ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLV, 22 SECTIO D 1990

Katedra i Zakład Histologii i Embriologii z Pracownią Cytologii Doświadczalnej. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. n. med. Irena Królikowska-Prasał

Maria MATYSEK, Tamara MAJEWSKA

Histochemical Investigations on the Influence of Vibramycin on the Kidneys of Rats

Badania histochemiczne nad wpływem wibramycyny na nerki u szczurów

In the present study the functional state of the kidneys was examined immediately after ten days of therapy with Vibramycin. It is supposed that sometimes the nephropathic influence does not depend on the drug itself, but on the products of its metabolism. In order to check this supposition Vibramycin with expired validity was used to find out whether it may have a nephrotoxic effet. The lack of experimental data concerning the influence of Vibramycin on the renal enzyme system prompted us to investigate some selected cell markers the localization of which in certain definite structures of the kidney may be evidence of tolerance to the drug applied.

MATERIAL AND METHODS

The experiments were performed with 10 white Wistar rats weighing 220—250 g. The animals were divided into 2 experimental groups (I, II) and were given Vibramycin („Polfa", Tarchomin). The group I received after fasting 2 ml of aqueous suspension of valid Vibramycin in a dose of 0.80 g/kg b.wt. intragastrically over a period of 10 days. Under the same conditions rats of group II were given Vibramycin with expired validity. The control group consisted of three animals receiving intragastrically 2 ml of water daily for 10 days.

Immediately after the experiment the rats were killed and the kidneys were taken for histological and histochemical examinations. Reactions for polysaccharydes were run on paraffin sections by the PAS method after McManus (8) and staining with haematoxylin and eosine. Other kidney sections, after fixation in Baker's solution served as material for histochemical reactions for alkaline and acid phosphatase activity after Gomori and detection of thiamine pyrophosphate phosphohydrolase (TPP-ase) after Novikoff and Goldfischer (8)

RESULTS

Serial examinations of the kidney preparations after haematoxylin eosin demonstrated in both experimental groups a shrinking of the glomeruli of the blood vessels of the renal bodies as compared with the control (Figs. 1—3). Moreover, the same examinations revealed in group II pronounced changes consisting in dilated segments of the convoluted tubules, focal lymphatic infiltration and distinctly widened blood vessels. Moreover, in the convoluted tubules, especially the distal ones the acidophilicity of cytoplasm was increased (Fig. 3). As compared with the control group, the reaction for glycogen was weaker in group I (Figs. 4, 5). After Vibramycin with expired validity the PAS reaction was modified and of diffusional character (Fig. 6). The reaction for alkaline phosphatase in the control animals and after valid Vibramycin was similar, whereas after the expired drug it was more intensive. In the structurally changed cortical part of the kidney the reaction for alkaline phosphatase occurred in the brush border of the convoluted tubules of first order. The activity of this enzyme appeared also in the basement membranes of the renal tubule epithelium. An intensive reaction was also observed in the renal glomeruli.

The pattern of acid phosphatase activity in the cortical part of the kidney in group I was similar to that in the controls (Figs. 7, 8). Active granules were seen above all in the neighbourhood of the peribasal convoluted tubules of first order. The intensive of the acid phosphatase reaction in the main tubules after expired Vibramycin was much stronger than in the other animals (Fig. 9). The pronounced great increase of the enzymatic marker in the widened renal tubules. Coloured grains were so densely packed that the tubules lumen was not visible.

The reaction for TPP-ase after the valid antibiotic was similar to that in the control. In group II the reaction was of diffusive character and irregular under the influence of the drug with expired validity. The convoluted tubules belonging to various nephrons differed from one another in the amount of coloured product and intensity of colouring.

DISCUSSION AND CONCLUSIONS

After oral application Virbamycin is rapidly and almost completely absorbed, 50% of this antibiotic is absorbed within 30 min and complete absorption reaches 95% (2). Tetracyclines are evacuated with urine or faeces, but the main route are the kidneys (7). According to literature data ca 70% of Vibramycin filtered through the renal glomeruli undergo resorption in the tubules. This explains the long subsistence of the drug in the peripheral blood and renal tissue (2).

Serial examination with the use of haematoxylin and eosin of kidney preparations from animals receiving the valid antibiotic and that with expired validity revealed shrinking of the renal glomeruli and leucocyte infiltrations close

to them. The blood renal vessels were distended. Comparison of the experimental data indicated that expired Vibramycin caused more severe nephropathy after application. Probably the products of its degradation interfered with the process of reabsorption, leading to dyselectrolytemia and tabular acidosis. This conclusion is supported by literature data (6).

Alkaline phosphatase participates in the processes of production and metabolic regeneration of nucleic acids and makes possible absorption in the proximal segment of the renal tubule. This reaction was greatly enhanced in the experimental group II. The reaction was diffusive and involved, beside the tubules, the endothelia and glomeruli. These results lead to the supposition that administration of expired Vibramycin disturbs the physiological alkaline phosphatase level. Such a level probably does not favour normal transport through the cell membranes. This is confirmed by the weak histochemical reaction of diffusive character for glycogen and polysaccharides.

Acid phosphatase is in general considered to be a histochemical marker of lysosomes. The acid phosphatase reaction may be assumed as a valuable indicator of both the present acid phosphatase activity of the phagocytes and sensitive indicator of intracellular lytic processes. In the present study the picture of acid phosphatase activity in group I was similar to that in the control group. It was distinctly different in group II where the reaction was very intensive. The coarse grains in the reaction for acid phosphatase suggested the appearance of larger lysosomes. The latter may arise in processes of enhancement of physiological metabolism, thus indicating increased pinocytosis and phagocytosis (5).

Specific phosphatase TPP-ase in known to be an enzyme functionally and structurally associated with the Golgi apparatus. It takes an active part in secretory processes of the cells. In the control group during this study an intensive positive reaction was observed in the kidneys for TPP-ase, indicating normal vital functions of the cells of proteins, blood vassels and convoluted tubules of first order (4). It was noticed in the experimental group I that the convoluted tubules of the first order differ from one another in the intensity of colouring of the product. These changes were more pronounced in group II moreover, there was a distinct diffusion reaction. These observations seem to indicate a participation of the Golgi apparatus, strictly speaking its thiamine pyrophosphatase marker in the carbohydrate transformations of the cell. This takes place by way of regulation transformation of thiamine and its phosphate derivative compounds, the suitable amount of which conditions, among other things decarboxylation of pyruvic acid in the process of glycolysis (6).

The observed results of weakened intensity of reaction for thiamine propylophosphatase correspond to the disturbances in the PAS reaction after expired Vibramycin. They may be the consequence of disorders in the physiological function of the Golgi structures involved in the metabolism of the examined tissues.

Conclusions

1. Vibramycin with the expired validity causes nephropathies consisting in shrivelling of the renal glomeruli, dilatation of tubule segments and focal lymphocyte infiltrations.

2. Acid phosphatase activity in the main tubules after 10 days of treatment with expired Vibramycin was much higher than in the other groups of animals.

3. The reaction for alkaline phosphatase in the control animals and in those after valid Vibramycin application was similar, whereas after the expired drug application the reaction was distinctly more intensive.

4. The picture of the reaction for TPP-ase after the valid antibiotic application was similar to the control animals, whereas in the group II — receiving the expired drug — the reaction was diffusive and irregular.

5. The reaction for glycogen was weaker in the experimental groups as compared with that in the control one. Moreover, after expired Vibramycin application the reaction was of diffusive character.

REFERENCES

1. Fabre J., Milek E., Kalfopoulos P., Mérier C.: Le comportement des tétracyclines chez l'homme. Schweiz. Med. Wschr. **101**, 625, 1971.
2. Gerkowicz T., Bekajowa B.: Leczenie odmiedniczkowego zapalenia nerek u dzieci Wibramycyną. Pol. Tyg. Lek. **26**, 832, 1971.
3. Kifer E.: Wpływ Lebacidu na histochemicznie oznaczaną aktywność enzymów w nerkach szczurów. Ann. Univ. M. Curie-Skłodowska, Lublin, Sectio D **36**, 32, 1981.
4. Staszyc J., Kifer E., Lipski M.: Badania cytofizjologiczne nad wpływem Diuramidu na nerki szczura. Ann. Univ. M. Curie-Skłodowska, Lublin, Sectio D **26**, 105, 1981.
5. Staszyc J.: Badania cytochemiczne nefronów po operacyjnym podwiązaniu tętnicy nerkowej u szczurów. Ann. Univ. M. Curie-Skłodowska, Lublin, Sectio D **27**, 11, 1982.
6. Tatoń J.:Kliniczna farmakologia niepożądanego działania leków. PZWL, Warszawa 1985.
7. Vibramycin Pfizer Comparation. Informacja kliniczna nr 4. Ośrodek Informacji Naukowej „Polfa" 1895.
8. Zawistowski S.: Technika histologiczna. PZWL, Warszawa 1986.

Otrzymano 1990.06.21.

STRESZCZENIE

Do badań użyto szczurów, samic rasy Wistar, którym przez 10 dni dożołądkowo podawano wibramycynę z aktualnym terminem ważności (grupa I) oraz przeterminowaną (grupa II). Zwierzęta kontrolne otrzymywały dożołądkowo wodę pitną. Bezpośrednio po doświadczeniu szczury dekapitowano, aby pobrać nerki do badań histologicznych i histochemicznych. Stosowano barwienie hematoksyliną i eozyną, histochemicznie wykrywano aktywność fosfataz: kwaśnej i zasadowej oraz obecność fosfohydrolazy pirofosforanu tiaminy, wykonano także reakcję PAS. Stwierdzono, że wibramycyna przeterminowana wywoływała ciężką nefropatię, objawiającą się zmianami w budowie histologicznej i zaburzeniami aktywności enzymów komórkowych nerek. Wyniki badań przeprowadzonych u zwierząt otrzymujących antybiotyk z aktualną datą ważności nie różniły się zasadniczo od uzyskanych u zwierząt kontrolnych.

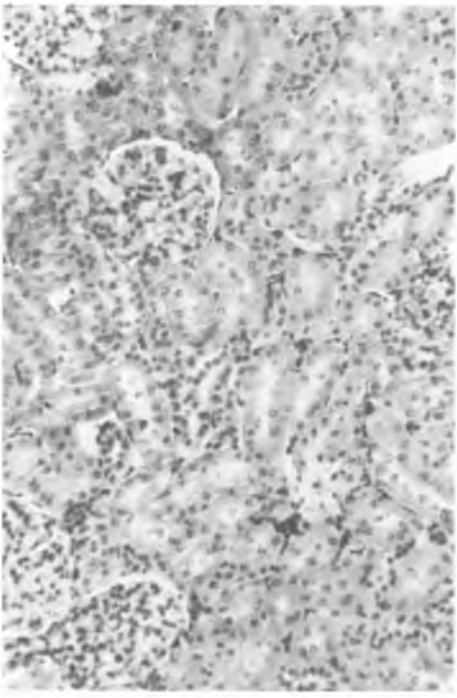

Fig. 1. Kidney of control rat. Hematoxylin and eosin staining. Magn. ca 100×

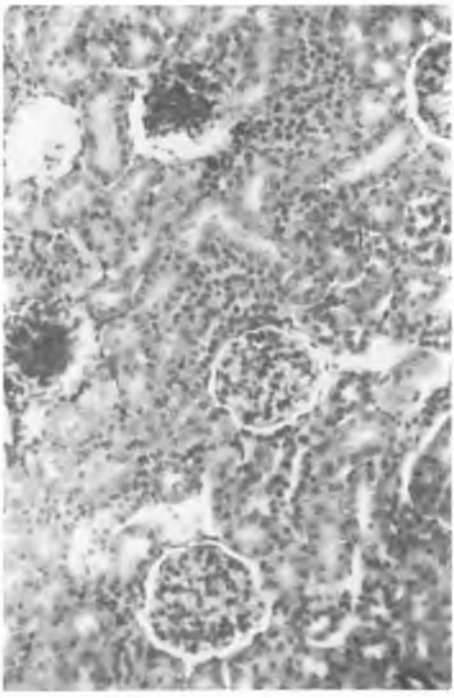

Fig. 2. Rat kidney experimental group I. Hematoxylin and eosin staining. Magn. ca 100×

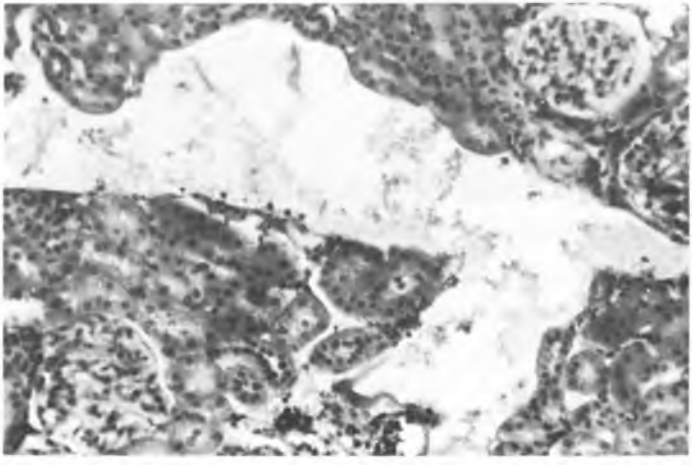

Fig. 3. Rat kidney. Experimental group II. Hematoxylin and eosin staining. Magn. ca 100×

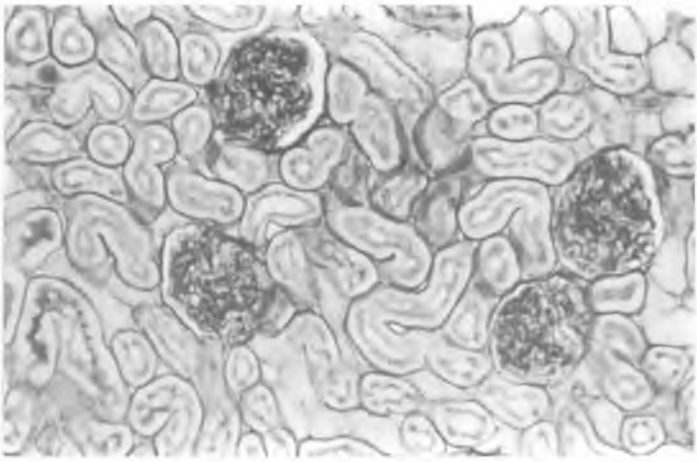

Fig. 4. Kidney of control rat. PAS reaction. Magn. ca 100×

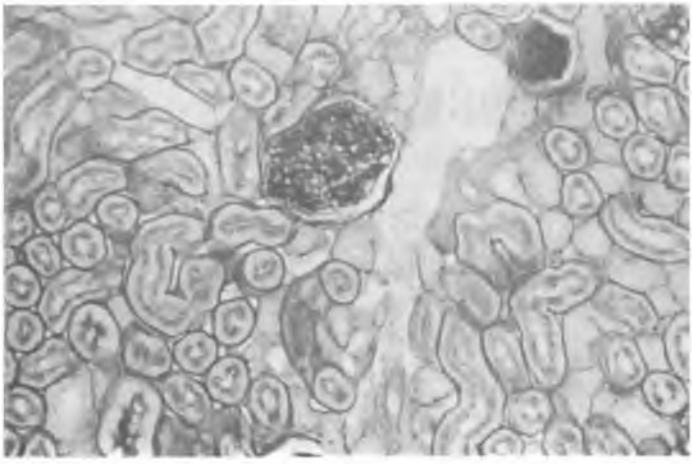

Fig. 5. Rat kidney. Experimental group I. PAS reaction. Magn. ca 100×

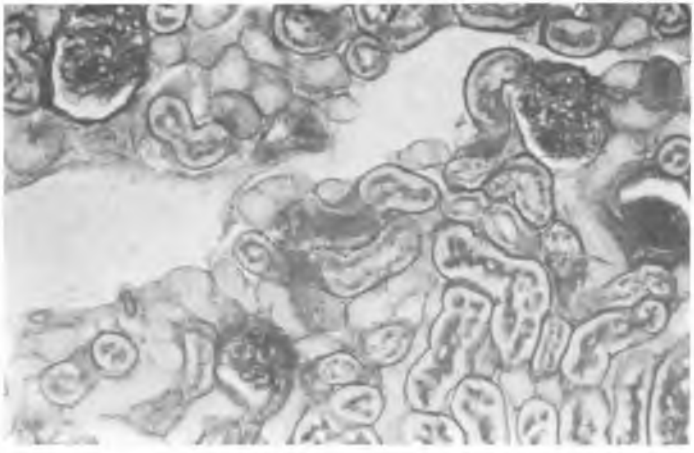

Fig. 6. Rat kidney. Experimental group II. PAS reaction. Magn. ca 100×

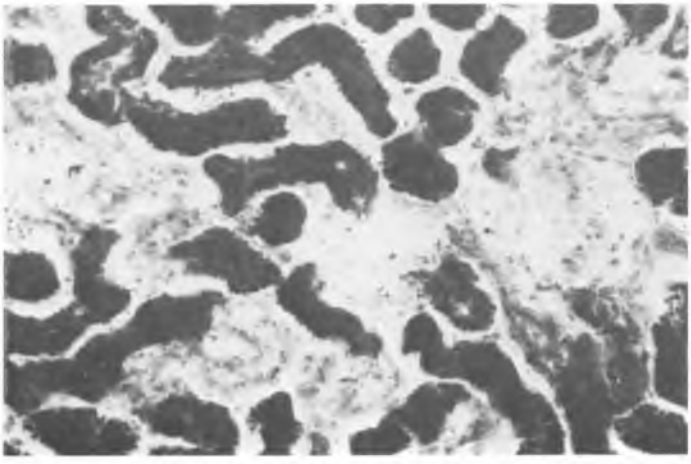

Fig. 7. Kidney of control rat. Reaction for acid phosphatase. Magn. ca 100×

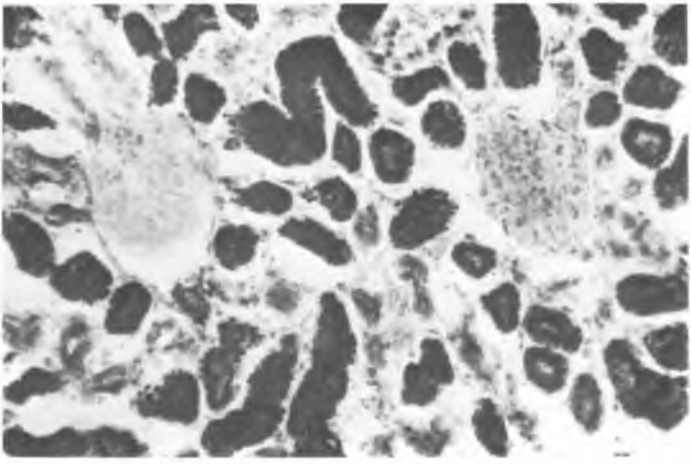

Fig. 8. Rat kidney. Experimental group I. Reaction for acid phosphatase. Magn. ca 100×

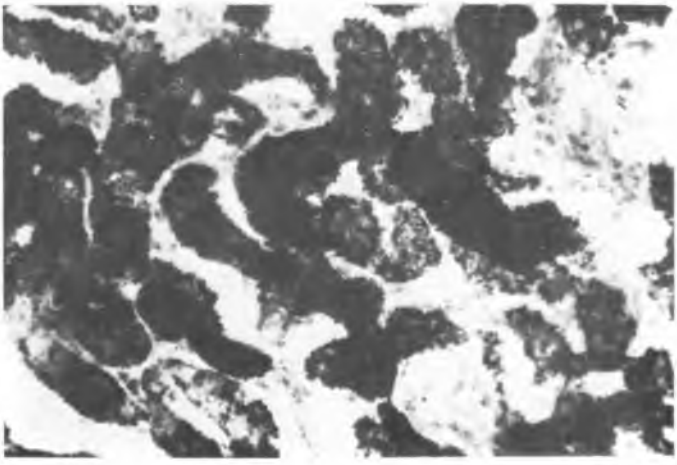

Fig. 9. Rat kidney. Experimental group II. Reaction for acid phosphatase. Magn. ca 100×