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Department of Histology and Embryology with the Lab of Experimental Cytology
Medical University of Lublin

DARIA HERNIK, MIROSŁAW ŁAŃCUT, JÓZEF VISCONTI,
AGNIESZKA PEDRYCZ

Ultrastructural changes in the liver of rat after experimental administration of rofecoxib

Cyclooxygenase (COX) is the enzyme which takes part in conversion of arachidonic acid (AA) to prostaglandins (PGs). COX exists in two isoforms: COX-1 and COX-2 (3). COX-1 is the constitutive enzyme, whereas COX-2 is considered to be the inducible enzyme, though it is expressed constitutively in some tissues. Inductors of COX-2 expression are cytokines, hormones, mitogens and growth factors (4). Until recently the role in homeostatic processes was attributed only to COX-1. Nowadays many scientists postulate an important regulating role of COX-2 in physiological conditions. They do research into mechanisms of carcinogenesis induction process in the liver and other organs, which can proceed with COX-2 participation. It is suggested that one of mechanisms of neoplasia in liver tissue is COX-2 overexpression leading to antiapoptosis, therefore inhibition of COX-2 by selective COX-2 inhibitors should promote apoptosis in neoplastic cells (6, 7, 8, 11). Another mechanism of carcinogenesis induction process can have some connections with the presence of chronic hepatitis or liver cirrhosis during which COX-2 occurs as the inducible enzyme (2, 10). Hepatotoxicity of selective COX-2 inhibitors is lower in comparison with classic non-selective COX inhibitors, nevertheless there is noted down the appearance of adverse effects in the form of cholestasis and hepatitis (9).

The following investigations were undertaken owing to lack of accurate literature reports regarding the influence of selective COX-2 inhibitors administered chronically on the liver of healthy individuals and natural apoptotic process in the liver.

MATERIAL AND METHODS

The experiment was carried out on 28 Wistar rat males weighting approximately 300 g. The animals were divided into one control group and three experimental groups. Each group numbered seven animals. The rats were fed with standard granulated fodder and they had water to drink in abundance. They were administered rofecoxib (the preparation Vioxx, MSD, N.Y., USA). The drug was given in the form of suspension in physiological saline by means of intragastric bougie, each day in the morning. The animals from experimental group I received rofecoxib in a single dose 1.25 mg/day for 4 weeks. This dose corresponds to ten times maximal therapeutic 24-hour dose for human being. The animals from experimental group II received rofecoxib in a single dose 0.125 mg/day for 8 weeks. This dose corresponds to the maximal therapeutic 24-hour dose for human being. The animals from experimental group III received rofecoxib in a single dose 1.25 mg/day for 8 weeks (5).

After the end of medicine application period the animals were decapitated. The liver specimens were fixed with buffered glutaraldehyde and 1% osmium tetroxide solution and then embedded in Epon 812. Ultrathin sections cut on ultramicrotome were contrasted with the aqueous solution of uranyl acetate and lead citrate according to the Reynold's method. Ultrastructural observations of the liver were led and pictures were taken in Tesla BS-500 transmission electron microscope.

RESULTS AND DISCUSSION

Rofecoxib is the drug metabolized in the rat's liver through reduction by cytosolic enzymes mainly to 5-hydroxyrofecoxib, with small participation of mitochondrial cytochrome P-450 system. Metabolites of rofecoxib do not show inhibition in relation to COX-2 (1). Expression of constitutive COX-2 is low in the liver (12). Only endothelial cells show somewhat higher COX-2 activity (4).

After 4 weeks' administration of rofecoxib in the dose 1,25 mg/day distinct congestion of the liver parenchyma was stated. Dilated liver sinusoids adhered directly to hepatocytes. Their delicate wall was invisible due to changes in endothelial cells. Moreover, the presence of extravascular exudations between hepatocytes was affirmed. Thicker cellular membranes were observed in some hepatocytes. Mitochondria were in low-energy state. They showed large electron density and well developed crests. There were visible single fatty vacuoles in some cells and also extracellularly. Cell nucleus, nucleolus and nuclear membrane did not show changes compared to control group.

The observed changes show that monthly administration of rofecoxib even in large dose does not damage hepatocytes, whereas cells which are very sensitive to rofecoxib administration as it turned out, are endothelial cells. The authors dealing with the investigation of selective COX-2 inhibitors influence on different organs report that disorder of COX-2 expression in endothelium can contribute to disorders in angiogenesis and in consequence activate neoangiogenesis process (11). Congestion of the liver parenchyma is connected with lowering of blood pressure and reduction of blood flow speed, and next this requires larger energy expenditure. The state of mitochondria pointing at hepatocytes metabolic mobilization is sign of this process (5). In the conducted experiment it was also observed the enlargement of cellular membranes thickness which can be the symptom of defensive mechanism.

After 8 weeks administration of rofecoxib in the dose 0.125 mg/day changes characteristic of the preliminary stage of cell damage in the form of considerably dilated spaces between endoplasmic reticulum membranes were observed. Considerably thicker cell membranes were affirmed in many hepatocytes in comparison with experimental group I. Present lipid drops were much more numerous and larger, whereas mitochondria demonstrated low-energy state like in experimental group I.

The observed changes show that 8 weeks' administration of rofecoxib in a smaller dose seems to be more pathogenic in relation to hepatocytes than 4 weeks' drug administration in a larger dose. Those changes are still reversible about what the state of mitochondria and cell nucleus testify (13).

The 8 weeks' administration of the drug in a large dose 1.25 mg/day caused the twofold kind of changes. In many cells vacuolized cytoplasm and strong disintegration of endoplasmic reticulum were visible. Cell nuclei had correct shape and strongly peripherally condensed chromatin (Fig. 1), whereas mitochondria showed condensed state. In other hepatocytes, in the presence of preserved endoplasmic reticulum, cell nuclei were strongly rugged, and mitochondria were very electron bright, turgent, without visible crests (Fig. 2). Moreover, in the liver of this experimental group a large number of macrophages was affirmed. However, a certain number of cells remained undamaged.

The first type of changes is characteristic probably of reversible damage of cell (13), whereas the enlarged number of macrophages supports the opinion that some cells undergo irreversible damage and are removed from the gland. Some researchers think that high COX-2 expression in the liver with

necrotic process is connected with extremely advanced necrotic and inflammatory changes (10). On the basis of conducted investigations it can be affirmed that similar changes can cause chronic selective COX-2 inhibitor administration in a large dose, which would suggest an important regulating role of COX-2 in homeostasis. The second type of changes which was stated in our experiment is the consequence of the remarkably lowered metabolism. Owing to the lack of signs of endoplasmic reticulum damage and mitochondria and also the presence of changes in cell nuclei structure (pyknotic cell nuclei, chromatin peripheral condensation) it resembles changes that occurred in cells undergoing apoptosis (7, 8, 12). The obtained result would point to apoptosis activation by rofecoxib or intensification of its natural rhythm.

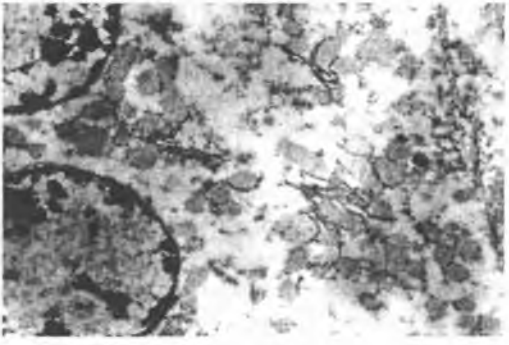

Fig. 1. Hepatocyte of the rat from experimental group III. Cell nucleus with strongly peripherally condensed chromatin and strong disintegration of endoplasmic reticulum is visible. Magn. 4000x

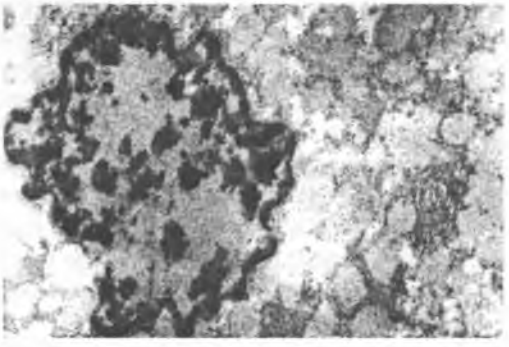

Fig. 2. Hepatocyte of the rat from experimental group III. Cell nucleus with altered chromatin structure and shape as a result of considerable rugosity of nuclear membrane is visible. Magn. 6000x

CONCLUSIONS

1. Rofecoxib administration in the dose 1.25 mg/day for 4 weeks: a) causes considerable damage of liver sinusoids endothelial cells, b) does not damage hepatocytes.

2. Rofecoxib administration in the dose 0.125 mg/day for 8 weeks except damage of liver sinusoids endothelial cells causes damage of hepatocytes in the form of considerably dilated spaces between endoplasmic reticulum membranes.

3. Rofecoxib administration in the dose 1.25 mg/day for 8 weeks except damage of liver sinusoids endothelial cells brings about: a) lowering of hepatocytes metabolism,

b) reversible damaging changes in many hepatocytes, c) changes characteristic of apoptosis in some hepatocytes.

4. Rofecoxib administration can induce or accelerate the natural process of apoptosis in the liver.

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SUMMARY

The Wistar rat males weighing about 300 g were administered rofecoxib according to the scheme: experimental group I – 1.25 mg/day for 4 weeks, experimental group II – 0.125 mg/day for 8 weeks, experimental group III – 1.25 mg/day for 8 weeks. After the end of drug administration period the animals were decapitated and the liver specimens were taken for investigations. Observations were made in transmission electron microscope. It was affirmed that rofecoxib administered for 4 weeks in a large dose causes damage of liver sinusoids endothelial cells, without damage of hepatocytes, rofecoxib administered for 8 weeks in a small dose leads to not a large reversible damage of hepatocytes, whereas rofecoxib administered for 8 weeks in large dose causes damage of hepatocytes or changes in undamaged liver cells which are characteristic of apoptosis.

Ultrastrukturalne zmiany w wątrobie szczura po doświadczalnym podawaniu rofekoksybu

Szczurom samcom rasy Wistar o masie ciała ok. 300g podawano rofekoksyb w schemacie: grupa doświadczalna I 1,25 mg/dobę przez 4 tygodnie, grupa doświadczalna II 0,125 mg/dobę przez 8 tygodni, grupa doświadczalna III 1,25 mg/dobę przez 8 tygodni. Po zakończeniu okresu podawania leku zwierzęta dekapitowano i pobierano do badań wycinki wątroby. Obserwacje prowadzono w mikroskopie elektronowym. Stwierdzono, że rofekoksyb podawany przez 4 tygodnie w dużej dawce powoduje uszkodzenie komórek śródbłonka naczyń zatokowych wątroby bez uszkodzenia hepatocytów, podawany przez 8 tygodni w małej dawce prowadzi do niewielkiego odwracalnego uszkodzenia hepatocytów, natomiast podawany przez 8 tygodni w dużej dawce powoduje uszkodzenie hepatocytów lub zmiany w nieuszkodzonych komórkach wątroby charakterystyczne dla apoptozy.