ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 110 SECTIO D 2004

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The proximal convoluted tubule of rats' nephron after experimental administration of gentamicin

Gentamicin is an antibiotic with a wide spectrum of action on G(-) and G(+) microbes. It is used in therapy of over 2% of patients and demonstrates nephrotic action (4). Reports of gentamicin nephrotic action (4, 5, 9, 11) led us to attempt observations of the histological and ultrastructural changes of proximal convoluted tubules of nephron cells of white rats directly after ten days' administration of the drug, and after three weeks' break in the drug's administration.

MATERIAL AND METHODS

The white rats (females) received intraperitoneally gentamicin in the dose of 16 mg/24h in 0.4 ml solution in a double dose for ten days. The using dose was 10 times the therapeutical dose. The rats of experimental group I were decapitated after the last injection. Rats of experimental group II were decapitated 3 weeks after the last dose. The animals of control group were decapitated simultaneously with the animals from experimental groups. All animals were fed with standard granulated fodder and water *ad libitum*. The paraffin samples of the kidney were stained with hematoxylin and eosin, with PAS–McManus method (on polysaccharides), and with the Brachet method (on RNA). The material for ultrastructural investigations was made with the standard method. Observations were made and photographs taken, using a JenaMed light microscope made by Carl Zeiss Jena. The ultrastructure of the cells was photographed with transmission electron microscope Tesla BS 500.

RESULTS

The kidneys of animals of experimental group I showed in the optic microscope various intensity of changes in individual samples. The kidneys of two rats showed a notable dilution of the cytoplasm of the proximal convoluted tubules cells and desertion in the basal part of cytoplasm (Fig. 1). Other individuals of this group showed localised clearing around cells nuclei and sporadic desertion in the cytoplasm. Besides some considerably changed cells, we observed cells with mitotic figures (Fig. 2). PAS reaction (on polysaccharides) showed nonequal staining of the brush border of proximal convoluted tubules in the kidney cortex. RNA stained with the Brachet method were located nonequally. The cells of some of proximal convoluted tubules did not include pironinophilic granules in basal or around nucleic parts of the cytoplasm.

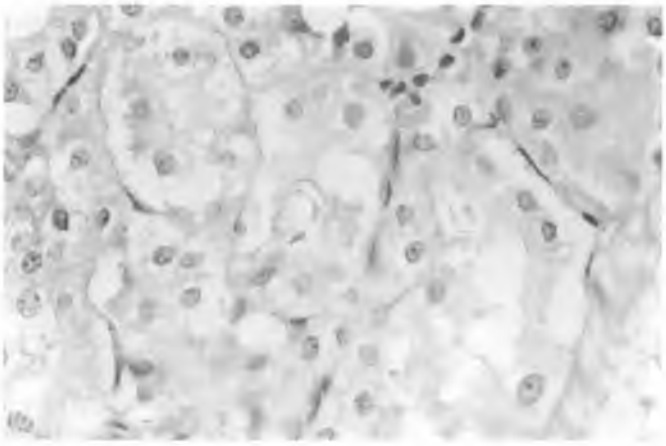

Fig. 1. Experimental group I. The kidney of the rat. Deserting in the basal part of the cytoplasm. Hematoxylin and eosin. Magn. 400x

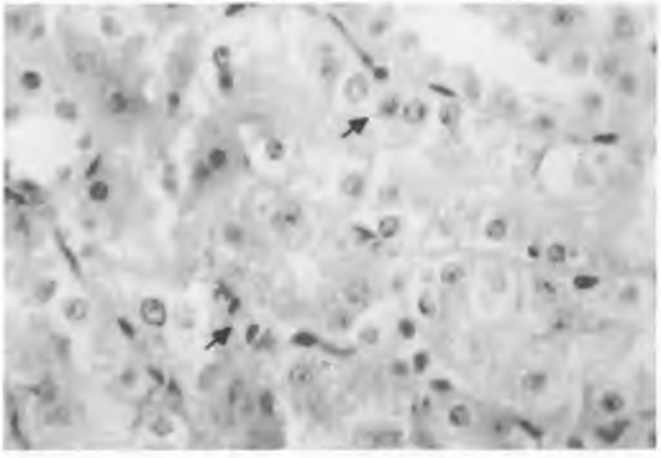

Fig. 2. Experimental group I. The kidney of the rat. The cells divsions – arrows. Hematoxylin and eosin. Magn. 400x.

Electron microscope photos of the cells of renal proximal convoluted tubules revealed changes of various intensity. Evident were numerous, various sized, sometimes large lysosomes containing substances of various electron density (Fig. 3). Mitochondria with no distinct cristae showed swelling features. The tubules of endoplasmic reticulum were widened forming sometimes large vacuoles in the basal part of the cytoplasm (Fig. 4).

The cells of proximal convoluted tubules epithelium stained with hematoxyllin and eosin, of animals of experimental group II resembled under the optic microscope the control group, but the lumen of the tubules often showed eosinophilic homogeneous material. The results of staining on polysaccharides and RNA were similar to the kidney of control animals.

In ultrastructure of cells were visible, sometimes insignificantly widened tubules of endoplasmic reticulum, as well as dilution of cytoplasm. The size of more numerous lysosomes was similar to that in lysosomes in proximal convoluted tubules cells of the kidney of control animals, while the structure of mitochondria was normal.

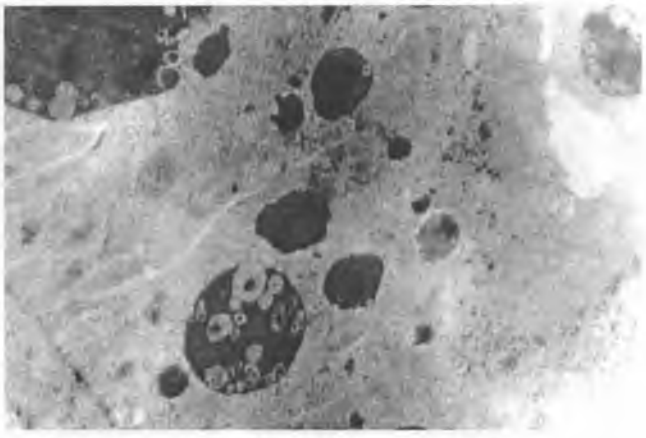

Fig. 3. Experimental group I. The kidney of the rat. The proximal convoluted tubule. A large lysosomes with material with various electron density. Magn. 6 000x

Fig. 4. Experimental group I. The kidney of the rat. The proximal convoluted tubule. A large vacuole in the basal part of the cytoplasm. Magn. 10 000x

DISCUSSION

In the experiment, after ten days' administration of gentamicin, the intensity of changes in proximal convoluted tubules of kidney of certain rats was quite various. The observed small clearing and considerable dilution and desertion in the cytopolasm found reflection in nonequal presention of RNA granules disclosed in the Brachet reaction. Similar results were presented by other authors (6, 7, 12). They observed, using various dosages of gentamicin, depending on the dose and time of action, clearing of cytoplasm and necrosis of tubules cells as well as their peeling (6, 8, 9). The vacuolar degeneration features of cells of proximal convoluted tubules and inflammatory infiltrations in young rats kidney were noted by R y m g a y ł ł o - K ą t s k a (11) after using lower dosages of gentamicin than those in our experiment. In the electron microscope photos evident were numerous, enlarged lysosomes, sometimes enormously so, filled with material with various electron density. Much enlarged lysosomes with lamellar bodies forming groups were noted by other authors after using gentamicin in various dosages. The intensity of changes was proportional to the using dose of gentamicin (8, 9, 11). Gentamicin, like other aminoglycosides penetrates in the way of endocytosis into the cytoplasm and accumulates

mainly in lysosomes (11). The myelin structures observed after administration of a large dose of gentamicin can show upon intensification of autophagocytosis (11) and also can result from lipids metabolism disturbances (5). An inhibiting action of gentamicin on phospholipase and sphingomy--elinidase activity (13) has been demonstrated.

The investigations of lysosomal enzymes activity in urine showed statistically an essential increase of activity of enzymes connected with sugar compound changes (1, 10). It has been observed that a small widening of the tubules of endoplasmic reticulum can result from increasing synthesis of enzymatic proteins not connected with lipids changes (13), but considerable inflation of reticulum tubules and the presence of large vacuoles and swelling of mitochondria show a decrease of oxidative phosphorilation and disturbances of electrolyte balance. The presence of large desertion in the cytoplasm can be an effect of the bursting of overloaded lysosomes. This could lead to irreversible changes, the death of cells and their elimination.

Disturbances of the structure and function of kidney produced by gentamicin can be detected and verified in biochemical investigations of urine and serum (1, 2, 3, 4, 5, 10). Nephrotoxic action of gentamicin can be increased by disturbances from other organs or by the simultaneous use of other drugs, e.g. captopril (9).

The kidneys of animals after 3 weeks' break in administration of gentamicin (experimental group II) showed only small disturbances of endoplasmic reticulum structure (the other organelles were normal). Above observations show a reversible character of the changes produced by gentamicin action (11). The cells in mitotic divisions with visibly damaged tubules testify to the large regenerative ability of tubules. Cell divisions of cells with damaged kidney tubules (after gentamicin administration) were observed by other investigators (11).

We must bear in mind that regeneration of damage caused by gentamicin within the kidney amounts to the return to normality of changes with minor intensity and to elimination within considerably damaged cells, which were replaced in regenerating processes (there were visible cells divisions). Our observations show an individual reaction of animals to nephrotoxic action of gentamicin, manifested by various intensity of changes.

CONCLUSIONS

1. Various intensity of observed changes in kidney of animals may be produced by individual coexistence of factors increasing gentamicin nephrotoxicity.

2. Little changes in the structure of cells of proximal tubules of rats' kidneys, after 3 weeks' break in gentamicin administration (experimental group II) testify to the reversible character of changes and to the regenerative ability of the epithelium of proximal convoluted tubules.

3. Gentamicin in therapy should be used with caution in view of its nephrotoxic side-effects.

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SUMMARY

The rats in this experiment received gentamicin in intraperitoneal injections in a dose ten times bigger than therapeutical. After ten days of drug administration, in the cells of proximal convoluted tubules of kidney there were observed the dilution of cytoplasm of various intensity, increases in amount and size of lysosomes, widening of endoplasmic reticulum tubules and swelling of mitochondria. The microscopic and ultrastructural pictures, after three weeks' break in gentamicin administration, showed fast regeneration of renal tubules. A large diversity of intensification of changes in particular individuals suggested individual reaction to gentamicin nephrotoxicity.

Kanalik proksymalny nefronu szczurów po doświadczalnym podawaniu gentamycyny

Szczurom podawano gentamycynę w iniekcjach dootrzewnowych w ilości odpowiadającej dziesięciokrotnej dawce terapeutycznej. Po dziesięciu dniach podawania leku w komórkach kanalików proksymalnych nerki obserwowano o różnym nasileniu rozrzedzenia cytoplazmy, zwiększenie ilości i wielkości lizosomów, poszerzenia kanałów siateczki śródplazmatycznej oraz obrzmienie mitochondriów. Obrazy mikroskopowe i ultrastruktury po trzytygodniowej przerwie w podawaniu gentamycyny wskazywały na szybką regenerację kanalików nerkowych. Duże zróżnicowanie w nasileniu zmian u poszczególnych osobników sugeruje indywidualną reakcję na nefrotoksyczność gentamycyny.