ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LIX, N 2, 104 SECTIO D 2004

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

JADWIGA ROMANOWSKA-SARLEJ, WŁODZIMIERZ MATYSIAK, EWA KIFER-WYSOCKA, KRYSTYNA CZERNY, TERESA MASŁYK, JANUSZ PISKÓRZ

Ultrastructure of the hepatocytes after application of azithromycin (Sumamed)

It is believed that macrolides, including semi-synthetic antibiotics, which are derivates of erythromycin, (clarithromycin, rocitamycin and azithromycin), damage the liver, however, as it seems, to a various extent. As most of the absorbed medicine is excreted in unchanged form, mainly in faeces, small quantities are metabolised in the liver (1), one can therefore suppose that azithromycin, which belongs to this group of antibiotics, will also have undesirable effect on the cells of the liver parenchyma (6, 7).

MATERIAL AND METHODS

Studies have been carried out on male Wistar rats of 300 g body mass. Animals subjected to experiments were given azithromycin as a preparation called Sumamed – (PLIVA Cracow), in the form of syrup (syrup forte 200 mg/5 ml), through a stomach tube. Animals were divided into two experimental groups and one control group (with five rats in each group). The rats of experimental group I were administered a 10-times bigger dose of the medicine than the therapeutic dose for an adult human – 20 mg of the medicine in 0.5 ml of distilled water per each rat on the first day and 10 mg in 0.25 ml per each for the next 4 days. The animals of the experimental group II were given a 100-times bigger dose – 200 mg of the medicine in 5 ml per each animal on the first day and 100 mg of the medicine in 2.5 ml per each for the next 4 days. The controls were given distilled water.

The fixed specimens of the liver (1 mm^3) were placed in Durcupan resin (ACM Fluka). Ultrathin sections were viewed and photographed in the transmission electron microscope Tesla BS 500.

RESULTS

Experimental group I. In comparison to preparations obtained from the control animals, in these experimental animals the liver cells with electronically lighter cytoplasm were observed. Mitochondria were more dispersed in the cytoplasm of the hepatocytes and they were less connected with the endoplasmic reticulum membrane system. In the whole cytoplasm agglomerations of glycogen grains were visible. Lysosomes were more numerous near the lumen of the biliary canaliculi. The lumen of the hepatic sinusoids was broad and showed numerous cross-sections of microvilli. The cytoplasm of Browicz-Kupffer cells revealed some areas of increased transparency and numerous phagosomes were found in it.

Experimental group II. In comparison to the image of hepatocytes in experimental group I, single swollen mitochondria were observed additionally (Fig. 1). In the cellular cytoplasm, adjoining the lumen of biliary canaliculi, usually numerous lysosomes were present (Fig. 2). Inside the canaliculi granular material could be seen (Fig. 2).

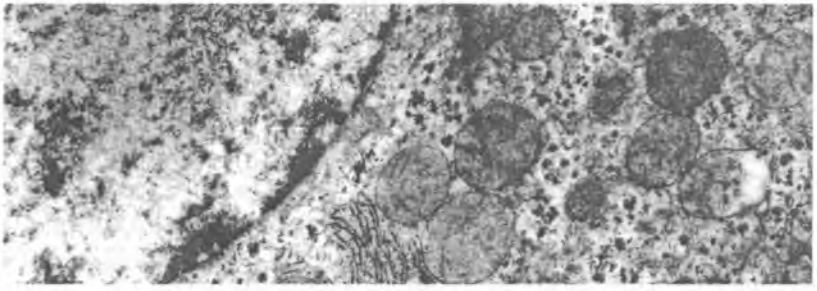

Fig. 1. Experimental group II. Single swollen miotochondria were observed in the cellular cytoplasm of hepatocytes. Magn. 8000x

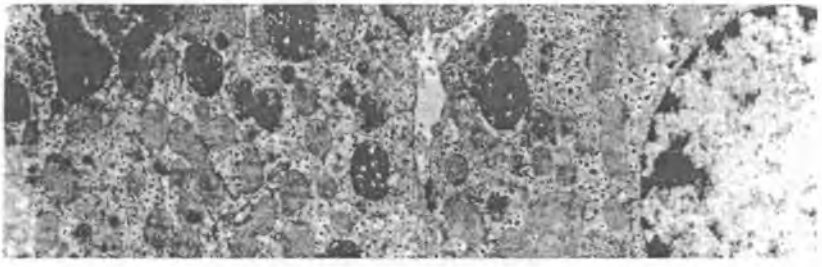

Fig. 2. Experimental group II. In the cellular cytoplasm, adjoining the lumen of biliary canaliculi, usually numerous lysosomes were present. Magn. 10000x

In the dilated lumen of the sinusoids bodies of mononuclear cells were found. The cytoplasm of Browicz-Kupffer cells, of lower electron density, contained more lysosomes and swollen mitochondria than in the previous experimental groups (Fig. 3).

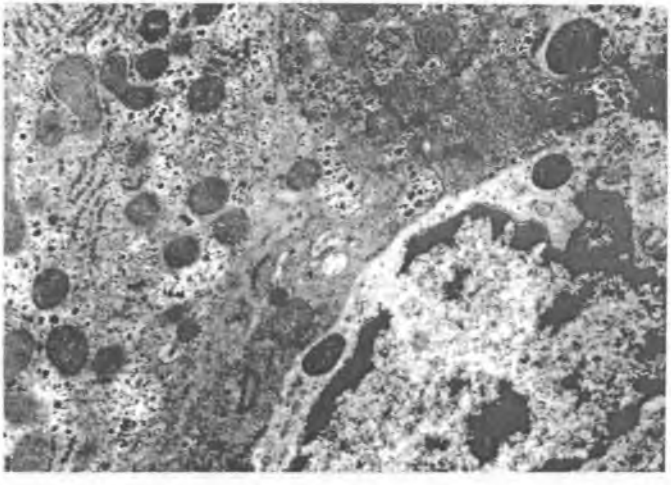

Fig. 3. Experimental group II. The Browicz-Kupffer cell. Magn. 8000x

DISCUSSION

The antibiotics applied in the therapy, irrespective of their beneficial effects, may also cause undesirable symptoms. Liver is a sensitive organ, which reacts to possible toxic effect of the administered antibiotic to various extent. Changes occurring in this organ may be of both morphological and functional character (1).

The application of two doses of azithromycin (10 and 100 times bigger than the therapeutic dose, in consideration of slight sensitivity of rats) enabled us to grasp the differences in the influence of these doses of the drug on the ultra structure of hepatocytes. The observed increase of the number of lysosomes in parenchymal hepatocytes and in Browicz-Kupffer cells, (secondary lysosomes), with heterogeneous content may result from the fusion of primary lysosomes with phagosomes. The observed changes in lysosome conformation could indicate the increased lytic activity of the enzymes present in lysosomes (firstly the acid hydrolase), as well as the stimulation of cellular endo- and exocytosis. The complete hydrolysis cannot always occur, so undigested residuals can often be found in the so-called residual bodies. The contents of residual bodies can be excreted through exocytosis into extra cellular space (in the case of liver – into the bile).

Additionally, in experimental group II, in hepatocytes and Browicz-Kupffer cells, a slight swelling of the mitochondria with increased transparency of the matrix was noted.

The changes found in the mitochondria may reveal the increasing energy requirement, probably related to the deficiency of ATP. The hepatocytic mitochondria are characterized by close vicinity of the rough endoplasmic reticulum (RER) – a kind of functional connection related to the synthesis of initial polypeptides for creation of, e.g. cytochrome P-450, cytochrome oxidase and/or ATP (3, 5). In this experiment the system of mitochondria and RER was disturbed.

In the oxidation reactions between various endo- and exogenous substances, which take place in the hepatocytic endoplasmic reticulum, with the participation of microsomal oxidation enzymes (oxidases) of mixed functions, which, for oxidation transformations require the presence of not only molecular oxygen (O_2), but also of a coenzyme in reduced form (NADPH), which is a biological reservoir of hydrogen for the cell. All these oxidation reactions occur with the participation of a cytochrome, which binds carbon monoxide (CO), also called cytochrome P-450. The hepatic cytochrome P-450 plays a significant role in microsomal transformations, connected, among others, with systemic detoxications (2, 4).

The presence of cytochrome P-450 is indispensable for binding molecular oxygen, as well as for transferring oxygen atoms during oxidation processes. One of the oxygen atoms is affixed to the drug or another endo- or exogenous substance, which undergoes detoxication, while the remaining atom of oxygen is used for the simultaneously formed H_2O (in the presence of NADPH – the hydrogen supplier). Scientific reports reveal that macrolidic antibiotics increase the concentrations of various molecular forms of the cytochrome P-450 (4).

CONCLUSIONS

1. Azithromycin administered to male rats in 10 and 100 times higher dose than the therapeutic dose (for the period of 5 days), causes slight changes in the morphology of liver cells.

2. Changes observed in the morphology of hepatocytes may reveal temporary violation of the functional balance in the liver.

3. The foregoing postulates do not forejudge the different effect of azithromycin on the liver cell in pathological conditions.

REFERENCES

1. Alvarez-Elcoro S. et al.: The macrolides: erythromycin, ciarithromycin, and azithromycin. Mayo Clin. Proc., 74, 613, 1999.
2. Bartłomowicz B.: Wątrobowy cytochrom P-450. Post. Bioch., 32, 459, 1986.
3. Brdiczka D. et al.: Mitochondrial matrix granules in soft tissues. Exp. Cell Res., 126, 127, 1980.
4. Kobylińska K. et al.: Formy molekularne cytochromu P-450 wątroby szczura. Post. Biochem., 40, 248, 1994.
5. Pickett C. B. et al.: The physical association between rat liver mitochondria and rough endoplasmic reticulum. Exp. Cell Res., 128, 343, 1980.
6. Tolman K. J. et al.: Chemical structure of erythromycin and hepatotoxicity. Ann. Int. Med., 81, 58, 1974.
7. Viluksela M. et al.: Cytotoxicity of macrolide antibiotics in a cultured human liver cell line. J. Antimicrob. Chemother., 38, 465, 1996.

SUMMARY

Azithromycin (Sumamed, PLIVA Cracow) was administered intragastrically to rats in doses 10 times and 100 times bigger than the therapeutic dose (according to the indications for a 5-day treatment). Ultrastructural examinations of the liver were performed. Changes observed in the morphology of hepatocytes and Browicz-Kupffer cells (swelling of mitochondria, more numerous lysosomes, weaker RER content and granular material inside biliary canaliculi) may indicate temporary violation of the functional equilibrium in the liver due to the application of azithromycin.

Ultrastruktura hepatocytów po zastosowaniu azytromycyny (Sumamed)

Azytromycynę (Sumamed, PLIVA Kraków) aplikowano szczurom dożołądkowo w dawce 10- i 100-krotnie większej od terapeutycznej (według wskazań pięciodniowej kuracji). Przeprowadzono badania ultrastrukturalne wątroby. Zaobserwowane zmiany w morfologii hepatocytów i komórek Browicz-Kupffera (obrzmienie mitochondriów, liczniejsze lizosomy, uboższa zawartość RER oraz materiał ziarnisty we wnętrzu kanalików żółciowych) mogą być przejawem przejściowego naruszenia równowagi czynnościowej wątroby wskutek aplikacji azytromycyny.