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*Histological examination of the extrabulbar segment of the optic nerve  
in experimental animals after administration of Cladribine*

Cladribine (2-chlorodeoxyadenosine) has a particular affinity for lymphoid cells because of their advantageous enzymatic configuration, which decides about its specific action on these cells and the low toxicity for other tissues (1, 2, 7). Cladribine is the most effective in a treatment of the hairy cell leukemia (10). Its immunosuppressive properties contributed to an application of this medicine in the experimental treatment of multiple sclerosis (5, 6, 9).

The aim of this research is to present the possible negative influence of Cladribine on the morphology of the extrabulbar segment of the optic nerve after the administration of the medicine to experimental animals.

#### MATERIAL AND METHODS

The experiment was carried out on 30 rabbits of New Zealand breed weighting about 3 kg. Rabbits received water and standard granulated fodder ad libitum. Animals were divided into three groups – one control and two experimental groups. The control group included animals receiving 0.9% NaCl (subcutaneously). 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 24hrs 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 optic nerve were collected for histological examinations. The obtained tissue material was fixed in 10%

neutral formalin, dehydrated in ethyl alcohol, cleared in xylene and embedded in paraffin. Then we performed the routine staining with hematoxylin and eosin, the staining with azan for the visualization of connective tissue fibers and P.A.S. reaction for the detection of neutral polysaccharides on paraffin 5  $\mu\text{m}$  thick sections. These staining procedures were performed simultaneously on the material of both experimental and control groups. 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 the extrabulbar segment of the optic nerve.

## RESULTS AND DISCUSSION

The regular morphology of the optic nerve was visualized in the control group after staining of slides with azan. The neuroglia colored red with oval nuclei which stain darker where presented. The cytoplasm was reddish. The structure of connective tissue fibers was regular. The connective tissue fibers were blue in slides stained with azan (Fig. 1). The evident changes in the extrabulbar segment of the optic nerve in animals of the experimental group I in comparison to the control group were not observed. The neuroglia (stained red) with the darker stained nuclei were visible. The structure of connective

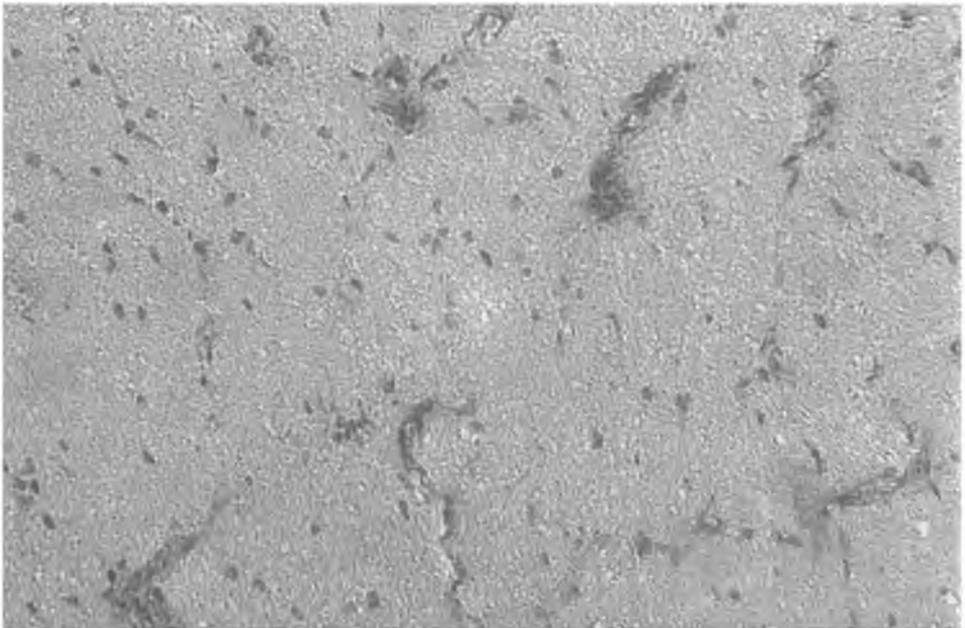


Fig. 1. The control group. The regular structure of the extrabulbar segment of the optic nerve. The nuclei of glial cells and connective tissue septa are visible.

Staining with azan. Magn. approx. 800x

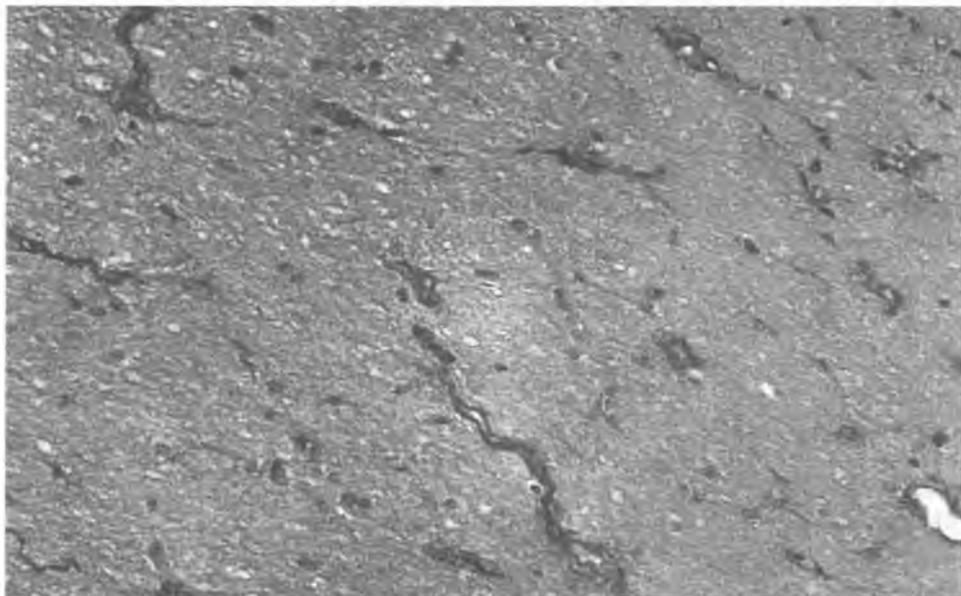


Fig. 2. The experimental group I. The structure of the extrabulbar segment of the optic nerve. Staining of connective tissue fibers with azan. Magn. approx. 800x

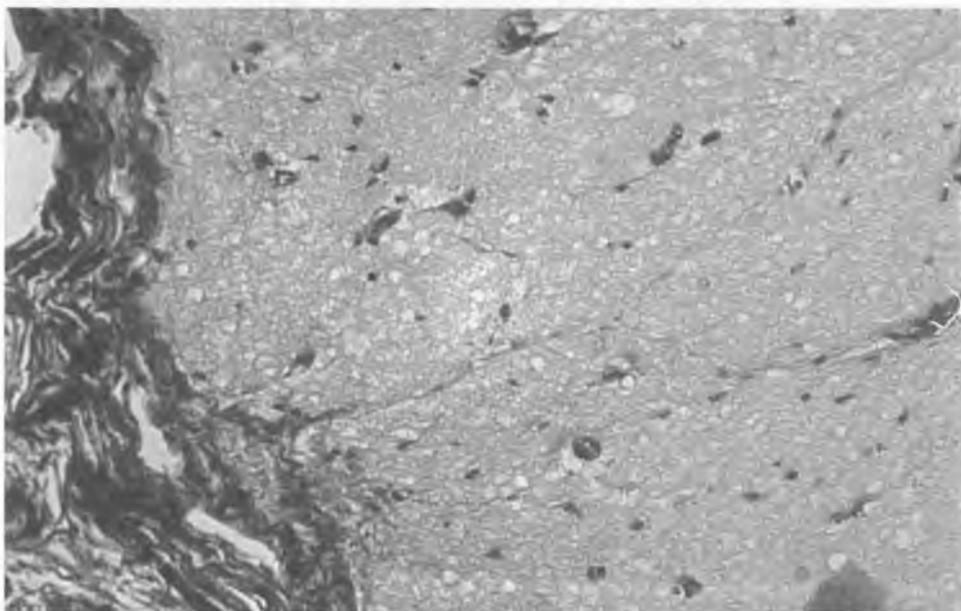


Fig. 3. The experimental group II. The structure of the extrabulbar segment of the optic nerve. Staining of connective tissue fibers with azan. Magn. approx. 800x

tissue fibers (stained blue) did not show the evident morphological changes (Fig. 2). The morphological changes were not observed in extrabulbar segment of the optic nerve in animals of the experimental group II in slides stained with azan. The red neuroglia with the darker stained oval nuclei were visible. The structure of connective tissue fibers (stained blue) did not show evident morphological changes (Fig. 3).

The available literature does not present papers concerning the influence of Cladribine on the optic nerve. Djaldetti et al. studied the influence of the high doses of 2-CDA on the peripheral nervous system in mice. They examined Schwann cells of myelinated and nonmyelinated peripheral nerve fibers. Authors concluded that Cladribine can cause the peripheral neuropathy damaging Schwann cells in doses much higher than the therapeutic doses (3). In our experiment we did not observe evident morphological changes in the examined structures in the light microscope after the administration of therapeutic doses of the medicine to experimental animals. However, the toxic influence of many cytostatic and immunosuppressive medicines on the optic nerve is observed. Green reported the pathological degeneration of the optic nerve induced with vincristine administered to the vitreous body (4). In the 1992 year Munier et al. described the case of the loss of vision caused by vincristine therapy administered for more than 7 months. They revealed the demyelination of the optic nerve (8).

## CONCLUSIONS

1. The administration of Cladribine in the doses corresponding to the therapeutic doses used in therapy of hairy cell leukemia and sclerosis multiplex does not cause evident morphological changes in the extrabulbar segment of the optic nerve on the level of the light microscope.

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

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#### SUMMARY

The experiment was carried out on rabbits, females of New Zealand breed, weighting about 3 kg. Rabbits from the experimental group I received Cladribine in the dose corresponding to the schema of the experimental treatment in the hairy cell leukemia and animals from the experimental group II, the dose corresponding to the experimental treatment in multiple sclerosis. The optic nerves were collected for histological examinations in the light microscope. It appeared that administration of Cladribine in the dose corresponding to the therapeutic dose used in therapy of hairy cell leukemia and sclerosis multiplex does not cause evident morphological changes in the extrabulbar segment of the optic nerve on the level of the light microscope.

Histologiczna ocena odcinka pozagalkowego nerwu wzrokowego po zastosowaniu leku immunosupresyjnego – Kladrybiny u zwierząt doświadczalnych

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 rozlanego. Do oceny histologicznej w mikroskopie świetlnym pobierano nerwy wzrokowe. Stwierdzono, że podawanie Kladrybiny w ilości odpowiadającej dawce leczniczej stosowanej u człowieka w terapii białaczki włochatokomórkowej i eksperymentalnym leczeniu stwardnienia rozlanego nie wywołuje morfologicznie uchwytanych zmian w odcinku pozagalkowym nerwu wzrokowego, stwierdzanych w badaniach przy użyciu mikroskopu świetlnego.