ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 1, 24 SECTIO D 2004

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Histomorphology of renal proximal convoluted tubules of kidney of experimental animals after Cladribine (2-CdA) administration

Cladribine (2-chlorodeoxyadenosine), 2-CdA as a antineoplastic and immunosuppressive nucleoside belongs to antimetabolites – purin analogs (1, 2, 12).

In the experimental treatment of sclerosis multiplex, Cladribine is often giving in a dose 0.07 mg/kg b.w./24 h for five successive days in six cycles with five weeks' break between each (the total dose – 2.1 mg/kg b.w.) or for six successive days in the same dose and in the same number cycles. A satisfactory effect with little side effects was usually observed after three cycles (5, 13).

2-CdA is of minor toxicity. The most common side effects are connected with its myelosuppressive action (granulocytopenia, anemia). With 2-CdA, there are observed side-effects that include opportunistic infection, skin reaction, digestive complaints, damage to autonomic nervous system, nephropathy (at larger dosage rates) (4, 9, 10).

Cladribine is excreted mainly through the kidneys (9).

Our research demonstrated that the changes in the tubules in the kidney caused by the administration of Cladribine (Biodribin), can be observed and monitored through the use of a light microscope.

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 control groups, including 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 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 being taken 24 hours following the last dose. In experimental group II, the animals were given Cladribine in the same dose and the same manner as in experimental group I, with the animals being 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; with the animals being killed 24 hours following the last dose. In experimental group IV, the animals were given Cladribine in the same dose and the same manner as 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 were stained with hematoxylin and eosin, by the PAS's method, the Masson's method, and by the Feulgen's method.

RESULTS

Control group. All staining techniques showed no deviation from the normal structure of the renal corpuscles in comparison with the control group.

Experimental group I. In experimental I group both areas with normal appearance (as in control group) and areas with large changes, were seen in the kidney sections. The epithelial cells of some of the proximal convoluted tubules looked "puffy". Their cytoplasm showed less reaction with acidic dies than in the control group. The lumen of some of proximal convoluted tubules were also filled with excretions. In the PAS met. staining, the lumen of some cells was narrowed and the intensity of staining of brush borders was similar to the control group. We also observed PAS (+) granules in the cytoplasm of the tubular epithelial cells.

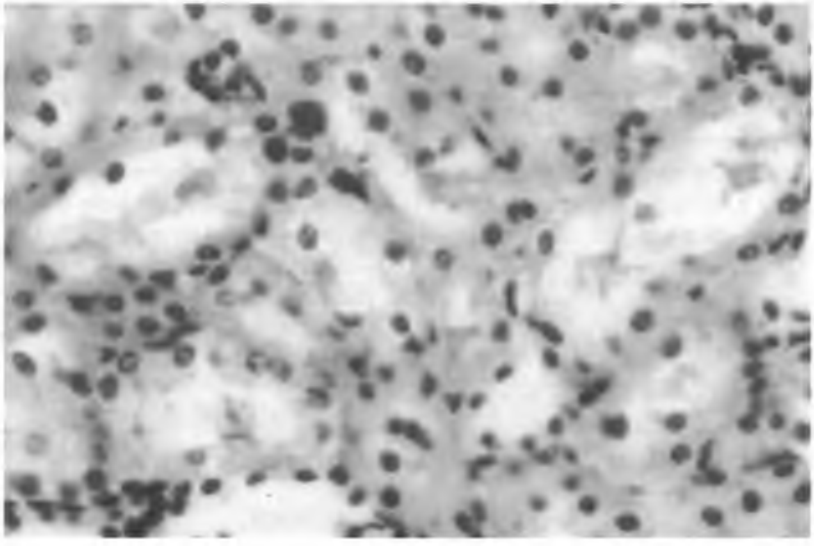

Fig. 1. Experimental group I. The rat's kidney. Sections through the proximal convoluted tubules; in their lumen – excretion. Hematoxylin and eosin staining. Magn. 800x

Experimental group II. The morphology of the kidneys of the animals of experimental group II reveal many changes in comparison with control group, in all of the staining methods used (H+E, the PAS method, the Masson's method, the Feulgen's method). We observed that some of the proximal convoluted tubules and their lumen were wider, and we observed secretions within the lumen of a few proximal convoluted tubules. In the cytoplasm of epithelial cells lining the proximal convoluted tubules, changes similar to hydropic degeneration were also seen (this is usually present when there is a lower kalium level in the blood. In the PAS met. staining, the brush border was lower and less intensively stained than in the control group and PAS (+) granules were visible in the cytoplasm of the epithelial cells.

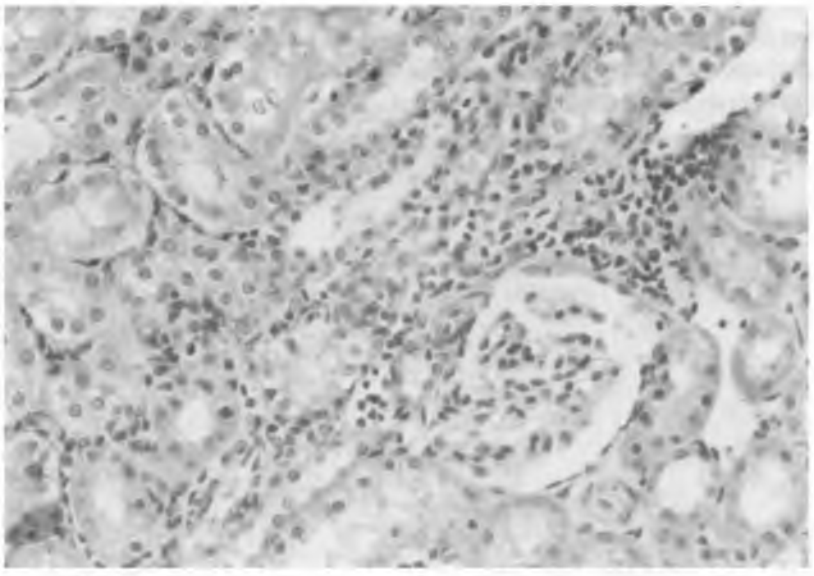

Fig. 2. Experimental group II. The rat's kidney. Sections through the renal corpusculus and proximal convoluted tubules; the vacuolar changes in cells of epithelium lining the proximal convoluted tubules. Hematoxylin and eosin staining. Magn. 400x

Experimental group III. The lumen of some proximal convoluted tubules was narrowed or was filled with a substance which inflated tubules and which was similar to hyalin. The use of the PAS staining method showed that the basement membrane of the epithelium lining the proximal convoluted tubules, was thickened , and the intensity of the brush border was a little less than in the control group. In the cytoplasm, the PAS (+) granules were observed.

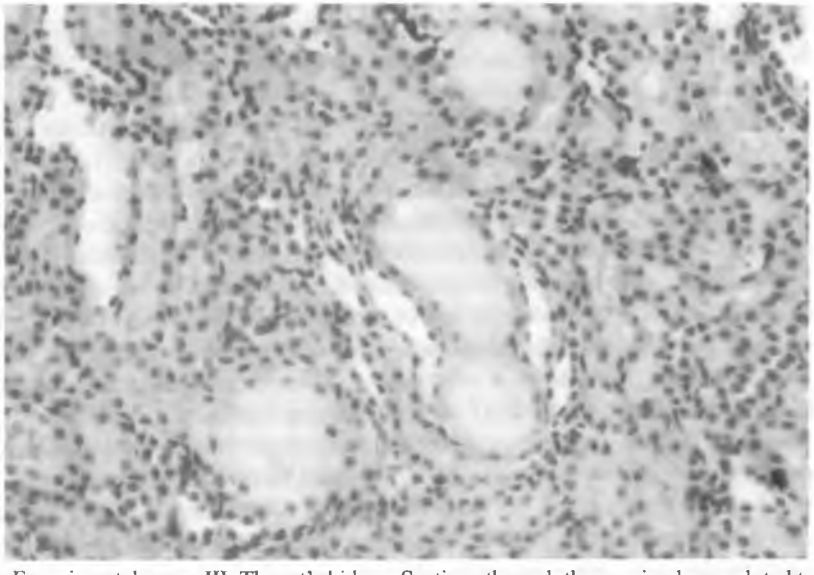

Fig. 3. Experimental group III. The rat's kidney. Sections through the proximal convoluted tubules filled with homogenous substance and through the very narrow tubules. Hematoxylin and eosin staining. Magn. 400x

Experimental group IV. In the proximal convoluted tubules, the cells of the epithelium were lower in number than in control group, thus, the lumen of the tubule was wider. Through out all of the cytoplasm of the epithelium cells, were numerous PAS (+) granules. The low brush border was also less intensively stained than in previous groups – probably due to damage.

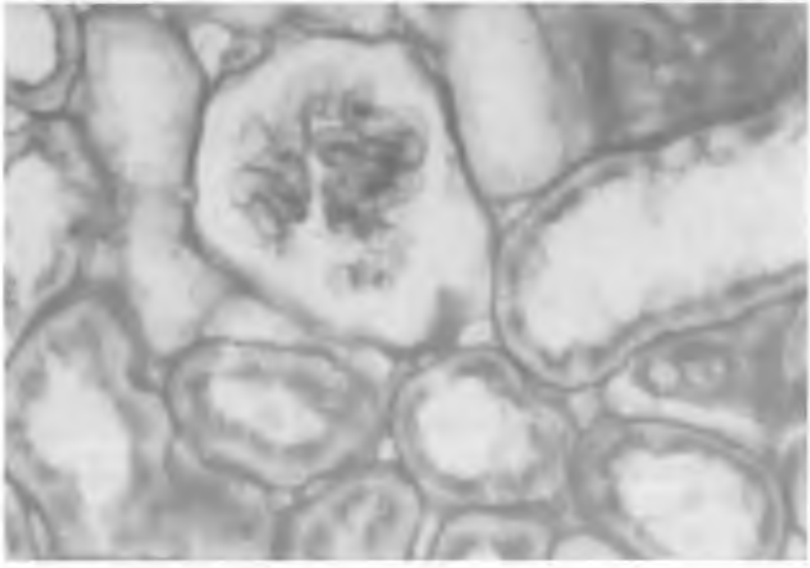

Fig. 4. Experimental group IV. The rat's kidney. Sections through the renal corpusculus and through the proximal convoluted tubules. The low and defect brush border. The cells of epithelium – low and lumen of the tubules – wide. PAS met. staining. Magn. 800x

DISCUSSION AND CONCLUSIONS

In the proximal convoluted tubules of the kidneys of animals of experimental group I, large changes were not visible. Only few proximal tubules were lined with epithelium "puffy" cells. As with cells treated with other purine or pirimidine analogs, in the proximal convoluted tubules, we observed "puffy" epithelial cells with vesicle-like nuclei, often separated from the basement membrane (6). In the cytoplasm of the epithelial cells lining the tubules, we observed numerous PAS(+) granules, which were probably glycogen granules. In addition, the lumen of some of the tubules was filled with excretions, of which the accumulation was dependent on treatment with this drug. Similar to administration with a high dose of Metotrexate, some of the tubules were wider with hyaline-like substance inside (11).

In experimental group II, damage of proximal convoluted tubules was more than in control and in experimental group I. In the cytoplasm of cells of the epithelium lining, the proximal convoluted tubules were seen to have large vacuoles forming a large part of the cytoplasm. These changes, known as "hydropic degeneration" were observed when there is a lower kalium level in the blood (7). With treatment with Adriamycin (cytostatic drug), we saw in the proximal and distal convoluted tubules, numerous nephrons that had visibly changed epithelial cells with "frothy" cytoplasm (hydropic degeneration). These cells, which were seen to be very flattened, rested on thick basement membrane. The irregular lumen of these tubules, was filled with PAS(+), homogenous substance. The borders between cells of the epithelium lining the tubules were not visible. In the cytoplasm, numerous large vacuoles and granules were seen to be present. In some places around the tubules, we observed infiltrations (8).

The large amount of glycogen granules in the cytoplasm of the cells of the epithelium lining proximal convoluted tubules, that were visible in PAS staining and in the electron microscope scanning, could be the result of increased accumulations within the cells or could be the result of weakened breakdown within their lysosomes. Disintegration of brush border (the blurring of borders between microvilli) could lead to disturbances in the physiological absorption of substances, This could be the partial explanation to the presence of excretions in the lumen of some of the proximal convoluted tubules. The blurred structure of brush border could also be the result of changes in the glycocalix which covers microvilli.

In experimental group III, changes in proximal convoluted tubules were more intensitive than in experimental group I or II. The lumen of most of proximal convoluted tubules was very narrow. This could be associated with lesser effectiveness of cells which pump ions (cells of epithelium of proximal convoluted tubules) as a result of unknown substances within the organism which are excreted by the kidney. We also noticed that some of the proximal convoluted tubules were wider and in their lumen were present substances which were composed of protein, according to Kruś (7). Some effects like the widening tubules and the interruption of the basement membrane, were reported by Erdinc et al., after administering the drug Cisplatin (3). We observed that when Ifosfamid was administered, proximal convoluted tubules showed damage (6). Levels of urine and creatinine were also raised, and in the blood, oliguria, glucosuria, aminoaciduria and electrolituria were also evident.

We noticed in our research that giving Cladribine at the dose of 0.1 mg/kg b.m./24 h for 7 successive days leads to small changes in the proximal convoluted tubules of kidney, but giving 2-CdA at the dose of 0.07 mg/kg b.m./24 h for 6 days in three courses leads to large changes in their epithelium. Changes increased with Cladribine administered in 3 cycles, and increased in examined groups 4 weeks after administration. This shows the necessity of examining kidney activity after finishing the normal multi-step treatment.

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SUMMARY

The proximal convoluted tubules of the kidney of the white Wistar rats were examined. The animals were given Cladribine (2-CdA) sub-cutaneously at the dose: 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's samples were taken for histological and histochemical examination and were stained with hematoxylin and eosin, using the Masson's, the PAS's, and the Feulgen's method. In experimental group I, we observed few changes (in comparison to the control group): cells of the epithelium of some of the proximal convoluted tubules were however, puffy. In a few tubules we observed some unknown substance and the lumen of some of these tubules was narrowed. In experimental group II, a few proximal convoluted tubules and their lumen were wider with an unknown substance within. We also observed hydropic degeneration. The brush border of these tubules was a little lower than in control group. In experimental group III, the cells of the epithelium of proximal convoluted tubules rested on a thicker basement membrane, the lumen of most of the proximal convoluted tubules being narrow and filled with some unknown substance. In experimental group IV, the lumen of the tubules was a little wider, and the epithelial cells were smaller than in the control group, thus the lumen of the tubules was wider. In the cytoplasm of epithelial cells we observed numerous PAS(+) granules. The low brush border appeared damaged.

Histomorfologia kanalików proksymalnych nerki zwierząt doświadczalnych po podawaniu Cladribine (2-CdA)

Badano kanaliki kręte 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 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. Massona, met. Feulgena. W grupie I obserwowano niewielkie zmiany (w porównaniu z kontrolą): komórki nabłonka niektórych cewek proksymalnych sprawiały wrażenie obrzmiałych. W niewielu kanalikach obserwowano zastój wydzieliny, a światło niektórych z nich było zwężone. W grupie II nieliczne kanaliki proksymalne, jak i ich światło były poszerzone z wydzieliną w środku, obserwowano zwyrodnienie wielkowodniczkowe; rąbek szczoteczkowy był nieco niższy niż w grupie kontrolnej. W grupie III komórki nabłonka kanalików proksymalnych spoczywały na pogrubiałej błonie podstawnej, światło większości kanalików było wąskie i wypełnione wydzieliną przypominającą wałeczki szkliste. W grupie IV światło kanalików było nieco poszerzone, komórki nabłonka wydawały się niższe niż w grupie kontrolnej, wobec czego światło kanalika było dość szerokie, w cytoplazmie komórek nabłonka widoczne były liczne PAS dodatnie ziarnistości. Niski rąbek szczoteczkowy był uszkodzony.