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Histological and morphometrical examinations of suprarenal glands in rats after experimental administration of atorvastatin and ethanol

All hormones synthesized by glandular cells of suprarenal glands have steroid structure. Cholesterol is the precursor for their synthesis. It may be derived from three sources: intracellular stores, de novo synthesis or cholesterol circulating in the blood (11). That is why an application of pharmacological preparations which decrease the total cholesterol level and cholesterol fractions may cause disturbances in the synthesis of suprarenal hormones. The preparation Sortis used in our experiment belongs to the group of statins. It contains an active substance – atorvastatin, which belongs to HMG-CoA reductase inhibitors (13).

The aim of our experiments was to assess whether different doses of atorvastatin and simultaneous administration of atorvastatin and ethanol induce changes in suprarenal gland. Our observations may contribute to more detailed knowledge about the mechanism of this medicine's action.

MATERIAL AND METHODS

The experiment was carried out on Wistar rat males weighing about 250g. The animals were divided into two control groups and three experimental groups, including 5 animals each. The rats from control group I received water *ad libitum* and standard granulated fodder. The animals from control group II received standard fodder and 20% ethanol instead of water. Each animal drank about 20ml of ethanol for 24h. The animals from experimental group I received Atorvastatin (the preparation Sortis PARKE-DAVIS GmbH, Berlin) in the dose of 0.28mg/24h for 6 weeks by means of the intragastric bougie, each morning before feeding. The animals from experimental group II received preparation Sortis in the dose of 2.8mg/24h for 6 weeks. The animals from experimental group III received both preparation Sortis in the dose of 0.28mg and 20% ethanol for 6 weeks. Each animal drank about 15ml of ethanol for 24h.

After 6 weeks the animals were decapitated. The suprarenal glands were fixed in 4% formalin for routine staining with hematoxylin and eosin and PAS method for detection of neûtral mucopolysaccharides. The right suprarenal glands were fixed in Baker's fluid for lipid staining according to Thomas's method (1).

Pictures were taken in the light microscope combined with Carl Zeiss Jena camera.

Moreover, the minimum and maximum diameters of cell nuclei were measured in the zona fasciculata with the use of a projection microscope and surface areas of the sections were assessed. Depending on the shape of nuclei the pattern πr^2 was used for a circle and the pattern πab was used for an ellipse. In each animal the surface areas of the section for 100 nuclei were assessed and then the average value of this parameter was determined for each group.

RESULTS AND DISCUSSION

In animals from control group II (receiving ethanol) a strong hyperemia of the suprarenal gland, especially in the zona fasciculata (Fig. 1), and a weaker reaction for lipids were revealed in comparison with control group I. This may indicate more intensive secretion of hormones than in animals from control group I. Farmer and Fabre (5) revealed that ethanol concentration of about

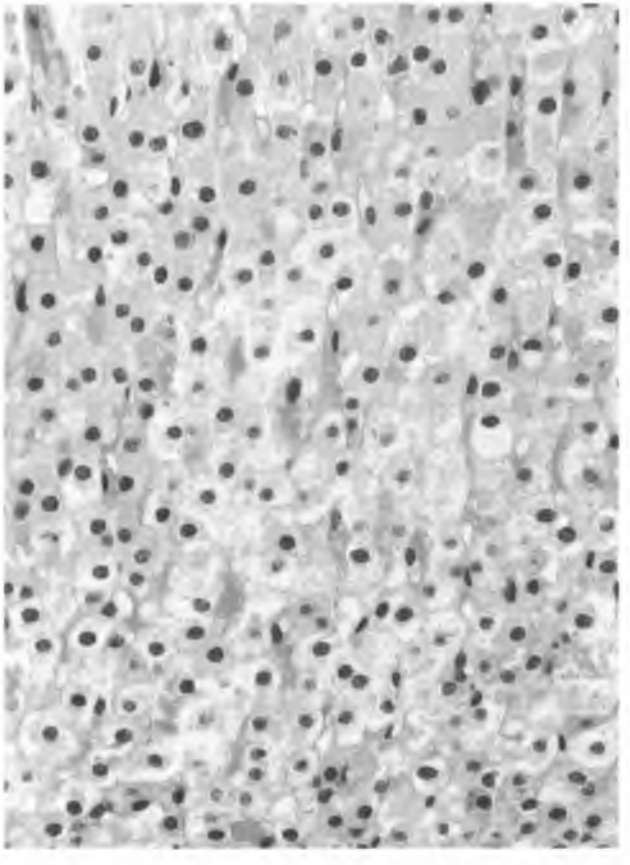

Fig. 1. Suprarenal gland of a rat from control group II (ethanol). Dilatated capillaries are visible in the zona fasciculata of the cortex. H+E staining. Magn. 500x

100ng/l exerts a stimulating influence on aldosterone secretion, however Santana et al. (9) on the basis of examination of beef suprarenal tissue revealed that 1% solution of ethanol inhibits aldosterone biosynthesis through the influence on conversion of corticosterone into 18-hydroxy-

corticosterone. Simultaneously, ethanol degradation can take place in adrenal cells modifying metabolism of cholesterol and steroid hormones. Sze (10) revealed an increased activity of alcohol dehydrogenase and catalase in mouse plasma after ethanol administration. The increased activity of these enzymes causes quicker detoxication of the organism. But the presence of glucocorticosteroids is necessary for the manifestation of increased activity of alcohol dehydrogenase and catalase. Ethanol tolerance rapidly decreases in the case of adrenal hypofunction (8).

In our experiment we revealed a decrease in the average surface area of cell nuclei sections in the zona fasciculata as much as 20% after ethanol administration (Tab.1).This fact can be explained by intensive cell metabolism leading to the decrease of nuclear hnRNA storage due to cellular degradation of ethanol as well as intensive secretion of corticosteroids. On the other hand, the decrease of cell nuclei with a lack of morphological changes in the parenchyma may indicate decreased cell activity caused by the action of ethanol.

6-week administration of atorvastatin in the dose of 0.28mg/24h, which corresponds to the maximum therapeutic dose in human did not cause distinct morphological changes in the parenchyma of the suprarenal gland. The observed changes were very delicate and they concerned a delicate decrease of lipid amount especially in the zona glomerulosa. In agreement with the feedback mechanism between suprarenal glands and pituitary, a decrease of lipid (steroid hormones) amount stimulated the ACTH secretion. As a result of this there was local moderate hyperplasia of the zona reticularis. However, the thickness of the zona glomerulosa, which is the least dependent on ACTH, was localy decreased in comparison with the control.

Table 1. Mean surface of cell nuclei section in zona fasciculate

Group	The average surface area of cell nuclei sections	Differences in comparison with control group I	Differences in comparison with control group I
Control I	19.405		
Control II (20% ethanol)	15.605	-3.800	-19.58
Experiment I (atorvastatin- 0,28mg/24h)	16.909	-2.496	-12.85
Experiment II (atorvastatin- 2,8mg/24h)	14.201	-5.204	-26.82
Experiment III (atorvastatin-0,28mg/24h + 20% ethanol)	14.886	-4.519	-23.29

Some authors (6) observed 20-60% decrease of LDL cholesterol level and 10-40% decrease of triglycerides level in the plasma after atorvastatin administration. It is known that a part of cholesterol for the production of triglycerides in the suprarenal cells derives from the plasma (11). Simultaneously, atorvastatin can inhibit the cholesterol synthesis directly in suprarenal cells like in hepatocytes (6). Insoluble atorvastatin metabolites present in the plasma and faeces are formed by its hydroxylation and first of all by β-oxydation. Cytochrome P-450 playes an important role in this process (2).

A small decrease of the surface area of nuclei sections in the zona fasciculata in animals from experimental group I indicates that the atorvastatin dose of 0.28g/24h did not cause important morphological changes of cell metabolism. After 6-week administration of 10 times higher ator-

vastatin dose (2.8mg/24h) distinct morphological changes were revealed in the suprarenal cortex. The total thickness of the zona glomerulosa was increased. This change was accompanied by distinctly weaker reaction for lipids and the surface area of nuclei sections in the zona fasciculata decreased as much as 27%. At the same time acidophilic degenerating cells forming agglomerations in the form of cords or clusters were present in the parenchyma (Fig. 2). On the basis of the observations it may be concluded that a high dose of atorvastatin significantly decreases corticosteroid production. As a consequence of this there was compensatory hyperplasia of the zona glomerulosa stimulated by juxtaglomerular apparatus.

Other authors' research revealed that pathophysiological indicators in the form of the increase of smooth endoplasmic reticulum in hepatocytes, cholangiohepatitis and cholecystitis appear after atorvastatin doses of 150mg/kg or higher applied for 2 weeks. Degeneration of hepatic cells, cerebral hemorrhage, demyelination of the optic nerve and necrosis of skeletal muscles were observed during the administration of higher doses – 280mg/kg for 12 weeks.

Concluding, it should be underlined that the higher dose of atorvastatin (2.8mg/24h) used in our experiment causes a damage of the suprarenal parenchyma. It is very likely that apart from liver atorvastatin is also metabolized by cytochrome P-450 bounded with the smooth endoplasmic reticulum membranes (4,7). It is also known that statins can cause mitochondrial damage through

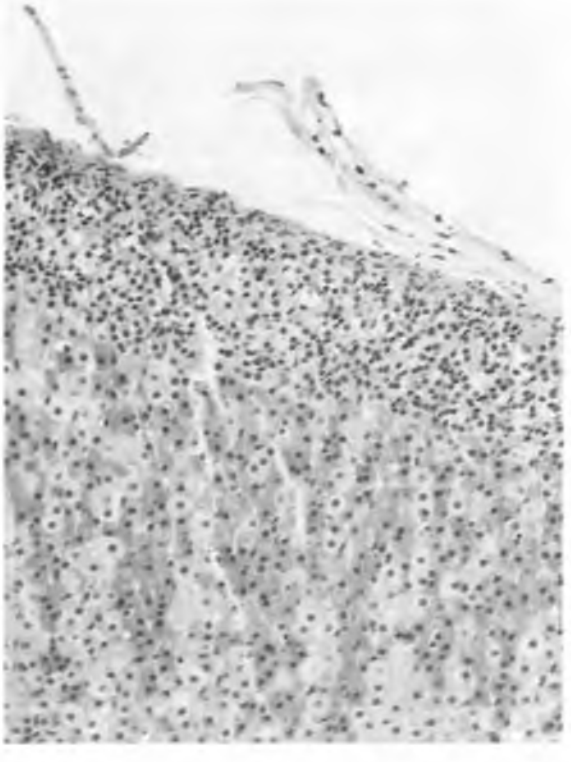

Fig. 2. Suprarenal gland of a rat from experimental group II (atorvastatin-2,8mg/24h). Thick zona glomerulosa and aggregations of degenerating cells in the zona fasciculata are visible. H+E staining. Magn. 250x

the inhibition of the activity of CoQ-10 ubichinone (3). This enzyme is an electron carrier in the oxydative phosphorylation, it stabilizes cell membranes and it is also an agent that eliminates free radicals. That is why it inhibits lipid peroxydation. Its lack is connected with numerous diseases, first of all cardiovascular ones. Statin action is less effective in the treatment of these diseases because simultaneously they decrease the level of CoQ-10. This phenomenon should be of interest to clinicians because a restoration of CoQ-10 level should be taken intro account during the statin therapy. Concomitant administration of a small dose of atorvastatin (0.28mg/24h) and ethanol caused degenerative changes in the suprarenal parenchyma like after a high dose of atorvastatin (2.8mg/24h) but the amount of damaged cells in the zona fasciculata was higher and their arrangement was irregular in many places. It proves the stimulation of connective tissue stroma that participates in the repair processes.

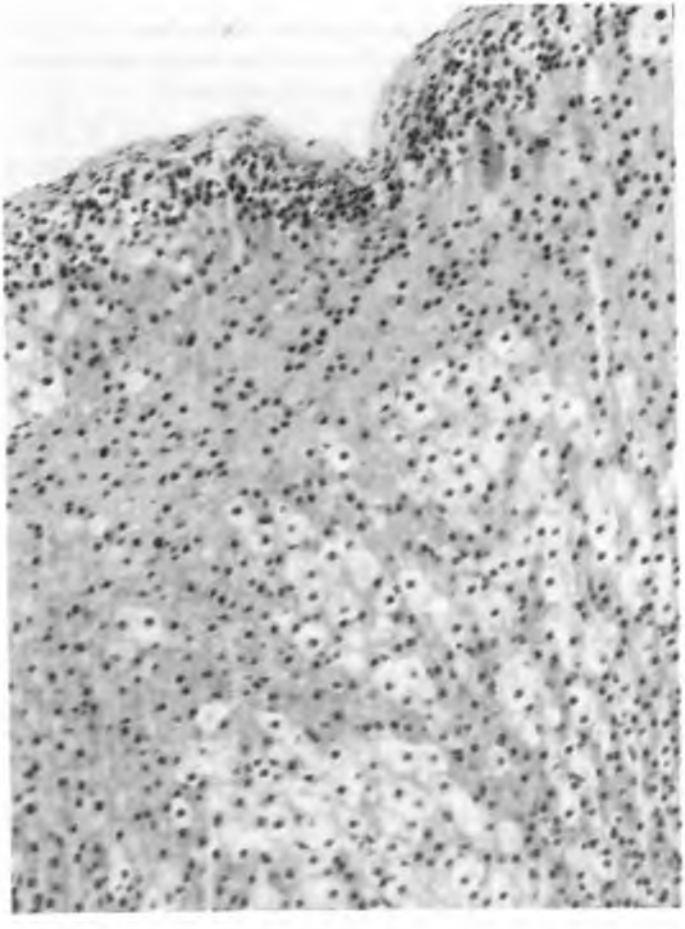

Fig. 3. Suprarenal gland of a rat from experimental group III (atorvastatin 0,28mg/24h+ethanol). Degenerative changes in a large part of the zona fasciculata in which cells are arranged irregularly. H+E staining. Magn. 250x

Our observations confirm the reports of Ucar et al. (12). They found an increase of the HMG-CoA reductase inhibitors myotoxicity in the case of their concomitant administration with other preparations (erythromycin, cyclosporines). Antibiotics probably inhibit metabolism of the HMG-CoA inhibitors through the influence on the cytochrome P-450.

Comparing the influence of ethanol itself and atorvastatin itself with the concomitant influence of both chemicals it may be concluded that their concomitant administration intensify toxic action of atorvastatin.

CONCLUSIONS

1. 6-week administration of 20% ethanol *ad libitum* causes strong hyperemia of suprarenal cortex in rats.
2. 6-week administration of atorvastatin in the dose of 0.28mg/24h causes delicate decrease of lipid amount in the zona fasciculata and zona reticularis and distinct in the zona glomerulosa of rat suprarenal cortex.
3. 6-week administration of atorvastatin in the dose of 2.8mg/24h causes the decrease of the zona fasciculata and zona reticularis thickness, a dilatation of the zona glomerulosa, distinct decrease of lipid amount and early degenerative processes with atypical arrangement of many cells of the zona fasciculata.
4. 6-week administration of 20% ethanol and atorvastatin in the dose of 0.28mg/24h causes the decrease of the total cortical thickness with concomitant dilatation of the zona glomerulosa, distinct decrease of lipid amount and degenerative changes with atypical arrangement of many cells in the zona fasciculata.
5. Concomitant administration of 20% ethanol and atorvastatin (0.28mg/24h) significantly increases its toxicity for the suprarenal cortex.

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SUMMARY

The experiment was carried out on Wistar rat males weighting about 250g. Animals from control group I received water and standard granulated fodder *ad libitum*. Animals from control group II received 20% ethanol instead of water. Animals from experimental group I received Atorvastatin in the dose of 0.28mg/24h, animals from experimental group II received Atorvastatin in the dose of 2.8mg/24h, animals from experimental group III received 20% ethanol + Atorvastatin in the dose of 0.28mg. After 6 weeks the animals were decapitated. H+E staining, PAS method for detection of neutral mucopolisaccharides and Thomas's reaction for detection of lipids were made on 6μm thick sections. The surface areas of the nuclei sections were assessed in the zona fasciculata. It was stated that ethanol causes strong hyperemia of suprarenal cortex, the high dose of atorvastatin – 2.8mg/24h and small dose of atorvastatin – 0.28mg/24h administered with ethanol cause the decrease of lipid amount in the whole suprarenal cortex and degenerative changes in many cells of the zona fasciculata.

Histologiczne i morfometryczne badania kory nadnerczy szczurów białych po podawaniu atorwastatyny i etanolu

Badania wykonano na szczurach-samcach rasy Wistar o masie ciała ok. 250 g. Zwierzęta grupy kontrolnej I otrzymywały wodę i standardową paszę granulowaną. Zwierzęta grupy kontrolnej II otrzymywały zamiast wody 20% etanol *ad libitum*. Zwierzętom grupy doświadczalnej I podawano atorwastatynę w dawce 0,28 mg/dobę, zwierzętom grupy doświadczalnej II - atorwastatynę w dawce 2,8 mg/dobę, zwierzętom grupy doświadczalnej III - 20% etanol + atorwastatynę w dawce 0,28 mg/dobę. Po 6 tygodniach zwierzęta dekapitowano. Na skrawkach o grubości 5μ wykonano barwienie H + E, reakcję PAS na mukopolisacharydy obojętne i reakcję Thomasa na lipidy. Oceniano również pola powierzchni przekroju jąder komórkowych w komórkach warstwy pasmowatej. Stwierdzono, że etanol wywołuje silne przekrwienie kory nadnerczy, natomiast duża dawka atorwastatyny - 2,8 mg/dobę oraz mała dawka atorwastatyny - 0,28 mg/dobę podawana łącznie z etanolem powoduje obniżenie poziomu lipidów w całej korze nadnerczy oraz zmiany degeneracyjne w dużej części komórek warstwy pasmowatej.