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Histomorphometry of nuclei of proximal convoluted tubules epithelium of kidney of experimental animals after Cladribine (2-CdA) administration

Cladribine (2-chlorodeoxyadenosine), 2-CdA is a potent chemo-therapeutic and immuno--suppressive nucleoside. It is an anti-metabolite – an analog of adenosine (purine) (2, 3). 2-CdA is equally toxic to dividing and to non-dividing cells in tissue culture. Cladribine, in dividing cells, is incorporated into the DNA strand, thus inhibiting the activity of enzymes which take part in DNA synthesis. Thus, these changes lead to disturbances of the cell's function and therefore to cell death. Cladribine may act by preventing the repair of DNA single-strand breaks. Cladribine, in non-dividing cells induces apoptosis – programmed cell death (1, 6, 8, 10, 17).

The changes in the cell which start the death program affect mainly the cell's nucleus. The chromatin in the nucleus condense and fragment, initially in the peripheral part of the nucleus. The nucleus is picnotic and then is endonucleases dependent on calcium and magnesium fragmentation on a 180 base pair or their multiplication. The fragmentation of chromatin leads to the development of apoptotic corpuscles which are bound with chromatin fragments surrounded with cytoplasm with organelles and a stabilized cell membrane (11).

The use of Cladribine is indicated in neoplasms originated from lymphoid tissue: hairy cell leukemia, chronic lymphocytic leukemia, non-granulated lymphomas of little grade, Waldenstrom macroglobulinemia, cutaneous T-cell lymphomas, and acute lymphocytic leukemia (13, 15). Recently, because of its immuno-suppressive activity, the drug has been trialed for therapy in the treatment of auto-immune disease such as multiple sclerosis (7, 14, 16).

2-CdA is slightly toxic. The most unwanted side-effects are due to its strong myelo-suppressive activity (granulcytopenia and anemia). Less common side-effects are opportunistic infections, fever, skin reactions, digestive complaints, damage to the nervous system, nephropathy – which occurs at great dosage rates (2, 3, 14, 15). The similar side effects occur during the therapy of other cytostatics (9, 12). Cladribine is excreted mainly through the kidneys (12, 14).

Our research demonstrates the differences between size of nuclei of cell's epithelium lining the proximal convoluted tubules in experimental groups after Cladribine administration.

MATERIAL AND METHODS

The experiment was carried out on female white rats weighing about 250–300 mg each. The animals were divided into four experimental groups and a control group, with five animals in each. In the control group the animals were given 0.9% NaCl in subcutaneous injection. In experimental group I, the animals were given Cladribine (Biodribin produced by The Institute of Biotechnology and Antibiotics (Instytut Biotechnologii

i Antybiotyków) in the dose of 0.1 mg /kg of body mass/24 h in subcutaneous injection, for seven successive days; samples for research was taken 24 hours following the last dose. In experimental group II, the animals were given Cladribine in the same dose and sampled in the same manner as in experimental group I, the animals were however, killed 4 weeks following the last dose. In experimental group III, the animals were given Cladribine in the dose of 0.07 mg/kg of body mass/24 h in sub-cutaneous injection for six successive days, in three courses with five weeks' break between each; the animals were then killed 24 hours following the last dose. In experimental group IV, the animals were given Cladribine in the same dose and in the same manner as those in experimental group III, with the animals being killed 4 weeks following the last dose.

The projection microscope enabled the experimentors to realize the areal measurement of the nuclei of the proximal convoluted tubules. The morphometric measurements have been done on the kidney samples stained with hematoxylin and eosin. The smallest and the largest nucleus diameters were measured. The surface section was then calculated using formulas πr^2 on the circle's surface and πab on the ellipse's surface. On fragments of each of the animals kidney, 100 nuclei were measured randomly. The examination results were worked out statistically using the Kołmogorow–Smirnow test for checking the hypothesis of regular break down, and then using a nonparametric test for two samples – (U-Mann's–Whitney's). For the comparison of few independent trials, Anova Kruskal-Wallis's test and the Median test were utilized. All the calculations were made with the help of the Statistica PL 6.0 packet from the Stat – Soft Polska Co (Ltd). The level of reality of verified hypothesis was established as $p < 0.05$.

RESULTS

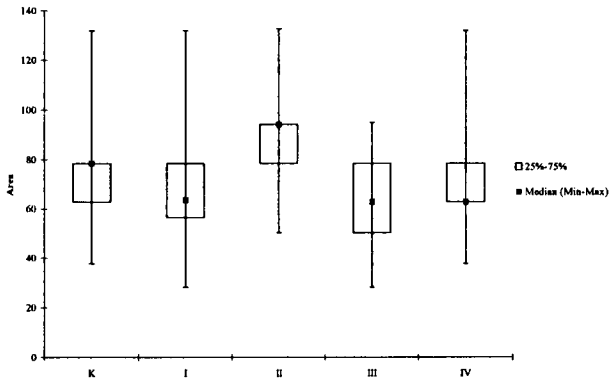


Fig. 1. Diagram of median in all groups

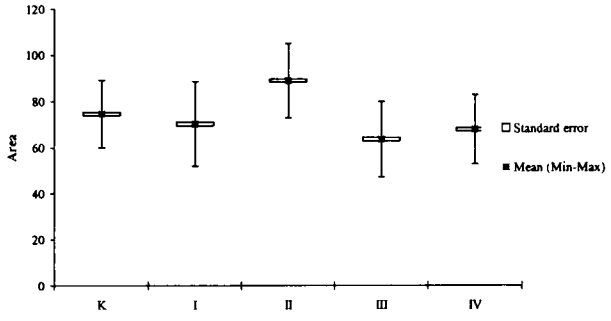
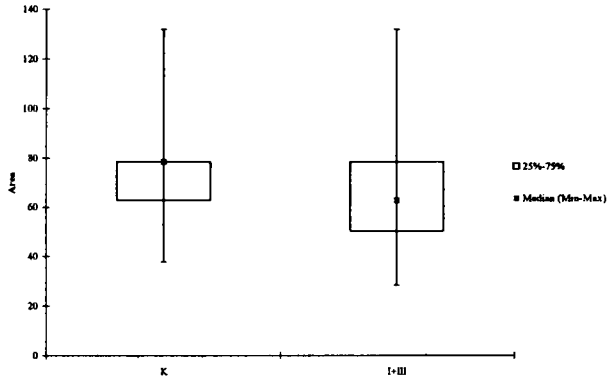
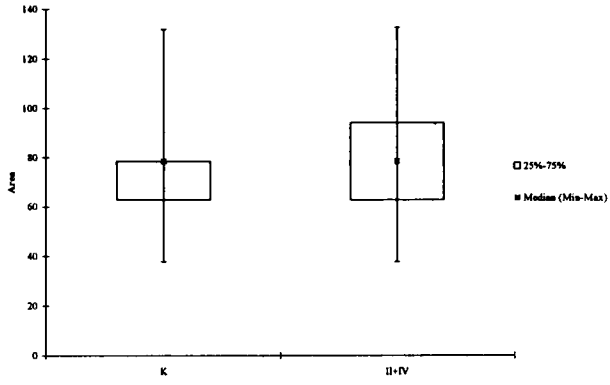


Fig. 2. Diagram of mean in all groups

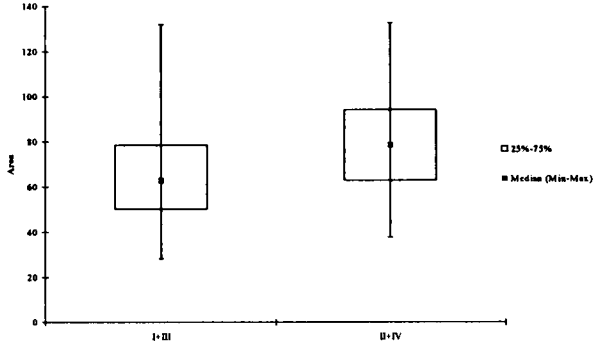
ANOVA rang the Kruskal-Wallis's test and the Median test show statistically essential differences in nuclei area between groups.



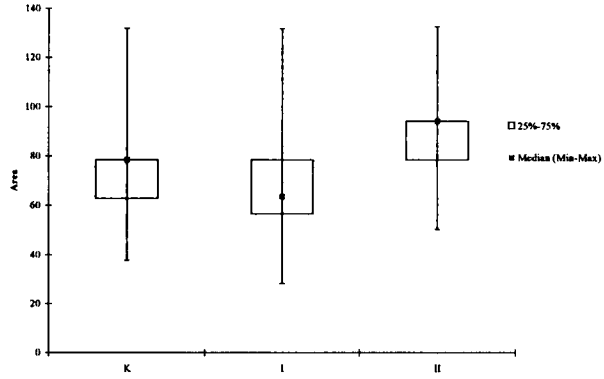
The comparison between control and experimental groups I and III affirmed that differences were statistically significant; in control group the surface's areas were significantly bigger.



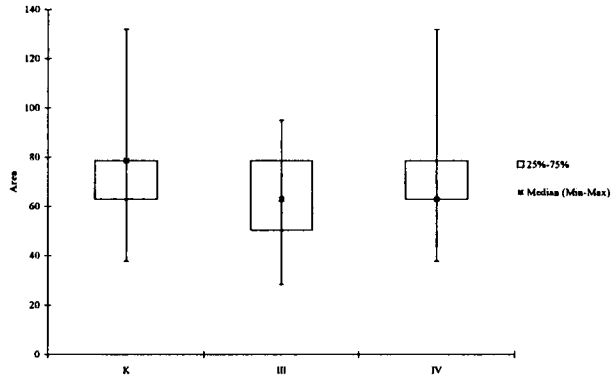
The comparison between control group and experimental groups II+IV I affirmed, according to the U-Mann Whitney's test that there existed significant differences between control group (the surfaces of nuclei were smaller) and experimental groups II+IV (the surfaces of nuclei were bigger).



The comparison between two pairs of groups showed significant differences: the surface area of the proximal tubules epithelium nuclei cross-sections in experimental groups II and IV were bigger than the surface area of the proximal tubules epithelium nuclei cross-sections in experimental groups I and III.



The comparison between experimental groups I and II and control groups proved that in experimental group I the size of surface of the nuclei significantly decreased and in experimental group II significantly increased.



The comparison betwen control and experimental groups III and IV affirmed that the surface areas of the proximal tubules epithelium nuclei cross-sections were smaller than in control groups. In experimental group III the surface areas of nuclei are smaller than in experimental group IV and in the control group.

DISCUSSION AND CONCLUSIONS

According to reports of Barbieri et al. and Ceruti et al., Cladribine led to apoptosis in mononuclear rested cells of peripheral blood (1) and in astrocytes culture (5).The most important and best observed changes are constrictions of the cell's nuclei in which apoptosis is observed (11). It is true that in histological and ultrastructure investigations no changes were observed that were characteristic of apoptosis, but statistical investigations show differences in the magnitude of nuclei of

the epithelium lining the proximal convoluted tubules. These differences arise probably from the change of intensity of metabolism and processes which occur in nuclei structures (12).

In experimental groups I and III the surface area of the proximal tubules epithelium nuclei cross--sections are smaller than in the control group. In experimental groups II and IV, the surface area of the proximal tubules epithelium nuclei cross-sections are bigger than in control groups.

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SUMMARY

The area of the section of nuclei of the epithelium cells lining the proximal convoluted tubules of the kidney of white Wistar rats was examined. The animals were given Cladribine (2-CdA) subcutaneously at the dosages of 0.07 mg/kg b.w./24 h for seven days and 0.1 mg/kg b.w./24 h for 6 days in three courses with 5 weeks' break between each. The animals were killed in each instance, 24 hours after the last dose of the drug and 4 weeks after the last dose. The mean area of the section of the cell's

nuclei was measured in projection microscope. Results of the examination were counted statistically. In experimental groups I and III the surface areas of the proximal tubules epithelium nuclei cross--sections are smaller than in the control grous. In the experimental groups II and IV the surface areas of the proximal tubules epithelium nuclei cross-sections are bigger than in the control group.

Badania histomorfometryczne jąder komórek nabłonka wyścielającego kanaliki proksymalne nerek zwierząt doświadczalnych po podawaniu Cladribine (2-CdA)

Badano statystycznie powierzchnię przekroju jąder komórek nabłonka wyścielającego kanaliki proksymalne w nerce szczurów rasy Wistar, którym podawano Cladribine (2-CdA) podskórnie w dawkach: 0,07 mg/kg m.c. przez 7 dni oraz 0,1 mg/kg m.c. przez 6 dni 3-krotnie w odstępach 5-tygodniowych. Materiał do badań pobierano w każdym przypadku 24 godz. po podaniu ostatniej dawki leku oraz 4 tygodnie po podaniu ostatniej dawki leku. Wykonane preparaty oglądano w mikroskopie projekcyjnym i mierzono powierzchnię przekroju jąder komórkowych. Do uzyskanych danych stosowano obliczenia statystyczne. W grupach doświadczalnych I i III pola powierzchni przekroju jąder komórkowych nabłonka wyścielającego kanaliki proksymalne są statystycznie istotnie mniejsze w porównaniu z grupą kontrolną, natomiast w grupach doświadczalnych II i IV są statystycznie istotnie większe w porównaniu z grupą kontrolną.