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Histomorphology of renal corpuscles of kidney of experimental animals after Cladribine (2-CdA) administration

Cladribine (2-chlorodeoxyadenosine), 2-CdA is a potent chemotherapeutic and immunosuppressive nucleoside. The structure of 2-CdA is identical to deoxyadenosine except for the substitution of chloride for hydrogen at the 2-position of the purine ring (1, 2, 15). This change prevents deamination of the adenine ring. 2-CdA resists the action of adenosine deaminase and it accumulates in cells with high deoxycytidine kinase and low 5'- nucleotidase activity, mainly in lymphoid cells (15). 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 (3, 5, 15).

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, acute lymphocytic leukemia (4, 12, 14). In the last years, because of its immunosuppressive activity, the drug has been trialed for therapy in treatment in autoimmune disease such as multiple sclerosis (7, 13).

2-CdA is slightly toxic. The most unwanted side-effects are connected with 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 (12, 14). Cladribine is excreted mainly through the kidneys (12, 14).

Our research demonstrates the technique of using a light microscope to observe the changes in renal corpuscle samples due to the administration of Cladribine (Biodribin).

MATERIAL AND METHODS

The experiment was carried out on white rats – females weighing about 250–300 mg each. The animals were divided into four experimental groups and a control group, with five animals in each. In 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 were 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 subcutaneous 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 right kidney samples were fixed in 10% buffered formalin. Paraffin sections 6 nm thick were histologically and histochemically evaluated. These samples were stained with hematoxylin and eosin, by PAS's method, by Masson's method, and by Feulgen's method.

RESULTS

Control group. All the stained samples showed no deviation from the normal structure of the renal corpuscles.

Experimental group I. In all the stained samples both areas with normal appearance (as in control group) and areas with large changes, were seen in the renal corpuscle sections. We observed few unchanged renal corpuscles – these were mainly in the external part of the cortex and we observed corpuscles with changed structure. These changed corpuscles showed vessels loops which were dense. The urinary space in these corpuscle sections were narrow. In addition, in these sections, the parietal layer of the Bowman's capsule and the basement membrane of capillaries (PAS met. staining) were segmentally thickened. A few sections were observed, in which the vessels loops were dilated and the urinary space was dilated as well. Slight lymphocyte infiltrations were observed around and inside the renal corpuscles. Large, dilated vessels in the stroma were filled with blood. In the Masson's method of staining, in some of the renal corpuscles, we observed more connective tissue than in the control group. With Feulgen's staining method, the nuclei of the endothelium cells appeared to be a little smaller, with chromatin more collected than in the control group.

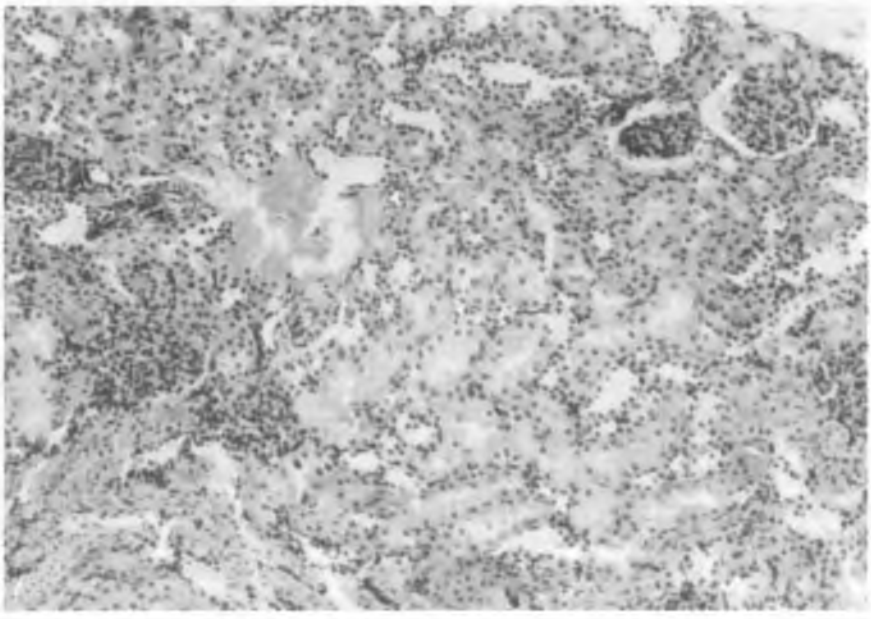

Fig. 1. Experimental group I. The rat's kidney. Section through the renal corpuscles with narrow or normal urinary space and through the proximal, distal and collecting convoluted tubules. Visible are wide blood vessels with the blood inside. Hematoxylin and eosin staining. Magn. 200x

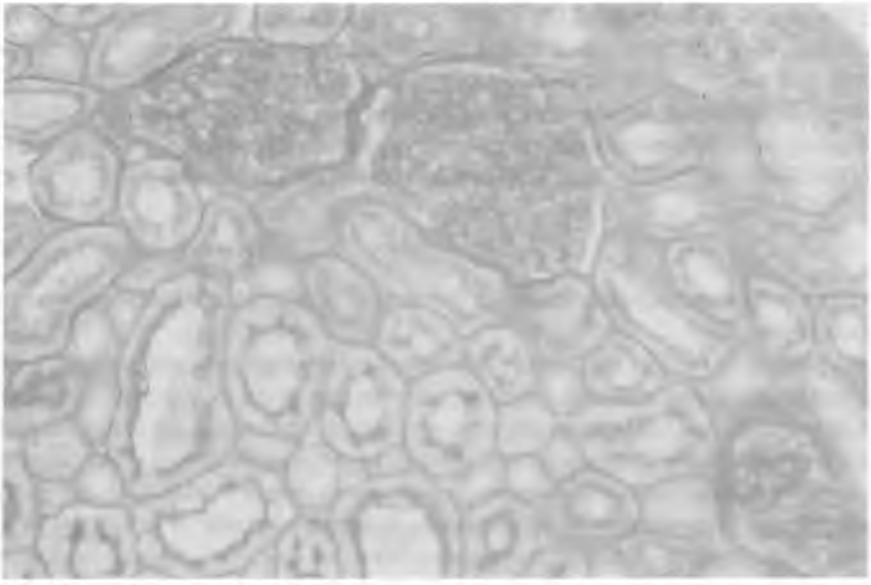

Fig. 2. Experimental group II. The rat's kidney. Sections through the renal corpusculus and tubules. The basement membrane of the parietal layer of the Bowman's capsule and capillaries – thicker. PAS met. staining. Magn. 400x

Experimental group II. The kidney corpuscles of animals of experimental II group reveal many changes in comparison with control group, no matter what staining methods was used (H+E, the PAS method, the Masson's method, the Feulgen's method). The changes were, however, less visible than in experimental group I, with most of the renal corpuscles looking normal. Those abnormal renal corpuscles had dense glomeruli and had narrowed urinary space. A few corpuscles were observed, in which the vessels loops were dilated in comparison to the urinary space.

In the kidney's stroma, cellular infiltrations were noticed. In some of the glomeruli, hyperaemia was visible. Large vessels were dilated and filled with blood. With PAS method staining, the parietal layer of the Bowman's capsule and the basement membrane of capillaries of some of the glomeruli were segmentally thickened. With the Masson's staining, in some of the glomeruli, more connective tissue than in the control group was observed. With the optic microscope, and using the Feulgen's method of staining, the nuclei were larger in comparison with those of control and experimental group I. The chromatin was also a little more dispersed than in the previous groups.

Experimental group III. In H+E staining, the vessels loops were often dense and the urinary space was not visible in some of the glomeruli. In large numbers of the renal corpuscles, the basement membrane of capillaries of the renal glomeruli and the parietal layer of the Bowman's capsule were seen to be thicker than in control group (PAS method). In the submedulla part of the cortex, normal renal glomeruli or glomeruli with wide urinary space were observed. Around some of the renal glomeruli and tubules, cellular infiltrations was visible. The dilated large vessels were filled by blood. In using the Masson's staining method, we observed in the renal corpuscles more connective tissue than in control group. These corpuscles were also much more congested. Around the large and dilated vessels, more connective tissue was visible than in control group.

When using the optic microscope and the Feulgen's staining method, the nuclei of the endothelium of the capillaries of the parietal layer of the Bowman's capsule, were seen to be smaller in comparison with control and experimental I groups. The chromatin was also a little more condensed than in the control group.

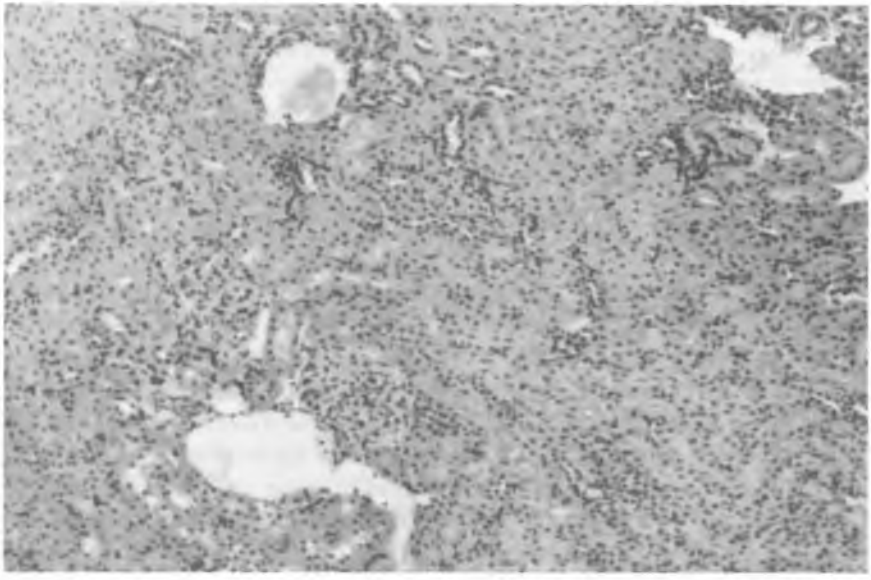

Fig. 3. Experimental group III. The rat's kidney. Sections through the renal corpuscles with no visible urinary space and tubules with very narrow lumen. Visible are the wide blood vessels. Hematoxylin and eosin staining. Magn. 200x

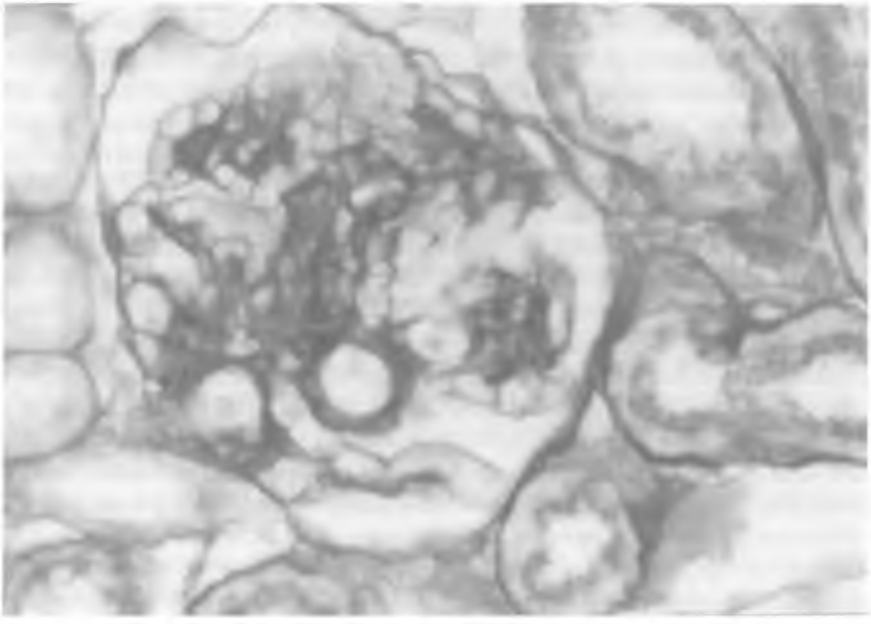

Fig. 4. Experimental group IV. The rat's kidney. Sections through the renal corpusculus and through the tubules. The thick basement membrane in capillaries of the glomerulus. PAS met. staining. Magn. 800x

Experimental group IV. In experimental group IV, beside glomeruli with normal appearance, there were a few corpuscles with dense vessels loops of glomeruli and narrowed urinary

space with the corpuscles having dilated capillary loops and wide Bowman's space. In comparison with the control group, these were much more numerous. The basement membrane of the capillaries of the glomeruli was also wider and the parietal layer of the Bowman's capsule was thicker than in control group (PAS method staining). In some parts of the kidney, mainly at the vessels' pole of the glomeruli, infiltrations were observed. In addition, a hyperaemia of the kidney parenchyma was noticeable, and the large blood vessels were filled with blood.

Using the Feulgen's staining method, the nuclei of the endothelium cells had an irregular shape, were smaller than in control group and their chromatin was denser, than in control group.

DISCUSSION AND CONCLUSIONS

Cladribine, according to reports seems bea valuable drug because when given in recommended dosages, few incidental effects occur, these being often in the form of myelo-suppression (1, 2, 12). Adverse side effects increase when the drug is given in a large dose. There are reports that Cladribine given at the dose > 0.3 mg/kg b.w./24 h for 7 days, is neuro- and nephrotoxic. Despite the minor toxicity of Cladribine (1, 2), excretion of the drug is based on patient's weight and the presence of a transfer factor, report K u t t e s c h and N e l s o n (9). In addition, large doses of Cladribine are a cause of neurologic and nephrologic dysfunctions (12, 14).

Because of the lack of detailed information about the effects of Cladribine on the kidney, and taking into consideration that the drug is excreted mainly through the kidney, we tried to determine the effect of Cladribine on the kidneys.

The experiment showed visible changes in the renal corpuscle samples of all of the experimental groups. The dilatation of blood vessels within the renal samples may be connected with increased blood pressure. This and the function of the glomeruli may influence aldosterone excretion. This may effect histamine activity. Histamine is secreted in large amounts mainly in reaction to inflammation and this inflammation is present in the organism after the administration of Cladribine. Thus administration of Cladribine leads to immunological changes, one of the effects of this possibly being blood vessel dilatation.

Beside the normal renal corpuscles that were visible, our research showed renal corpuscles with narrowed urinary space and constricted capillaries, and renal glomeruli with wider urinary space. The different appearance of renal glomeruli suggested a difference in their contribution to blood filtration and suggested non-equal sensitivity to the given drug. The narrowed urinary space suggested dysfunction of filtration in the glomerulus, because of functional or organic changes. The widening of urinary space in other corpuscles were probably compensation in proportion to the narrowed space in other corpuscles. The widening of urinary space may be due to increased filtration in corpusculus and with pathologic accumulation of protein in the space (10, 11). Thickening of basement membrane is often connected with dysregulation of protein transformation (8), so the widened urinary space and the thickened basement membrane in this experiment, is possibly an effect of proteinuria. Infiltrations around renal corpuscles and tubules are characteristic of kidney inflammation due to administration of many different drugs such as cytostatic and immunosupressive drugs, e. g. Interferon α i γ (10, 11).

The observed changes in all the experimental groups were similar, but of different intensity. The greatest intensity was observed in groups, in which the kidneys were taken for investigation 24 h after the last dose, and following 4 weeks' break in the administration of Cladribine.

Our results indicate the need for investigations in and search of, preparations and procedures which would decrease Cladribine nephrotoxicity.

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SUMMARY

The renal corpuscles 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 7 days and 0.1 mg/kg b.w./24 h for 6 days in 3 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 kidney samples were taken for histological and histochemical examination, then stained with hematoxylin and eosin, using the Masson's, PAS, and Feulgen's methods. In all of the groups, changes were observed but the intensity differed, with widening or narrowing of the urinary space, thickening of the basement membrane of the parietal layer of the Bowman's capsule and the basement membrane of capillaries, and density changes in capillary vessels. Hyperaemia in renal glomeruli and in all parenchyma, and infiltrations around the tubules and renal glomeruli, were also observed.

Histomorfologia ciałek nerkowych w nerce zwierząt doświadczalnych po podawaniu Cladribine (2-CdA)

Badano ciałka nerkowe szczurów rasy Wistar, którym podawano Cladribine (2-CdA) podskórnie w dawkach: 0,07 mg/kg m.c. przez 7 dni i 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. Stosowano barwienia H+E, met. PAS, met. Masona, met. Feulgena. W każdej z grup obserwowano podobne zmiany, ale o różnym nasileniu: poszerzenie lub zwężenie przestrzeni moczowej, poszerzenie błony podstawnej torebki Bowmana oraz błony podstawnej naczyń włosowatych, zbite pętle naczyń włosowatych, przekrwienie w kłębkach nerkowych oraz w całym narządzie, nacieki okołocewkowe oraz wokół ciałek nerkowych.