

Fig. 2. Experimental group. Ganglion cell – numerous indentations of nuclear envelope into the interior of the nucleus. The swelling of the mitochondria. Magn. 8000x

tation of the nuclear envelope into the interior of the nucleus is observed during the histological analysis of the cell nucleus (Fig. 2). This alteration has various intensity. The mitochondrial swelling is visible in single neurons (Fig. 2).

Performed ultrastructural examinations revealed evident but not intensive changes in single ganglion neurons in comparison with the control group. Morphological changes of mitochondria having the character of the organelle swelling were observed. The disturbances in structure of these organelles can be connected with the disturbances in the ionic balance (4). The influence of the Cladribine on some respiratory enzymes connected with the redox system which can lead to disturbances of the oxygen mechanism cannot be excluded because the depletion of cell NAD stores appears as the result of an action of the medicine. NAD is the coenzyme responsible for the transport of hydrogen ions from the carboxylic acid cycle on the mitochondrial respiratory chain (11). Its depletion leads to an inhibition of ATP synthesis in the respiratory chain and to the breakdown of the cell energetic processes (3).

An indentation of the nuclear envelope to the interior of the nucleus was observed during examinations of the cell nucleus ultrastructure in the experimental group. This morphological change was of various intensity, which can suggest a decrease in the cell metabolism due to disturbances in the DNA structure by an incorporation of Cladribine into its chain.

The available literature does not present papers concerning the influence of Cladribine on the organ of vision. Matyja et al. studied the effects of the exposition of the organotypic human malignant glial-like cell cultures on the 2-chloro-2'-deoxyadenosine and related 2-bromo-2'- deoxyadenosine. The cell cultures were derived from the surgical biopsy of ten gliomas. After 6-10 days of the growing *in vitro*, the cultures were incubated with 2-CDA and 2-BDA in concentrations 0.3-10 μM for 1 to 10 days. They observed swelling of mitochondria and mitochondrial crista atrophy which were dependent on the dose and the term of an exposition. The signs of cytotoxicity appeared only in the high-anaplastic cells. They did not reveal the differences between the effect of 2-CDA and 2-BDA (5).

The toxicity of many cytostatic and immunosuppressive medicines was observed. Green demonstrated the atrophy of retinal ganglion cells with mitochondrial swelling and the depletion of cell organelles due to the intravitreous administration of vincristine in the dose of 0.1 μg. The large cysts bordered on the processes of Muller's cells which were filled with the cell remnants were visible at the place of ganglion cells. Morphological changes included the neuronal swelling, a depletion of organelles and microtubules and an accumulation of fibro-granular material (2). However, Szymankiewicz-Rak et al. observed the toxic influence of glucocorticosteroids in the concentration higher than 125 μg/ml resulting in the damage of nerve fibers, the synapse depletion and a compensatory formation of new synaptic structures (9).

CONCLUSIONS

1. Cladribine administered in therapeutic doses used in therapy of hairy cell leukemia causes morphological changes in ultrastructure of retinal ganglion cells which are classified as reversible in cytophysiology.
2. The presence of even such slight changes in nerve structures of the eye inclines to the undertaking detailed examinations of other eye structures.
3. Regular ophthalmological examinations are indicated during Cladribine treatment.

REFERENCES

1. Avery T. L. et al.: Biochemical pharmacology of 2-chlorodeoxyadenosine in malignant human hematopoietic cell lines and therapeutic effects of 2-bromodeoxyadenosine in drug combinations in mice. Cancer Res., 4972, 49, 1989.

2. Green W. R.: Retinal and optic nerve atrophy induced by intravitreous Vincristine in the primate. Trans. Am. Ophthalmol. Soc., 73389, 1976.
3. Góra-Tybor J., Robak T.: Farmakologia kliniczna 2-chlorodeoksyadenozyny (Kladrybiny). Przegl. Lek., 614, 53, 8, 1996.
4. Kawiak I. et al.: Podstawy cytofizjologii. PWN, Warszawa 1997
5. Matyja E. et al.: Mitochondrial damage following exposure of organotypic cultures of human malignant gliomas to 2-chloro- and 2-bromo-2-deoxyadenosine. Folia Neuropath., 31, 34, 1, 1996.
6. Robak T. et al.: 2-chlorodeoxyadenosine (2-CdA) in 2-hour versus 24-hour intravenous infusion in the treatment of patients with hairy cell leukemia. Leuk. Lymph., 107, 22, 1996.
7. Robak T. et al.: 2-chlorodeoksyadenozyna w leczeniu nowotworów układu chłonnego – wyniki wieloośrodkowych badań klinicznych w Polsce. Acta Haematol. Pol., 105, 28, 2, 1997.
8. Saven A. et al.: Long-term follow-up of patients with hairy cell leukemia after Cladribine treatment. Blood, 1918, 92, 6, 1998.
9. Szymankiewicz-Rak B. et al.: Glucocorticoid effects on axonal regeneration of retinal ganglion cells *in vitro*. Restor. Neurol. Neurosci., 83, 6, 2, 1994.
10. Tallman M. et al.: Treatment of hairy-cell leukemia: Current views. Sem. Hematol., 155, 36, 2, 1999.
11. Zhu Ch. et al.: Enhanced cytotoxicity of nucleoside analogs by overexpression of mitochondrial deoxyguanoside kinase in cancer cell lines. J. Biol. Chem., 14707, 273, 24, 1998.

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SUMMARY

The experiment was carried out on rabbit females of New Zealand breed weighing about 3 kg. The animals from the experimental group received Cladribine in the dose of 0.1 mg/kg for 7 days. Specimens of retina were collected for ultrastructural examinations. It was revealed that administration of Cladribine in the dose corresponding to the therapeutic dose used in human for the treatment of the hairy cell leukemia causes morphological changes in ultrastructure of retinal ganglion cells which are classified as reversible in cytophysiology. The necessity of periodic, regular ophthalmological examinations during Cladribine treatment was indicated.

Zmiany ultrastrukturalne komórek zwojowych siatkówki królików rasy nowozelandzkiej po doświadczalnym podaniu 2-CDA (Kladrybina)

Badania wykonano na królikach samicach rasy nowozelandzkiej o masie ciała ok. 3 kg. Zwierzętom grupy doświadczalnej podawano Kladrybinę w dawce 0,1 mg/kg m.c./dobę przez 7 dni. Do oceny histologicznej w transmisyjnym mikroskopie elektronowym pobierano fragmenty siatkówki. Stwierdzono, że podanie Kladrybiny w ilości odpowiadającej dawce leczniczej stosowanej u człowieka w terapii białaczki włochatokomórkowej wywołuje zmiany w budowie ultrastrukturalnej komórek zwojowych siatkówki, zaliczane w cytofizjologii do odwracalnych. Wskazano również na potrzebę okresowych, regularnych badań okulistycznych.