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Ultrastructural changes of pancreas of white rats after experimental administration of ethanol and cephalexin

Despite the fact that the toxicity of cephalosporins is small, it is claimed that sporadically the damage of haematopoietic tissue and liver occurs. Cephalexin combines with plasma proteins and rapidly penetrates tissues. It is not metabolized in the organism but is wholy excreted through kidneys in unchanged shape. However, in patients with insufficiency of these organs psychotic reactions were described after administration of cephalexin (3). Ethyl alcohol decreases the absorption of beta-lactam antibiotics in the digestive tract (4, 11), but on the other hand it increases the toxic action of many drugs. Considering a lack of data regarding the action of cephalexin on the pancreas, we decided to investigate wheather this antibiotic administered separately and together with alcohol has a toxic influence on exocrine cells in this gland. The observation of changes in electron microscope may contribute to more detailed understanding of the cytomechanism of this drug action.

MATERIAL AND METHODS

The experiment was carried out on rats inbreeding Wistar race males weighing about 200 g. The animals were divided into three experimental groups and one control group (including five animals each). Rats from the control group received standard granulated fodder and water *ad libitum*. In the experimental group I, rats received standard granulated fodder and 20% ethyl alcohol instead of water for 10 days. Rats from experimental group II received cephalexin (Lilly, Florence, Italy) in a single dose 42 mg/24 h. This dose corresponded to tenfold minimal therapeutic dose in human. The drug was adminis-

tered each day morning for ten days as suspension in 0.9% NaCl. Animals from experimental group III received cephalexin in the same way like animals from experimental group II. Moreover, instead of water they received 20% ethyl alcohol *ad libitum*. Each animal from experimental group I drank about 20 ml of alcohol and from experimental group III – about 15 ml of alcohol per 24 h. After 10 days animals were gillotined. Specimens of pancreas 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 Reynold's method. Ultrastructural observations were led and electron micrographs were taken in the Tesla BS-500 transmission electron microscope.

RESULTS AND DISCUSSION

The decline in number of ribosomes connected with membranes and cytoplasmic rybosomes were revealed in many pancreatic exocrine cells of animals receiving ethanol. The number of zymogen granules was smaller than in the control and cell nuclei were irregular in shape. Moreover, a dilatation of the spaces between membranes of endoplasmic reticulum were observed in many places. In some cells, mitochondria showed the signs of oedema and even the presence of myelinic structures (Fig. 1). The decline in

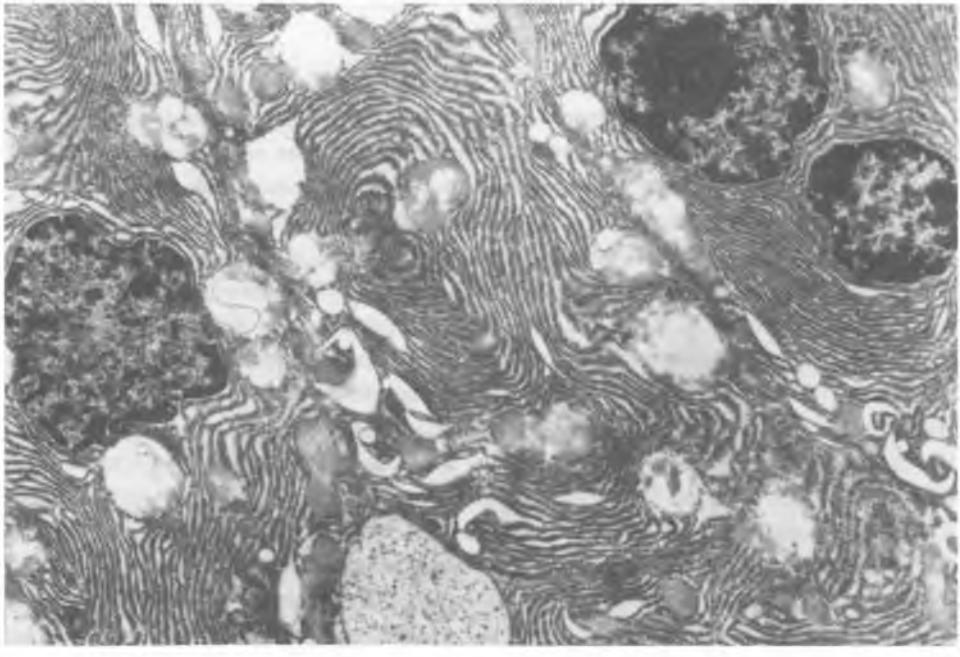

Fig. 1. Pancreas of a rat from experimental group I. The decreased amount of ribosomes, nuclei irregular in shape and damaged mitochondria are visible. Magn. 4000x

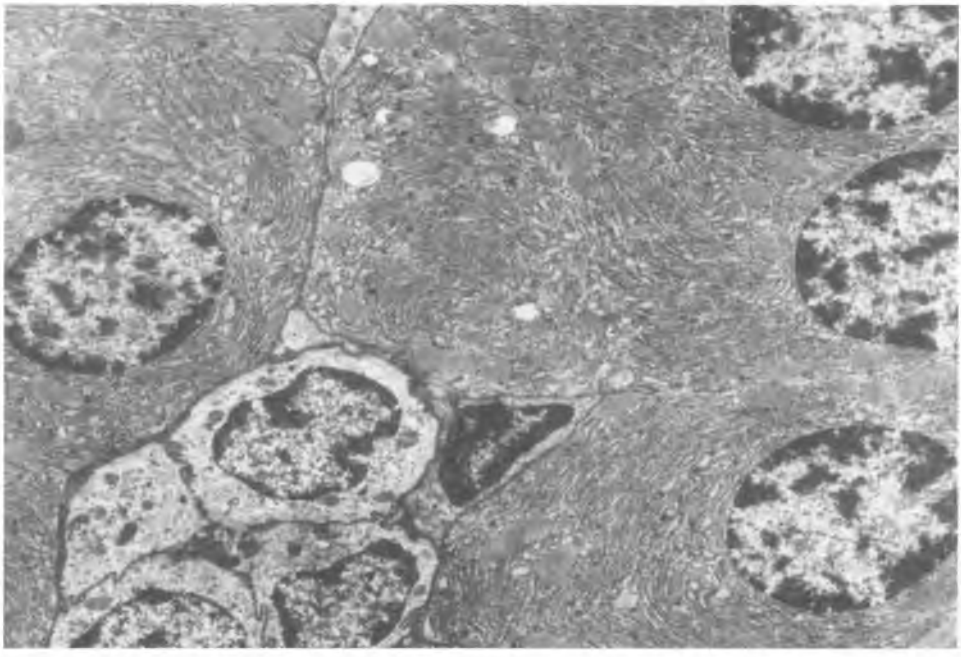

Fig. 2. Pancreas of a rat from experimental group II. The increased amount of rough endoplasmic reticulum membranes, which fill the whole cell is visible. Magn. 4000x

number of rybosomes leads to reduction of the activity in cell secretion. Other authors' investigations (1, 2) also revealed that ethanol inhibits the RNA synthesis and decreases the processes of protein synthesis. It is very likely, that the decrease in number of ribosomes was connected with the transformation of rough endoplasmic reticulum into the smooth endoplasmic reticulum, which is responsible for cell detoxication (8, 9, 10). The dilatation of spaces between membranes of endoplasmic reticulum is characteristic of early changes leading to cell damage (13). The increase in the number of rough endoplasmic reticulum membranes which fill the whole cell was observed after administration of cephalexin (Fig. 2). That is why the amount of zymogen granules in apical parts of cells was smaller than in the control. In many cells zymogen was not observed at all. Apart from ribosomes connected with membranes, a large amount of ribosomes was present in the spaces between membranes. It can be supposed that 10-day administration of cephalexin in the dose of 42 mg/24 h of body mass stimulates the formation of rough endoplasmic reticulum membranes and ribosomes. Similar reaction of pancreatic cells was observed after partial hepatectomy and the administration of Biseptol 480 and it is the sign of mobilization of the gland to the intensive secretion after administration of the antibiotic. At the same time the decrease in thickness of basal membrane surrounding serous acini was observed in many places. It may be that it was the defense reaction against antibiotic diffusing from small periacinar blood vessels. The basal lamina plays an

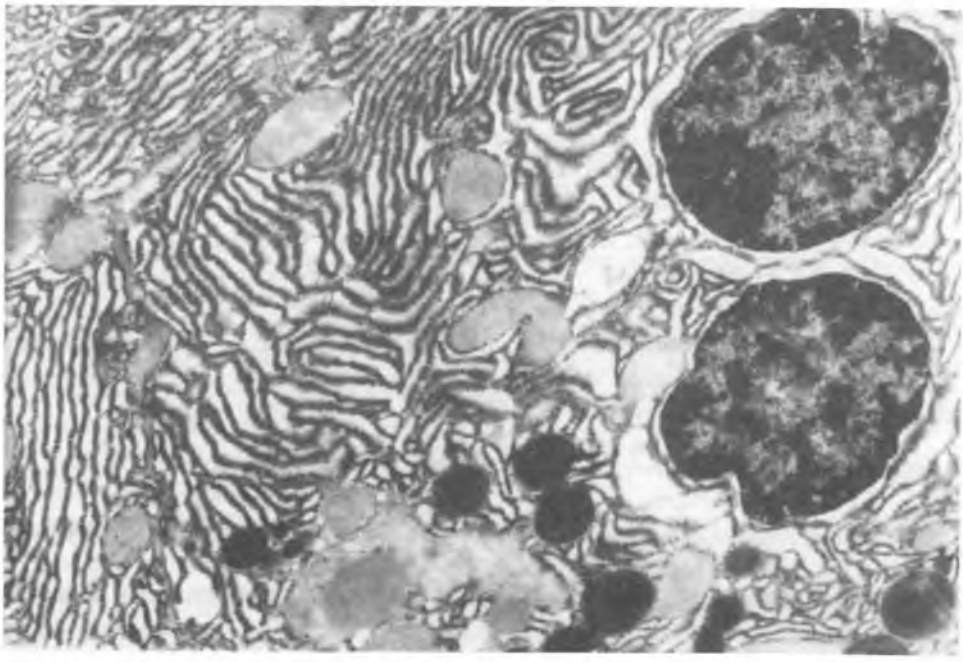

Fig. 3. Pancreas of a rat from experimental group III. Distinct decrease of the amount of ribosomes. Membranes of rough endoplasmic reticulum resemble smooth endoplasmic reticulum. Magn. 4000x

important role in the interchange between blood vessels and glandular cells. The increase in thickness of many membranes observed in electron microscope was certainly connected with the change of their biochemical composition. It may be that protein substances present in the secretion that was observed in many periacinar spaces accumulated within these membranes. On the other hand, the observed changes were typical functional changes and they did not indicate the cell damage. It can be supposed that rat exocrine pancreatic cells are resistant to the action of cephalexin administered in the dose of 42 mg/kg/24 h.

After administration of cephalexin and ethanol a decrease in the amount of ribosomes connected with membranes and free ribosomes was observed and abundant rough endoplasmic reticulum resembled the smooth one (Fig. 3). At the same time the amount of zymogen granules was similar to that in the case of animals receiving only alcohol. The signs of mitochondrial damage were similar to those in the case of animals receiving only ethanol, but they were more intensely marked. Lash et al. report the changes in the bioactivity of cytochrome P-450 and mitochondrial dysfunction induced by cephalosporins (5). These drugs decrease mitochondrial oxydative processes through the inhibition of the uptake of substrates (e.g. butandioic acid) entering the citric acid cycle.

At the begining these changes are reversible, until the acylation of transporters takes place (12).

Morphological changes observed in this experimental group indicated the decline of secretory activity and, on the other hand, the increased detoxication. Barrio-Lera et al. (1) examined the influence of ethanol on the absorption and secretion of beta-lactam antibiotics in the small intestine of rats. They showed that 2-month administration of 15% ethanol decreases the absorption of cephalexin in the intestine and its secretion with urine. Probably ethanol modulates the processes of the transport through membranes in different way for each antibiotic (2, 4).

On the basis of carried aut observations one can assume that the administration of both alcohol and cephalexin in used dosages for 10 days leads to early degenerative changes, which are yet reversible in this stage.

CONCLUSIONS

1. 10-day administration of 20% ethanol *ad libitum* causes a decrease in the number of ribosomes and zymogen granules and changes in some mitochondria.

2. 10-day administration of cephalexin in the dose of 42 mg/kg/24 h does not exert a toxic influence on pancreatic exocrine cells. It causes the increase in the amount of rough endoplasmic reticulum membranes and free ribosomes and the decrease in the amount of zymogen granules.

3. 10-day administration of both ethanol and cephalexin causes reversible degenerative changes in pancreatic exocrine cells of rat which include the decrease in the number of ribosomes, swelling of many mitochondria and the presence of myelinic structures inside cells.

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SUMMARY

The experiment was carried out on Wistar rat males weighting about 200 g. Animals from experimental group I received 20% ethanol for drinking (*ad libitum*), animals from experimental group II – cephalexin in the dose of 42 mg/24h, animals from experimental group III – cephalexin and ethanol in the mentioned doses.

After 10 days the animals were decapitated and pancreases were collected for ultrastructural examinations in electron microscope. The performed experiments showed that 10-day administration of ethanol causes mainly the decrease of amount of ribosomes and zymogen granules in pancreatic exocrine cells, whereas cephalexin causes increase of amount of these organelles. Administration of both ethanol and cephalexin causes revers-

ible degenerative changes including distinct decreasing of the number of ribosomes, swelling of many mitochondria and the presence of myelinic structures inside cells.

Zmiany ultrastrukturalne trzustki szczurów białych po doświadczalnym podawaniu etanolu i cefaleksyny

Badania wykonano na szczurach, samcach rasy Wistar o masie ciała ok. 200g. Zwierzęta I grupy doświadczalnej otrzymywały 20% etanol do picia (*ad libitum*), zwierzęta II grupy doświadczalnej - cefaleksynę w dawce 42mg/24h, zwierzęta III grupy doświadczalnej - cefaleksynę i etanol w wymienionych dawkach.

Po 10 dniach zwierzęta dekapitowano i pobierano trzustkę do badań w mikroskopie elektronowym. Stwierdzono, że 10-dniowe podawanie etanolu powoduje przede wszystkim zmniejszenie liczby rybosomów i ziaren zymogenu w komórkach egzokrynowych trzustki, natomiast cefaleksyna powoduje wzrost liczby tych struktur. Łączne podawanie alkoholu i cefaleksyny powoduje odwracalne zmiany degeneracyjne w postaci wyraźnego zmniejszenia liczby rybosomów, obrzmienia wielu mitochondriów i obecności w nich struktur mielinowych.