ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LVII, N 2, 137 SECTIO D 2002

Department of Histology and Embryology with the Lab of Experimental Cytology
Medical University of Lublin, Department of Histology and Embryology
Agricultural University of Lublin

MAŁGORZATA KWIETNIEWSKA, REGINA CYBULSKA,
PIOTR KWIETNIEWSKI, JOANNA SEKITA-KRZAK,
KRYSTYNA CZERNY

# *Histological examination of the retinal ganglion layer in rabbits after experimental administration of the new immunosuppressive medicine – Cladribine*

Cladribine is the new medicine used in the therapy of neoplasms derived from the lymphatic system. The best results were achieved in patients with the hairy cell leukemia (1, 10). 2-CDA shows strong immunosuppressive properties which are not connected with an elimination of immune system cells (9). This contributed to its application in the experimental treatment of multiple sclerosis which is the idiopathic inflammatory-demyelinating disease (2, 7).

The aim of the research was to show the possible negative influence of Cladribine on morphology of retinal ganglion cells after its administration in experimental animals.

## MATERIAL AND METHODS

The experiment was carried out on 30 rabbit females of New Zealand breed weighting about 3 kg. The rabbits received water and standard granulated fodder *ad libitum*. The animals were divided into three groups: one control and two experimental groups. The control group included animals receiving 0.9% NaCl in the appropriate dose. The experimental group I included rabbits receiving Cladribine in the dose corresponding to the schema of experimental treatment in the hairy cell leukemia and the experimental group II – the dose corresponding to the schema of experimental treatment in multiple sclerosis. 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 the optic disc were collected for histological examinations. The obtained tissue material was fixed in glutaraldehyde and $OsO_4$ and embedded in Epon 812. 1 μm thick semithin sections were stained with methylene blue and azure II. The slides were observed and the photos were taken in the light microscope Janamed with the photo-camera (Carl Zeiss, Jena). We performed the histological analysis of retinal ganglion cells.

## RESULTS AND DISCUSSION

The retinal ganglion layer of the rabbits from the control group consisted of the large, spherical nuclei of ganglion cells with the large, intensely basophilic perykarions. Dendrites of the optico-ganglional neurons arborized in the internal plexiform layer and their axons formed the layer of nerve fibers (11). The structures of the retinal ganglion cells in animals from the experimental group I did not show evident morphological changes in comparison with the control group (Fig. 1). The evident deviations were not observed in the retinal ganglion cell layer during the light microscope examinations. Their amount, proportions and localization were similar to the control group. Large

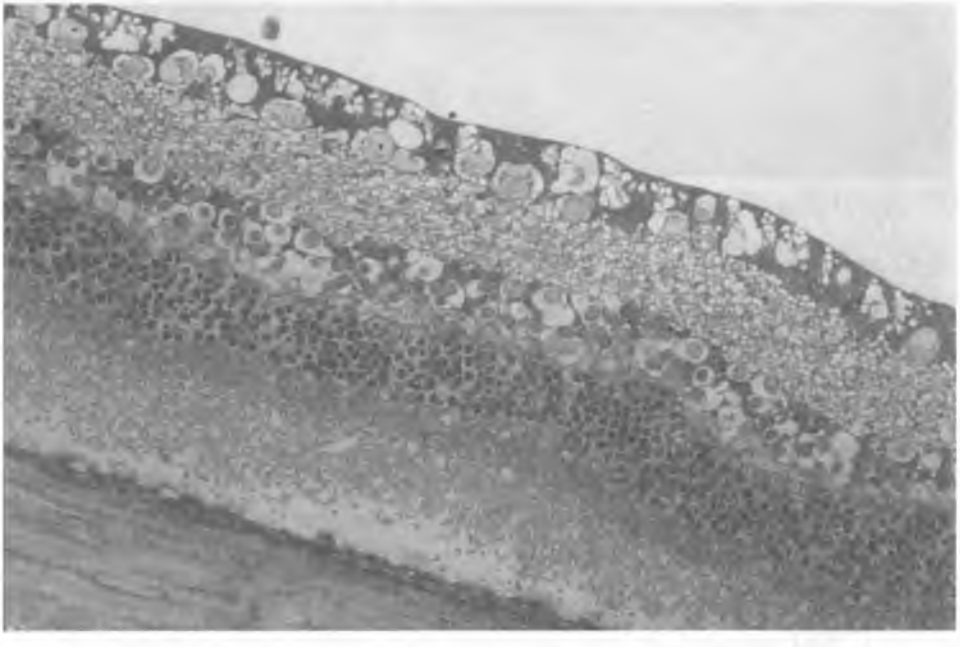

Fig. 1. Experimental group I. The structure of retina on the transverse section. Staining with methylene blue and azure II. Magn. 800x

perykarions of ganglion cells containing spherical nuclei were observed. Their dendrites were located in the internal plexiform layer and their axons formed the layer of nerve fibers. The group of retinal ganglion cells of the experimental group II did not show evident pathological changes during the light microscope examinations in comparison with the control group (Fig. 2). The differences in morphologic pictures of this group were not observed in comparison with the experimental group I. Ganglion cells possessed large, spherical nuclei and large perykarions.

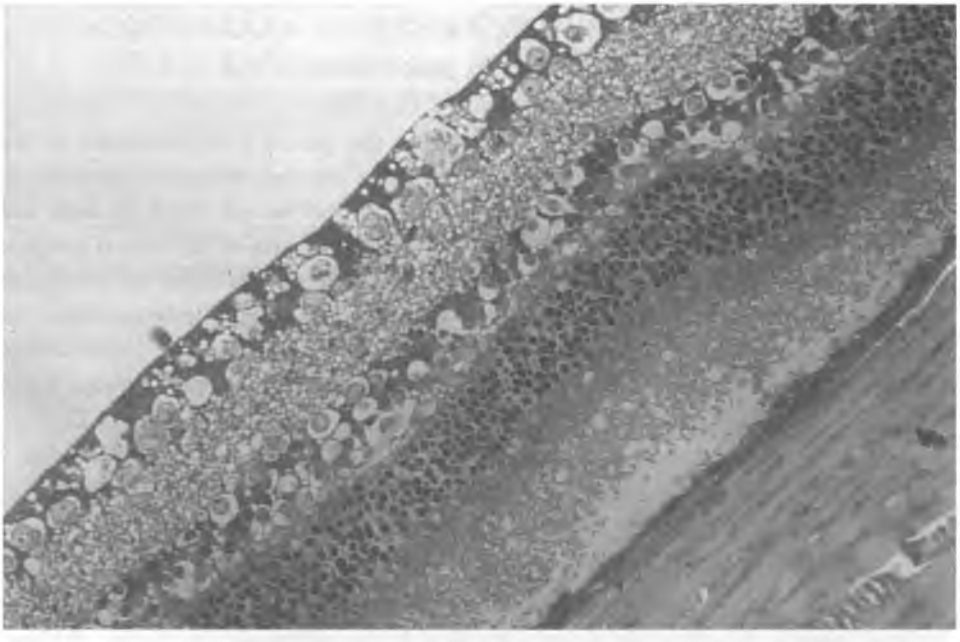

Fig. 2. Experimental group II. The structure of retina on the transverse section. Staining with methylene blue and azure II. Magn. 800x

In the recent years, the toxicity of many cytostatic and immunosuppressive medicines for the retinal ganglion neurons was demonstrated. It suggested the necessity of an analysis of the Cladribine influence on these structures. Green reported the pathological degeneration of retinal ganglion cells caused by the intravitreous administration of vincristine in the dose of 0.1 μg. The large cysts bordered on the processes of Muller's cells filled with the cell remnants were visible at the place of ganglion cells. In 1992, Munier et al. described the case of vision loss caused by vincristine treatment administered for more than 7 months. They revealed the total retinal ganglion cell atrophy during the histological examination (8).

Daniels and Moore performed *in vitro* examinations on the chick embryos following the direct exposure to the folic acid antagonist – methotrexate. After 16 hrs incubation with MTX the retinal ganglion cells showed the pyknotic nuclei and mitotic figures

(3). In 1987, Dharma et al. have published their research regarding the toxicity of the single doses of interferon administered into the vitreous body in rabbits. They did not cause evident histological changes in the light microscope (4). The research work of Karacorlu regarding alpha-interferon toxicity after its intravitreous administration showed neither evident histological changes in the eye tissues nor inflammatory changes (6).

## CONCLUSIONS

1. Cladribine administration in the dose corresponding to the therapeutic dose used in human for the treatment of hairy cell leukemia and multiple sclerosis does not cause evident morphological changes in retinal ganglion cells during the light microscope examinations.

2. It seems needful to take subsequent examinations on the ultrastructural level.

## REFERENCES

1. Armer E. S. J.: On the phosphorylation of 2-chlorodeoxyadenosine (CdA) and its correlation with clinical response in leukemia treatment. Leuk. Lymph., 225, 21, 1996.
2. Bryant J. et al.: Systematic review of immunomodulatory drugs for treatment of people with multiple sclerosis: Is there good guality evidence on effectiveness and cost? J. Neurol. Neurosurg. Psychiatry, 574, 70, 2001.
3. Daniels E., Moore K. L.: Early chick neuroretinal responses following direct exposure to Methotrexate. J. Morphol., 307, 150, 1976.
4. Dharma S. K. et al.: Toxicity of intravitreally administered alpha-interferon. Ophthalmic Surg., 51, 18, 1987.
5. Green W. R.: Retinal and optic nerve atrophy induced by intravitreous Vincristine in the primate. Trans. Am. Ophthalmol. Soc., 73389, 1976.
6. Karacorlu M. A. et al.: Lack of toxicity of intravitreally administered interferon alpha-2a. Ophthalmic Surg., 833, 23, 1992.
7. Lucchinetti C. F. et al.: Multiple sclerosis: lessons learned from neuropathology. Semin. Neurol., 337, 18, 1998.
8. Munier F. et al.: Perte des cellules ganglionnaires de la retine secondaire a la prise de vincristine. Klin. Mbl. Augenheilk., 550, 200, 1992.
9. 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.

10. Tallman M. et al.: Treatment of hairy-cell leukemia: Current views. Sem. Hematol., 155, 36, 1999.
11. Zawistowski S.: Zarys histologii. PZWL, Warszawa 1990.

2002.05.04

SUMMARY

The experiment was carried out on rabbit females of New Zealand breed weighing about 3 kg. Animals from the experimental group received Cladribine according to the schema of experimental treatment in the hairy cell leukemia and rabbits from the experimental group II – according to the schema of experimental treatment in multiple sclerosis. Specimens of retina were collected for histological examinations in the light microscope. 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 and multiple sclerosis does not cause evident morphological changes in retinal ganglion cells on the level of light microscope.

Histologiczna ocena warstwy zwojowej siatkówki królika po doświadczalnym podaniu leku immunosupresyjnego - Kladrybiny

Badania wykonano na królikach, samicach rasy nowozelandzkiej o masie ciała ok. 3 kg. Zwierzętom grupy doświadczalnej I podawano Kladrybinę według schematu leczenia białaczki włochatokomórkowej, a królikom grupy doświadczalnej II – według schematu eksperymentalnego leczenia stwardnienia rozsianego. Do oceny histologicznej w mikroskopie świetlnym pobierano fragmenty siatkówki. Stwierdzono, że podawanie Kladrybiny w ilości odpowiadającej dawce leczniczej stosowanej u człowieka w terapii białaczki włochatokomórkowej i eksperymentalnym leczeniu stwardnienia rozsianego nie wywołuje morfologicznie uchwytnych zmian w komórkach zwojowych siatkówki, stwierdzanych w badaniach przy użyciu mikroskopu świetlnego.