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The ultrastructure of kidney renal corpuscles 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 similar to a deoxyadenosine (1, 2, 3, 7). 2-CdA is equally toxic to dividing and to non-dividing cells in tissue culture. Cladribine, in non-dividing cells induces apoptosis – programmed cell death (6, 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 (1, 2, 7). In the last years, because of its immuno-suppressive activity, the drug has been trialed for therapy in treatment in auto-immune disease such as multiple sclerosis (8).

2-CdA is slightly toxic. The most unwanted side-effects are connected with its strong myelo--suppressive activity (granulocytopenia 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 (1, 2, 3, 14). Cladribine is excreted mainly through the kidneys.

Our research demonstrates 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 groups, 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 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 subcutaneous injection for six successive days, in three courses with 5 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 four weeks following the last dose.

Specimens of right kidneys were fixed with buffered glutaraldehyde and OsO_4 and then embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate according to the Reynolds' method. Ultrastructural observations were led and electron micrographs were taken in the Tesla BS-500 transmission electron microscope.

RESULTS

Control group. In control group, ultrastructure of the renal corpuscles of the kidney showed no deviation from normal structure.

Experimental group I. The basement membrane of capillaries was thicker. The large blood vessels were filled with erythrocytes and plasma. Between the podocytes (the visceral layer of the Bowman's capsule) and parietal layer there was either thicker or thinner urinary space filled with homogeneous material. The basement membrane of parietal layer of the Bowman's capsule was thicker and a little fibrous.

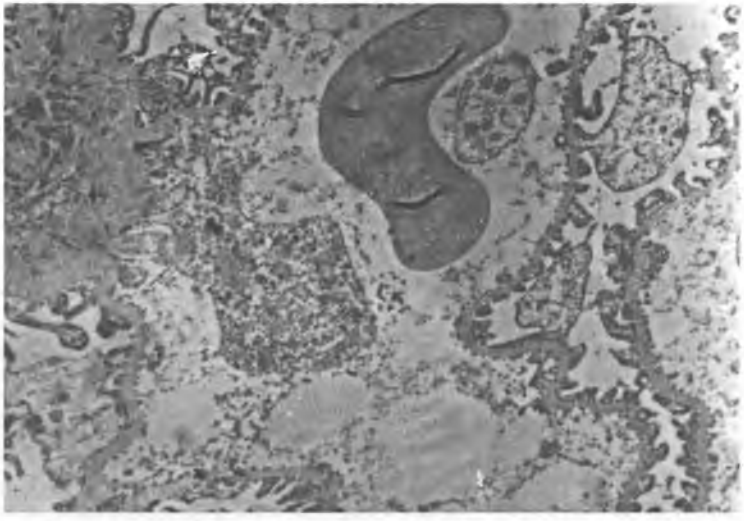

Fig. 1. Experimental group I. The rat's kidney. Section through the renal corpusculus. Visible are wide blood vessel with blood inside.The basement membrane is thick.TME. Magn. 6000x

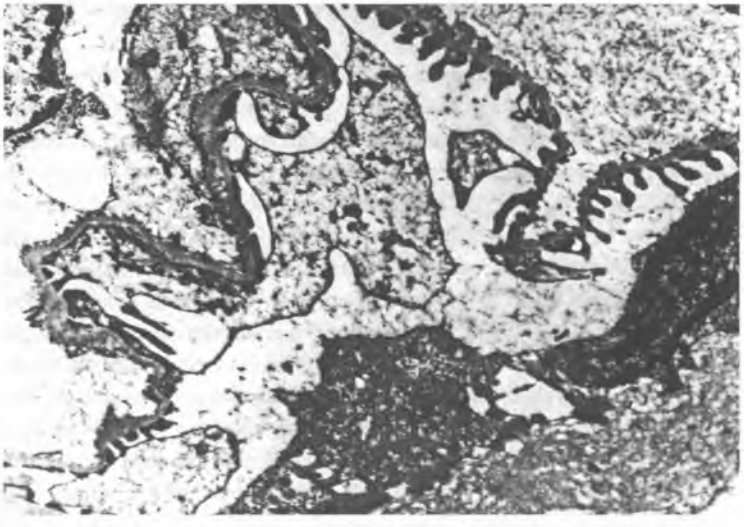

Fig. 2. Experimental group II. The rat's kidney. Sections through the renal corpusculus. The basement membrane of parietal layer of the Bowman's capsule and capillaries – thicker. TME. Magn. 6000x

Experimental group II. Changes in ultrastructure of renal corpuscles of animals of experimental group II were noticeable when observed under the electron microscope. The basement membrane of capillaries of renal glomeruli was a little thicker. The blood vessels were dilated and filled with blood. The urinary space was hardly wider and an excretion was noticeable.

Experimental group III. In experimental group III, the basal membrane of capillaries was wider, but similar to the other experimental groups. The lumen of dilated capillaries included numerous erythrocytes (hyperaemia in renal glomeruli). The width of the urinary space appeared less than in control group.

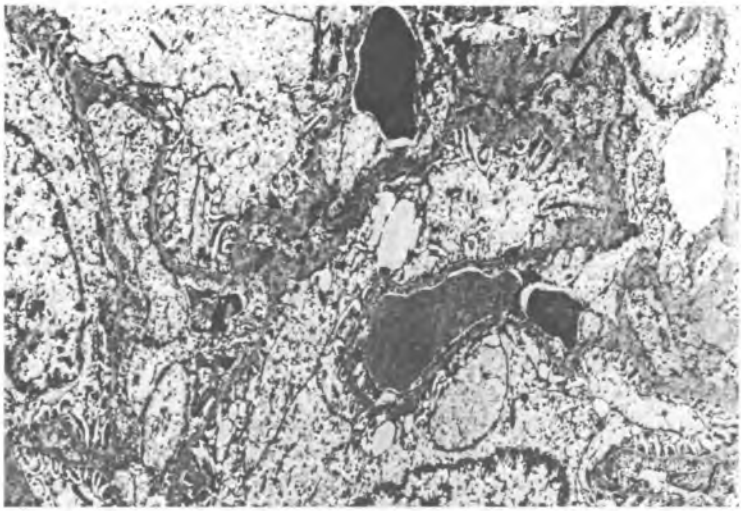

Fig. 3. Experimental group III. The rat's kidney. Sections through the renal corpusculus. Visible are the widened blood vessels. The urinary space is narrow. TME. Magn. 4000x.

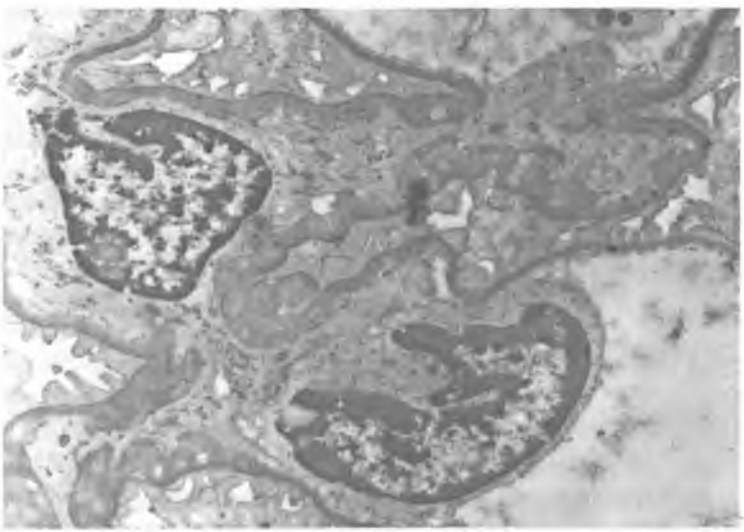

Fig. 4. Experimental group IV. The rat's kidney. Sections through the renal corpusculus. The basement membrane in capillaries is thick. The foot processes of podocytes are connected. The nuclei of endothelium are picnotic. TME. Magn. 6000x

Experimental group IV. In this group, in the renal glomeruli, the basement membrane of the capillaries was marked, thickened, and electron dense. In the lumen of dilated capillaries were erythrocytes and particle material. The nuclei of the endothelium cells showed picnotic features. The nuclear envelope was invaginated inside the nucleus, and chromatin was more electron dense than in the other experimental groups. The foot processes of the podocytes were widened and connected. The nuclear envelope of the nuclei of the podocytes formed deep invaginations into the nucleus, giving an irregular shape. The width of the urinary space was similar to that in control group.

DISCUSSION AND CONCLUSIONS

Despite the minor toxicity of Cladribine (1, 2, 14), excretion of the drug is based on patient's weight and the presence of a transfer factor, report Kuttesch and Nelson (12). In addition, large doses of Cladribine are a cause of neurologic and nephrologic dysfunctions (14).

The experiment showed visible changes in the renal corpuscle samples of all of the experimental groups. The dilation 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, which in turn, 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 dilation.

The thickening of basement membrane is often connected with dysregulation of protein transformation (11, 13), so the widened urinary space and the thickened basement membrane seen in this experiment is possibly an effect of proteinuria.

The changes of vessels and parenchyma were observed following the administration of the maximum tolarated dose (3–5 g/kg) of Methotrexate to rats. Fushevag et al. reports about focussed changes: the cells of epithelium lining capillaries in glomeruli, like in other vessels, swelling and breaking the basement membrane forms and disrupting processes within the vessels. We noticed that some of the nuclei of epithelium were constricted, so their shape was irregular. In the lumen of vessels and around it we also observed erythrocytes, thrombocytes, leukocytes and the small arterioles were constricted (5).

In experimental group IV, the changes affected only the podocytes and their foot processes. The podocyte had irregular shape and their nuclear envelope formed numerous invaginations. The foot processes were lower, wider and joined to one another. These changes of podocytes are the cause of syndroma nephroticum for children and induce minimal nephropatic changes (13). The changes in localisation of podocytes appear in different kidney diseases and after the administration of drugs: Adriamycin, Mitomycin and lead to proteinuria, mainly albumin (4, 9, 10, 13).

The hyperaemia of kidney and large, dilated blood vessels were signs of the effects of swelling.

We noticed in our research that giving Cladribine at the dose of 0.1 mg/kg b.m./24 h for seven successive days leads mainly to changes in renal glomeruli and blood vessels. Giving 2-CdA at the dose of 0.07 mg/kg b.m./24 h for six days in three courses leads to changes more extensive than does giving Cladribine in one course.

The changes created following administration of Cladribine at the dose of 0.1 mg/kg b.m./24 h for 7 successive days are reversible within the structures of renal corpuscles. Thus changes created during giving 2-CdA at the dose of 0.07 mg/kg b.m./24 h for 6 days in three courses intensify in renal glomeruli four weeks after administration and affect blood vessels, podocytes and nuclei of the epithelium.

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SUMMARY

The renal glomeruli of the kidney of white Wistar rats were examined. The animals were given Cladribine (2-CdA) subcutaneously at dosages of 0.07 mg/kg b.m./24 h for 7 days and 0.1 mg/kg b.m./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 or 4 weeks after the last dose. The kidney samples were taken for ultrastructural examination. In all of the groups, changes were observed but the intensity differed: 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 as well as infiltrations around the renal glomeruli. Most changes were observed in experimental group IV: the picnotic nuclei of epithelium, widening and fusion of the foot processes of podocytes (the narrowing of the filtration spaces) and numerous invaginations of the nuclear envelope of damaged podocytes.

Ultrastruktura ciałek nerkowych nerek zwierząt doświadczalnych po podawaniu Cladribine (2-CdA)

Badano ciałka nerkowe 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 elektronowym. 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, nacieki wokół ciałek nerkowych. Największe zmiany obserwowano w grupie IV: pyknotyczne jądra komórek śródbłonka, poszerzenie i połączenie wypustek stopowatych podocytów (zwężenie przestrzeni filtracyjnych), liczne wpuklenia otoczki jądrowej jąder uszkodzonych podocytów.