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Histological picture of intestinal mucosa of rats after simultaneous administration of atorvastatin and ethyl alcohol

Atorvastatin – a synthetic compound from the statin group, is a drug which effectively decreases the cholesterol level (7, 9, 10). Like other statins,it can lead sometimes to serious defectsin parenchymatous organs and to injury of skeletal muscles (1, 4, 9, 14). The most common side-effects following atorvastatin use are minor gastrointestinal disturbances (9). It is known that excessively consumed ethyl alcohol has a negative influence on the organism when alcohol is consumed while taking this drug (2, 5, 11, 12, 13).

The purpose of the paper was to investigate the histological effect on mucosa of rat intestinum of rats administered simultaneously atorvastatin and ethanol.

MATERIAL AND METHODS

The experiment was carried out on white rats weighing about 250 mg each. The animals were fed with standard granulated fodder and water *ad libitum*. The animals from experimental groups I–IV received the drug (atorvastatin – Sortis – from Parke-Davis GmbH, Germany) in water emulsion in the dose of 1 ml/24 hours by stomach-tube for 6 weeks. Experimental group I: the maximal therapeutical dose for human 80 mg/24 h (for rat 0.28 mg/24 h). Experimental group II: the animals received atorvastatin in the same manner as the animals of experimental group I, plus 20% solution of ethyl alcohol in their drink-straws instead of drinking water. Experimental group III: rats received the drug in the dose 10x higher than the therapeutic dose. Experimental group IV: the drug was given in the same dose as in experimental group III and 20% solution of alcohol in drink-straws. Experimental group V: the rats received 20% solution of alcohol instead of drinking water.

In all of the experimental groups, 24 hours after the last dose of the drug or alcohol, five rats were killed by decapitation; the material for investigations being taken. The other five rats in all groups were killed after 30 days after the last dose of the drug or alcohol. In this way subgroups: IA, IIA, IIIA, IVA, VA were formed. The rats of the control group which were fed with standard granulated fodder were killed simultaneously with rats of the experimental groups (five rats) and with rats of subgroups A (five rats).

The intestinum samples fixed with 10% formalin were stained with H+E, with Masson's method, with PAS by McManus method. Observations were made and photographs taken using a JenaMed light microscope made by Zeiss Jena firm.

RESULTS

The intestinal mucosa of rats of experimental groups I and II taking atorvastatin in the therapeutical and 10 x higher dose did not show any essential differences in comparison with the control group.

In the intestinum of rats receiving atorvastatin in the therapeutical dose and drinking ethanol (experimental group II) essential changes were observed in the structure of intestinum villi. H+E staining showed light vesicle-like spaces and shrunken stroma of the villi under epithelium covering the villi (Fig. 1). In Masson's method staining the stroma of the villi in subepithelial part showed essential loosening. PAS reaction showed locally decreasing amount of polysaccharides on the villi. On the top of some villi we did not observe a positive reaction to polysaccharides.

Fig. 1. Experimental group II. The intestinum of the rat, showing vesicle-like products under the epithelium of the villi. Hematoxylin and eosin. Magn. 400x

In the group of animals receiving atorvastatin in the dose 10 x higher than therapeutical and drinking alcohol (IV group), changes were stronger than in experimental group II. The epithelium showed no connecting with stroma of villi. The top part of some villi was covered by epithelium (Fig. 2). In the upper part of the villi a small amount of mucous cells (PAS method) was observed. There was observed hyperaemia of connective tissue stroma of villi in Masson's staining (Fig. 3).

Fig. 2. Experimental group IV. The intestinum of the rat, showing the lack of the epithelium on the top of the villi. Hematoxylin and eosin. Magn. 400x

Fig. 3. Experimental group IV. The intestinum of the rat, showing hyperaemia of stroma of the villi and wide spaces under the epithelium. Masson's method. Magn. 400x

In experimental group V (drinking alcohol) there were recorded changes like in experimental II group of rats (atorvastatin in the therapeutical dose and alcohol). The stroma of the villi was shrunken, under the epithelium there were located vesicle-like free spaces. The epithelium locally did not show connecting with stroma of the villi, while sometimes on the top of villi, there were observed squamous, separated enterocytes, which showed the lack of positive PAS reaction on the apical part of villi.

In rats of all experimental groups, after 30 days' break in giving atorvastatin and/or alcohol (subgroups A) the microscopic picture of mucosa of intestinum was similar to that observed in the control group. But in subgroups IVA (10 x higher than the therapeutical dose of atorvastatin + alcohol) there were often visible hyperaemia of the stroma of the villi and light, vesicle-like spaces under the epithelium (Fig. 4). There were observed no changes in intestinum glands in any of the groups.

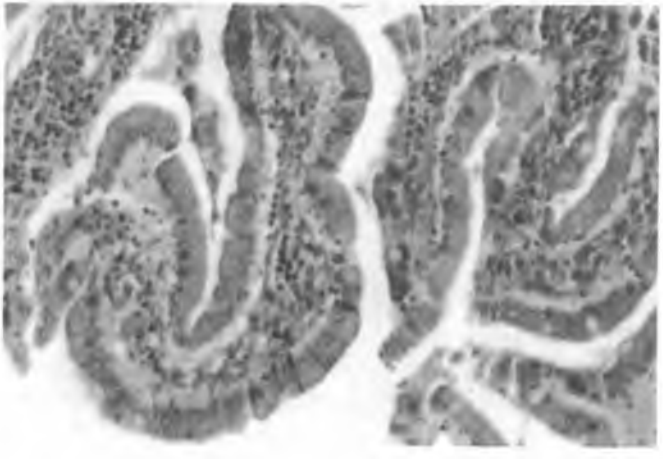

Fig. 4. Experimental group IV. Subgroup A. The intestinum of the rat, showing hyperaemia of stroma of the villi. Masson's method. Magn. 400x

DISCUSSION

In the experiment no changes in the structure of mucosa of intestinum of rats receiving only atorvastatin were found. These observations are confirmed by investigations of other authors (3, 8, 14). Walsh et al. (14) confirmed that atorvastatin dosages to 80 mg/kg applied in dogs during 12 weeks did not induce pathological changes in the histological picture of various organs, and in the intestinum. The higher doses of atorvastatin cause changes in parenchymatous organs, in cerebrum and necrosis of skeletal muscles (14). Kloss et al. (8), investigating drugs of statin groups, observed no changes in the intestinum of rodents. The biopsy investigations of the intestinum carried from patients with hypercholesterolaemia using reductase HMG-CoA inhibitors, did not show changes in proportion of the length of villi to the length of intestinum cryptes (6).

Changes in intestinum villi in rats occurred only in ethanol or ethanol and atorvastatin subepithelial deserting, separated epithelium fragments – as observed in our investigation, were described by other authors. Dinda et al. (5) using various dosages of ethyl alcohol observed in intestinum villi subepithelial accumulation of liquid in the form of vesicles and with increasing of concentration of alcohol solution, the separation in epithelium from villi stroma. The investigations carried out *in vivo* and *in vitro* suggested that changes did not depend on microcirculation in the intestinum.

In rats receiving the drug with alcohol or only alcohol, weak positive PAS reaction was seen on the surface of villi epithelium. This or the local lack of positive reaction indicate damage of the protective mucosal barrier. The damage of mucosal barrier and epithelium can be a cause of the inflammatory state of mucosa of intestinum and a place of ulceration. The expression of inflammation state is either hyperaemia of mucosa which is observed in rats after a higher dose of the drug and alcohol, and still showed even after a month after the exclusion of these factors from the diet. The present observations suggest that simultaneous activity of high doses of atorvastatin and ethanol notablably influence damage of mucosa of intestinum.

The toxic influence of alcohol on mucosal barrier of intestinum increases chronic exposure of the organism on bacterial antigens and can cause pathological increases of production of IgA by the intestinal system of monocytes and macrofages and wrong protein synthesis and thus inability to normally repair intestinal damage (11, 12).

Wrong absorption caused by activity of alcohol can be evident in the liquid of lipid layer of membrane of enterocytes microvilli and can influence the connection with membrane enzymatic proteins (2, 13). Atorvastatin inhibits cholesterol synthesis and has influence on the change of composition of lipids fraction (7, 10) and this might influence these changes.

CONCLUSIONS

1. Atorvastatin, after six weeks' administration at the therapeutical dose and 10 x higher, did not cause changes in the histological structure of mucosa of rats' intestinum.

2. The observed damage of epithelium and changes in the stroma of intestinal villi after simultaneous acting of atorvastatin and 20% ethanol show mainly in damaging alcohol activity.

3. Changes which were induced by alcohol remain longer at simultaneous activity at the higher dose of atorvastatin (10 x therapeutical dose), which can mean that atorvastatin delays the recovery from post-alcohol changes.

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SUMMARY

The rats received atorvastatin in the therapeutical dose and 10x higher, 20% solution of ethanol and ethanol with atorvastatin for 6 weeks. The microscopic investigations (H+E staining, Masson's method, PAS method by McManus) showed no changes in histological picture of intestinal mucosa after giving atorvastatin. The rats receiving alcohol and alcohol with atorvastatin showed similar changes, but different than the first groups. Under the epithelium of intestinal villi there were observed light, vesicle-like spaces, separation of epithelium from connective tissue stroma, also lack of the epithelium of the top of villi and hyperaemia of stroma of villi. The observed changes were stronger in rats receiving simultaneously alcohol and atorvastatin in the dose 10x higher than therapeutical. Hyperaemia of connective tissue of stroma of villi was still visible after month period of administration.

Obraz histologiczny błony śluzowej jelita cienkiego szczurów
po jednoczesnym podawaniu atorwastatyny i alkoholu etylowego

Przez okres sześciu tygodni podawano szczurom atorwastatynę w dawce terapeutycznej i dziesięciokrotnie większej, 20% roztwór etanolu oraz etanol łącznie z atorwastatyną. Badania mikroskopowe (barwienie H+E, wg met. Massona i reakcja PAS wg Mc Manusa na polisacharydy) nie wykazały zmian w obrazie histologicznym błony śluzowej jelita cienkiego po podawaniu atorwastatyny. U szczurów otrzymujących alkohol oraz alkohol z atorwastatyną wystąpiły podobne zmiany. Pod nabłonkiem kosmków jelitowych obserwowano jasne, pęcherzykowe przestrzenie, oddzielenie nabłonka od zrębu łącznotkankowego, brak nabłonka na szczycie kosmków oraz przekrwienie zrębu łącznotkankowego kosmków. Obserwowane zmiany silniej wyrażone były u szczurów otrzymujących jednocześnie alkohol i atorwastatynę w dawce dziesięciokrotnie większej od terapeutycznej. Przekrwienie zrębu łącznotkankowego kosmka utrzymywało się jeszcze po miesięcznym okresie karencji.