ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 1, 27 SECTIO D 2004

Department and Institute of Histology and Embryology, Skubiszewski Medical University of Lublin

MARTA LIS-SOCHOCKA, MIROSŁAW ŁAŃCUT, KRYSTYNA CZERNY

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

Cladribine (2-chlorodeoxyadenosine), 2-CdA – a potent chemo-therapeutic and immuno-suppressive nucleoside belongs to antimetabolites, purine analogs. The group includes e.g. pentostatin (2'-deoxycoformicin, dCF) and fludarabine (FAMP) (7, 15). 2-CdA is equally toxic to dividing and to non-dividing cells in tissue culture (4). 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 (2, 3, 15). Cladribine, in non-dividing cells induces apoptosis – programmed cell death (1, 6, 9, 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 (2, 3, 14). Recently, because of its immuno-suppressive activity, the drug has been trialed for therapy for the treatment of auto-immune diseases such as multiple sclerosis (8).

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, and nephropathy – which occurs at great dosage rates (14).

Cladribine is excreted mainly through the kidneys.

Our research shows the changes in proximal convoluted tubules during administration of Cladribine (Biodribin).

MATERIAL AND METHODS

The experiment was carried out on female 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 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 four 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 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 using the Tesla BS-500 transmission electron microscope.

RESULTS

Control group. In the control group the ultrastructure of the proximal convoluted tubules of kidney showed no deviation from normal structure.

Experimental group I. The proximal convoluted tubules were not changed in comparison with the control group.

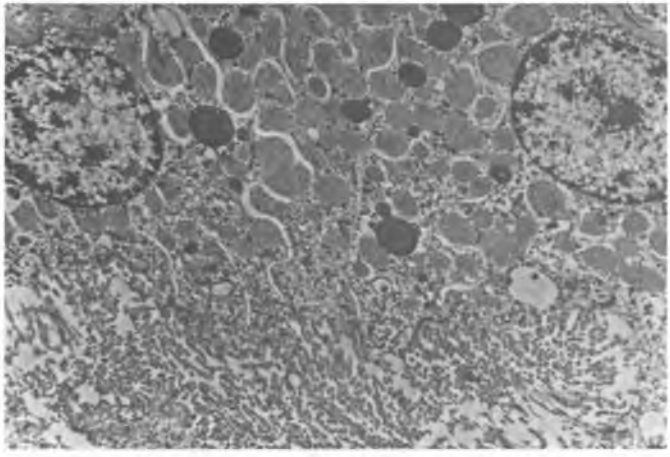

Fig. 1. Experimental group I. The rat's kidney. Section through the cells of the epithelium lining the proximal convoluted tubules – the normal appearance. TME. Magn. 6000x

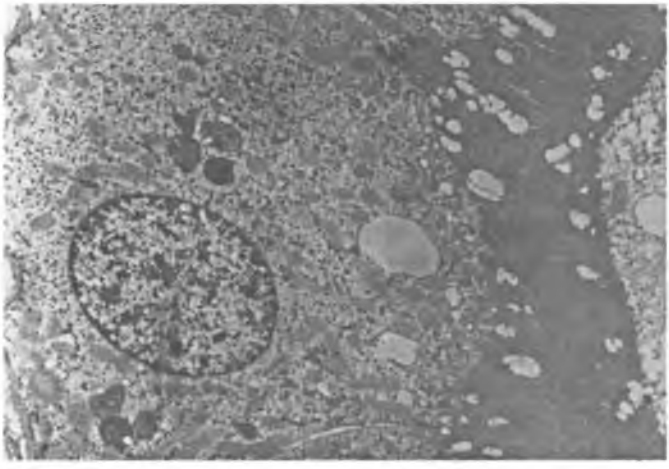

Fig. 2. Experimental group II. The rat's kidney. Section through the cells of the epithelium lining the proximal convoluted tubules. The decreased amount of irregularly located mitochondria and increased amount of glycogen granules are visible. Diminished border of the microvilli of brush border and no homogenous substance in the lumen of the tubule. TME. Magn. 6000x

Experimental group II. In the cytoplasm of the epithelium cells, in the upper part of the cell (near the lumen) were large vacuoles filled with a homogenous substance. In the brush border, which covered all surface of the epithelium cells, striping was also less distinct than in control group. In the basal part, the mitochondria were irregularly composed. The glycogen granules were very numerous.

Experimental group III. In the proximal convoluted tubules, changes were distinctly visible. The basement membrane of the epithelium was thicker than in control group. Mitochondria, less numerous than in control group, were also irregularly composed in the cytoplasm. In the mitochondria were quite evident mitochondrial cristae and the mitochondrial matrix was electron dense. There was also little diluted cytoplasm around the nucleus. In the cytoplasm, fibrous material was seen. Throughout the cytoplasm were small glycogen granules, but they were less numerous than in the other experimental groups. The brush border was however, thinner, and there was hyperaemia of the kidney.

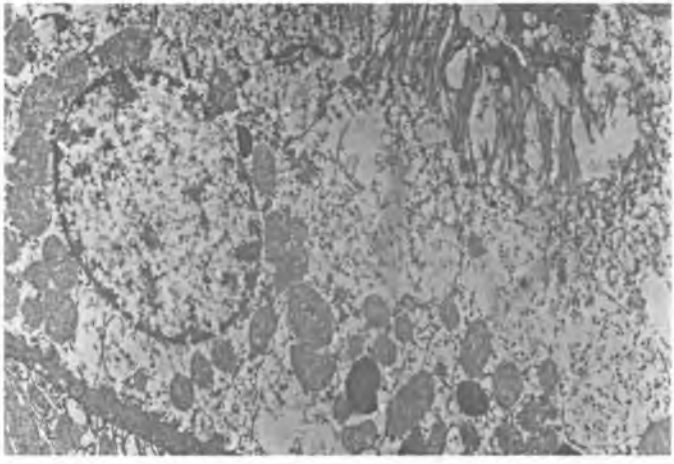

Fig. 3. Experimental group III. The rat's kidney. Section through the cells of the epithelium lining the proximal convoluted tubules. The thickened basement membrane, the decreased amount of irregularly located mitochondria, the damaged brush border are visible. The lumen of the tubule is filled with substance. TME. Magn. 4000x

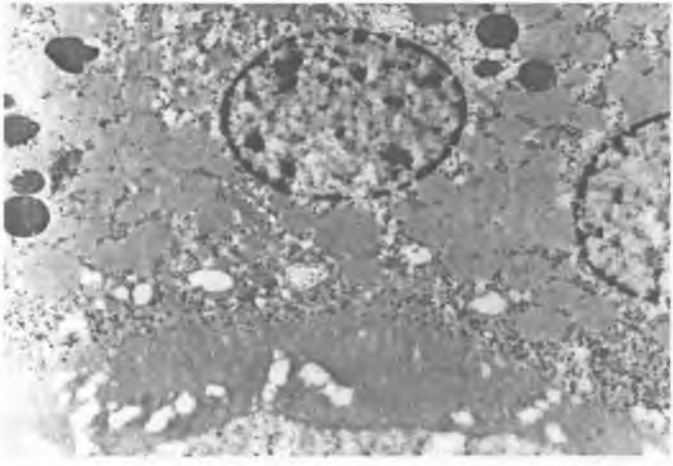

Fig. 4. Experimental group IV. The rat's kidney. Section through the cells of the epithelium lining the proximal convoluted tubules. The decreased amount of irregularly located mitochondria, numerous glycogen granules, the damaged, low, brush border are visible. TME. Magn. 6000x

Experimental group IV. In the basal part of the cell were more mitochondria than in experimental group III. Mitochondria were also filled with electron dense mitochondrial matrix. In the cytoplasm of the epithelial cells, secondary lysosomes were visible, while in the apical part of the cell were middle magnitude vacuoles and numerous glycogen granules. Around the nuclei was a diluted cytoplasm.

DISCUSSION AND CONCLUSIONS

Despite the minor toxicity of Cladribine (2, 3, 7, 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), like other cytostatics (10, 13).

The proximal convoluted tubules of animals of experimental group I were not changed in comparison with control group. In experimental group II the vacuoles located in the apical part of the cytoplasm, filled with the undifferentiated substance, showed intensified pinocytosis due to the change of initial urine composition and a gain due to glomerulal damage, and damage of the brush boundaries. The irregular position of the mitochondria in the basement layers of the labyrinth, as well as their much reduced quantity showed the lowered metabolic efficiency of the lining cells. The multitude of brushes and electron-dense parenchyma in these structures reveals that the intensity of their metabolic activity was greater when their numbers were lowered. The excessive amount of glycogen granules in the cytoplasm of the lining cells of the proximal tubules which was noticeable in the PAS staining method as well as under the electron microscope, could have been due to its greater accumulation in the cells or due to the faulty breakdown in lysosomes. Disintegration of the brush border showed as a diminished border of the microvilli could lead to the faulty physiological absorption, which can explain partially the presence of secretions in the lumen of the proximal convoluted tubules. It can be thought that the vanished structure of the border brushes was due to the changes in glycocalix covering the microvilli. It is quite probable that the lessened metabolic efficiency of cells shown by the diminished numbers of mitochondria was due to the changes in the production of glycocalix. This caused the vanishing of the borders between the microvilli and at the same time the disappearance of striping. In experimental group III, the widening of the lining of the basement membrane could be the start of the faulty active transportation of ions across the lining membrane. The mitochondria with their thick mitochondrial content showed clearly visible mitochondrial combs that were irregularly scattered. Such form of mitochondria – the so-called low energy state, can be observed in the cells with quite intense metabolic processing and having a high requirement for energy. During the intensive work, the amount of ATP is diminished and the concentration of ADP is increased in these structures (11, 13). The cells of the proximal tubules are metabolically very active. Therefore such form of condensed mitochondria seems to be very suitable for these cells. The diminished amount of mitochondria, requires a substitute increase in the respiratory process of the unaffected mitochondria, thus, is revealed in the electronically dense filling and the great amount of mitochondrial combs.

The thinning of the cell cytoplasm and the presence of the fine thread-like material observed surrounding the nucleus, could be due to the swelling of the cells connected with the accumulation of water in the cell due to faulty transportation.

The appearance of secondary lysosomes in cytoplasm of experimental group IV, can be due to the intensified phagocytotic and pinocytotic processes.

Slightly different changes in some distal tubules could be observed after the administration of methotrexate (10 mg/kg) in guinea pigs. There was observed swelling of the cells lining the tubules, with the changed organelles, increased and enlarged vacuoles and electron-dense substances in the upper part of the cytoplasm and disorganization of the microvilli (5).

The administration of Cladribine in the dosage of 0.1 mg/kg of body weight/24 h for 7 days does not lead to instant changes in the ultrastructure of the proximal convoluted tubules; changes appear after four weeks of post-administration of the drug. Administration of Cladribine in the dosage of 0.07 mg/kg body weight per 24 h for 6 days in three cycles leads to quite, visible changes in the kidney tubules. However, these changes are not intensified after four weeks' post-administration.

REFERENCES

1. Barbieri D. et al.: Apoptosis by 2-chloro-2'-deoxy-adenosine and 2-chloro-adenosine in human peripheral blood mononuclear cells. Neurochemistry International, 32, 5-6, 493, 1998.
2. Beutler E.: Cladribine (2-chlorodeoxyadenosine). Lancet, 340, 17, 952, 1992.
3. Beutler E.: New Chemotherapeutic Agent: 2-Chlorodeoxyadenosine. Seminars in Hematology, 31, 1, 40, 1994.
4. Carson D. A. et al.: Specific toxicity of 2-chlorodeoxyadenosine toward resting and proliferating human lymphocytes. Blood, 62, 4, 737, 1983.
5. El-Badawi M. G. et al.: Nephrotoxicity of low-dose Methotrexate in guinea pigs: an ultrastructural study. Nephron, 73, 462, 1996.
6. Genini D. et al.: Deoxyadenosine analogs induce programmed cell death in chronic lymphocytic leukemia cells by damaging the DNA and by directly affecting the mitochondria. Blood, 96, 10, 3537, 2000.
7. Góra-Tybor J., Robak T.: Farmakologia kliniczna 2-chlorodeoxyadenozyny (Kladrybiny). Przegl. Lek., 53, 8, 614, 1996.
8. Grieb P., Stelmasiak Z.: Leczenie stwardnienia rozsianego przy pomocy nowego immunosupresanta Kladribiny (2-CdA). Neurologia i Neurochirurgia Polska, 29, 1, 69 1995.
9. Guchelaar H. J. et al.: Apoptosis and necrosis-inducing potential of cladribine, cytarabine, cisplatin, and 5-fluorouracil *in vitro*: a quantitative pharmacodynamic model. Cancer Chemotherapy and Pharmacology, 42, 77, 1998.
10. Kintzel P. E.: Anticancer drug-induced kidney disorders. Drug Safety, 24, 1, 19, 2001.
11. Kruś S.: Patomorfologia nerek. PZWL, Warszawa 1986.
12. Kuttesch J. F., Nelson J. A.: Renal handling of 2'-deoxyadenosine and adenosine in humans and mice. Cancer Chemotherapy and Pharmacology, 8, 221, 1982.
13. Orłowski T.: Choroby nerek. PZWL, Warszawa 1997.
14. Robinson C. P.: New products: Cladribine. Drugs of Today, 29, 6, 379, 1993.
15. Szmigielska-Kapłon A., Robak T.: Mechanizmy działania cytostatycznego nowych analogów puryn. Nowotwory, 50, 5, 523, 2000.

SUMMARY

The proximal convoluted tubules 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 three courses with 5 weeks' break between each. The animals were decapitated 24 hours after the last dose of the drug and 4 weeks after the last dose. The kidney samples were taken for ultrastructural examination. Giving Cladribine at the dose of 0.1 mg/kg b.m./24 h for 7 successive days does not lead to instant changes in the ultrastructure of the proximal convoluted tubules. Changes noticed 4 weeks after Cladribine administration are the following: decrease in amount of mitochondria, the presence of numerous vacuoles, changes in the structure of the brush border, presence of numerous glycogen granules, and the presence of a diluted cytoplasm around the nucleus.

Giving 2-CdA at the dose of 0.07 mg/kg b.m./24 h for 6 days in three courses leads to similar changes in proximal convoluted tubules with more extensive damages of the brush border. These were no more intensive after 4 weeks' break in Cladribine administration.

Ultrastruktura kanalików proksymalnych nerki zwierząt doświadczalnych po podaniu 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. Próbki materiału biologicznego 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. Podawanie Cladribine w dawce 0,1 mg/kg m.c./ 24h przez 7 dni nie prowadzi do natychmiastowych zmian w budowie ultrastrukturalnej cewek proksymalnych. Zmiany te występują po 4 tyg. przerwy w podawaniu leku i polegają na zmniejszeniu liczby mitochondriów, obecności licznych wakuoli, zaburzeniu w strukturze rąbka szczoteczkowego, obecności licznych ziaren glikogenu, rozrzedzeniu cytoplazmy wokół jądra. Podawanie Cladribine w dawce 0,07 mg/kg m.c./24h przez 6 dni w trzech cyklach prowadzi do podobnych zmian w kanalikach nerkowych z jeszcze większym uszkodzeniem rąbka szczoteczkowego. Nie nasilają się one po 4 tygodniach przerwy w podawaniu leku.