

Estrogen-induced hepatotoxicity in rats

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Abstract

Estrogens, primarily in the form of oral contraceptives, are responsible for several pathological conditions, including hepatocellular changes, cholestatic injury, vascular lesions and adenomas. The aim of this study was to determine the influence of estrogen on the morphology of the liver. The experiment was conducted on female Wistar rats. Oestradiolum benzoicum was administered intramuscularly for 8 weeks in 3 different doses (0.00075-0.003 mg/kg). All animals were sacrificed after 9 weeks and livers were examined macroscopically, and on the histological and ultrastructural levels. The most common features were as follows: peliosis hepatis, fatty changes, adenomas, nodular regenerative hyperplasia, canalicular cholestasis, and mitochondrial damage. It can be concluded that long-term estrogen administration causes toxic damage in the liver of rats.

Key words

liver, hepatotoxicity, morphology, estrogen, fatty changes, vascular disorders, peliosis hepatis, tumors

INTRODUCTION

The liver plays an important role in the metabolism of estrogen conjugates, such as: cellular uptake of compounds, intracellular metabolism and release of the metabolites to the sinusoidal or to the canalicular side of the liver cells [1]. Hepatic metabolism of estrogens has been extensively studied because such knowledge is fundamental for an understanding of the normal bio-availability of natural and synthetic estrogens, and the molecular alteration in pathological conditions such as fatty changes [2], vascular disorders [3], fibrosis, cirrhosis [4] and liver tumors [5].

Ethinyl estradiol, a synthetic estrogen, is an important component of combined oral contraceptives (OCs). Such therapy has been used daily by more than 50 million women around the world for the prevention of unwanted pregnancy [6, 7]. There are many health benefits of taking birth control pills, but there are also risks associated with the action of the components of OCs (a synthetic estrogen and a progestin), and current literature contains many examples of the negative influence of estrogens on: coagulation process, carbohydrate metabolism, glucagon deposition [8], breast disease [9, 10], cervical neoplasia [11], endometrial neoplasia [12], ovarian tumors [13] and also liver disorders [14]. The effects of estrogen on the liver has been described as a types of acute hepatic injury and as the chronic hepatic injury, the former being manifested by cholestasis, hepatocellular necrosis or steatosis. Vascular lesions, such as peliosis hepatis [15], Budd-Chiari syndrome [16, 17], venoocclusive disease [3], sinusoidal dilatation, portal hypertension [18]; cholestatic injury, steatosis [19] and neoplasm [20], are the chronic complications.

It has been estimated that drugs causing hepatotoxicity have been classified into 2 categories: intrinsic and idiosyncratic hepatotoxins. The majority of drugs that cause hepatotoxicity appear in the second group. They are host-dependent, often dose-independent, and difficult to reproduce in animals. However, the hepatotoxicity caused by intrinsic hepatotoxins, such as estrogens, is generally host-independent, dose-dependent, and reproducible in animals [21].

In the present work, the influence of long-time estrogen administration on liver morphology was studied in rats.

MATERIAL AND METHODS

The entire experiment was based on an animal experimental model in accordance with the general principles for animal experimentation [15], and under the guidelines of the Bioethical Committee of the Medical University in Lublin, Poland.

The study was conducted with outbred female Wistar rats. The animals (180-300 g) were housed in standard laboratory plastic cages (max. 5 rats per cage) at a room temperature of 20±3 °C in a daylight cycle. Standard laboratory chow LSM® (AGROPOL; Motycz, Poland) and municipal filtered tap water were provided *ad libitum*. Food and water consumption were monitored daily.

After a 2-week acclimation period, the animals were gathered in 8 experimental groups (Tab. 1). Oestradiolum benzoicum (Jelfa, Jelenia Góra, Poland) was administered intramuscularly for 8 weeks. Additionally, 2 control groups were designed: K0 – untreated animals (n=20), K1 – animals that received the adequate quantity of oleum *pro injectione* (n=20).

Immediately after decapitation, fragments of the liver were investigated during autopsy. Liver sections were separated during autopsy and routinely processed for further histological and ultrastructural studies. Histological slides were stained with hematoxyline + eosin, methylen blue +

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Table 1. Group characteristics

Experimental group	Dose oestradiolum benzoicum (mg/kg body weight)	Time of administration
E1	0.00075	Once per week
E1.1	0.00075	Every three days
E2	0.0015	Once per week
E2.1	0.0015	Every three days
E3	0.003	Once per week
E3.1	0.003	Every three days

azur, and periodic acid-Schiff, and examined under a light microscope. Ultratime slides contra-stained with osmium tetroxide were evaluated under a Tesla BS-500 transmission electron microscope.

RESULTS AND DISCUSSION

During the study, no behavioral changes were observed. In the control groups, no animal deaths were noted. In the described experiment, the quantity of dead rats in each group was subjected to statistical analysis. In the experimental groups, animal deaths were determined as 7.3% (8 rats).

Histological evaluation revealed the presence of irregular staining of cells, and the circular nucleus showed different stainability (K0, K1, E1, E2), while hepatic triad was clearly visible. Similarly, the cytoplasm staining using the H+E method did not show any significant changes in groups K0, K1, E1 and E2. In all groups of animals, the sites of regular lobulated structure were visible. The liver cells were organized into clusters with not clearly visible borders. The lumen of the vessel was dilated and the clusters of erythrocytes inside the vessel and blood extravasation within the triads were noticed (E1, E2) (Fig. 1).

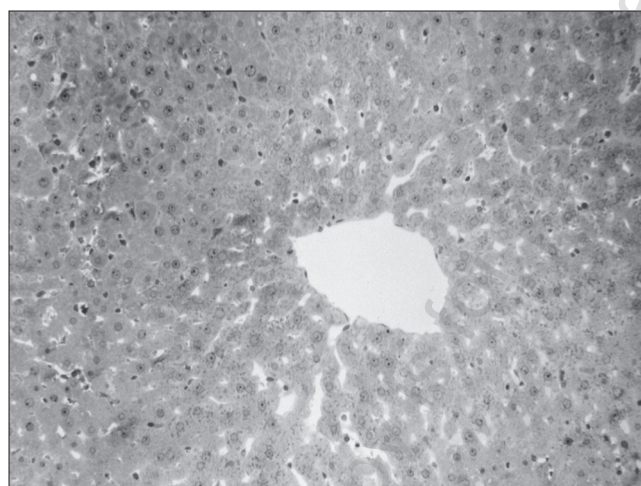


Figure 1. Dilated lumen of central vein. E1 group (methylen blue+ Azur II, magn. approx. 200 x)

In the cases of animals treated with higher doses of estrogens (E2, E3), large inflammatory infiltrations and vasculitis involving the small caliber vascular channels were observed (Fig. 3).

Numerous, diffusely distributed rounded spaces with occasionally clotted bloody fluid inside, were also observed.

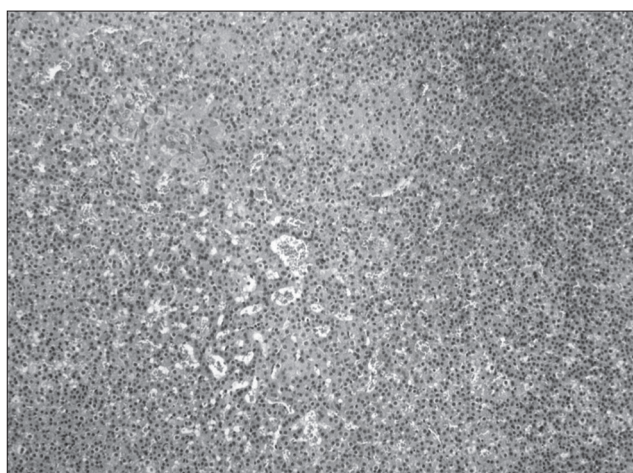


Figure 2. Sinusoidal dilation with congestion. E3.1 group (H+E) (magn. approx. 100 x)

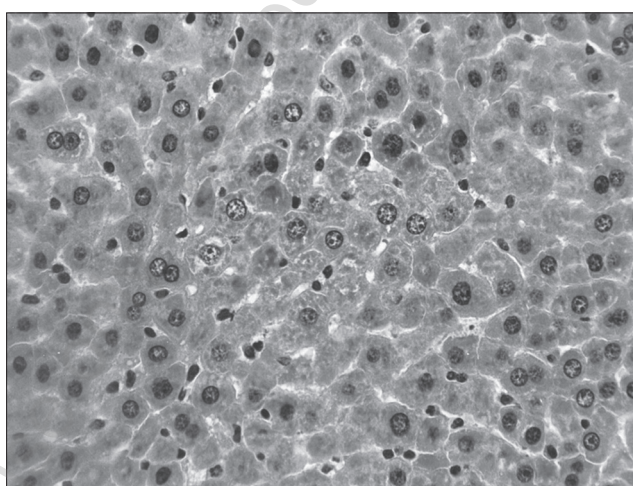


Figure 3. Eosinophilic degeneration of the hepatocytes. E 3 group (H+E) (magn. approx. 400 x)

In single cases, the foci of parenchymal hemorrhage of some spaces and peliosis hepatis, sinusoidal dilation were revealed (Fig. 2). Neighbouring hepatocytes were swollen and microvesicular fatty changes were clearly visible. Furthermore, in 5 cases, amorphous eosinophil masses were noted (E3.1).

According to the criteria of [22], the normal sinusoidal endothelial cells were identified, recognized by their thin cytoplasmic extensions, oval nucleus, sparse cytoplasmic organelles, and a constant location in the sinusoidal lining. Such cells were observed in the less inflamed areas (E1, E2) (Fig. 5). In areas with inflammatory infiltration, the sinusoidal endothelial cells showed changes. For this type of endothelial cell, Bardadin and Desment proposed the term 'active endothelial cell' [23]. This criterion was accepted for the presented study. The active endothelial cells were found in the groups of animals which received the highest doses of estrogen (E2, E3, E3.1). They were characterized by a decrease in the number of glycogen, enlargement of the Golgi complex, increase in size and number of mitochondria, increase in the number of free ribosomes, and increase in the smooth endoplasmic reticulum (Fig. 4).

Occasional bile canaliculi from the livers of the estrogen-treated animals (E3 and E3.1) showed only mild canalicular dilatation with microvilli diminished in number. Evagination

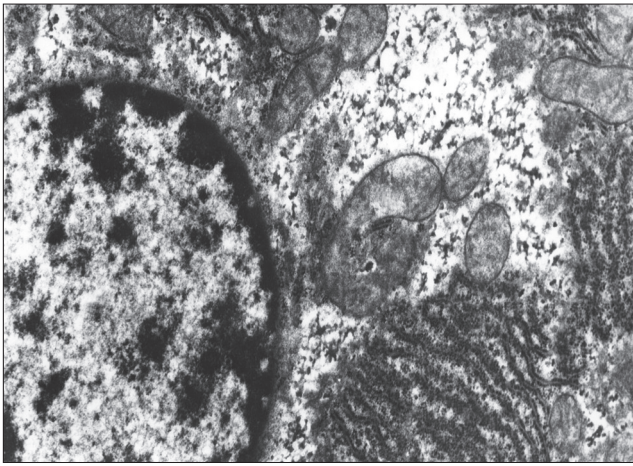


Figure 4. Electron micrograph of rat liver. E3.1 group. Swelling of mitochondria and sparse glycogen granules in the cytoplasm of the hepatocytes (magn. approx. 32,000×)

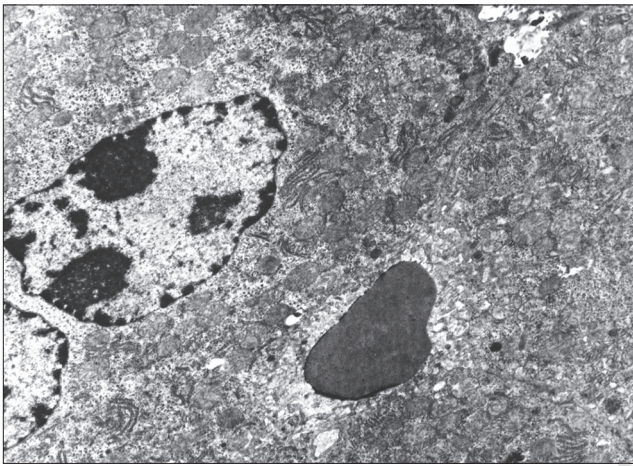


Figure 5. Electron micrograph of rat liver. E2 group. Mild bile canalicular dilatation with microvilli diminished; erythrocytes inside the space of Disse (magn. approx. 8,000 ×)

or disruption of portions of the canalicular membrane were not observed. In 6 samples from animals group E3.1, Ito cells were found.

In the groups of estrogen-treated animals, vascular disorders were clearly visible. Sinusoidal dilatation were noted in the samples from the E3 and E3.1 groups of animals. In these last mentioned groups, round, blood-filled spaces connecting with the hepatic sinusoids were observed. Peliotic spaces containing blood and with erythrocyte extravasation were often observed. There were occasional foci of parenchymal hemorrhage and thrombosis of some spaces. Sporadically in groups E3.1 (3 cases), a focal nodular hyperplasia was founded.

The active endothelial cells, as well as hepatocytes changes, such as necrosis and inflammatory infiltrations, observed in groups E2.1, E3, E3.1, could be interpreted as drug-induced. The results show that administered estrogen is intrinsic, and a mild hepatotoxicant capable of producing fatty changes. The highest doses of estrogen initiated vascular disorders, mainly vasculitis and peliosis hepatis, and led to a dramatic form of sinusoidal dilatation. Furthermore, the study shows that long-term estrogen administration has been incriminated in hepatic vein thrombosis, and changes described as focal

nodular hyperplasia (5 cases). The changes observed in our experiment seemed to be types of chronic hepatic injury: fatty changes, peliosis hepatis, vascular lesions, sinusoidal dilatation, and adenoma.

Electron microscopy provides an important advance in the study of the microstructure of the liver by enabling the surface of the hepatocytes to be exposed so that details of intracellular spaces and sinusoidal endothelial cells, mitochondrial, and endoplasmic reticulum (ER) are visible. The normal electron picture of the liver has been described previously [24, 25]. Since then, the study of liver structure is on-going [26]. Our ultrastructural observation confirmed the changes observed [26] in 12 liver biopsies from patients with chronic active hepatitis. The active endothelial cells with nuclei which were oblong, sometimes with a slight indentation, were also identified by examination of semi-serial sections from multiple blocks from the some needle biopsy in the study of [26]. Furthermore, the chromatin was dispersed in course floculations and showed condensation near the nuclear membrane. Mitochondria were roughly similar in size. These authors observed the fibroblastic reticulum cells in sinusoids and portal tracts. They were often seen in areas with abundant amorphous basement-membrane-like material, and reticulin fibres in the space of Disse. These were interpreted by the authors as fibroblasts. We also observed dilatation of the space of Disse and the Ito cells within the latter. We did not observe cells with myofilaments from the fibroblastic reticulum cell group, described in a previous study on the hepatotoxic effects of drugs by [27]. The active endothelial cells observed in our study, and the fibroblastic reticulum cells excluded by the above-mentioned authors, may play a role in protecting hepatocytes against a toxic effect. These cells, observed by indirect immunofluorescence and described by these authors, could be responsible for the synthesis of collagen types I, III, IV and V, as well as fibronectin. Further study is needed on this phenomenon

One of the most common responses of the liver to estrogen insult is the accumulation of fat in the parenchymal cells, most often in the periportal zone. Estrogen, like other chemicals, interferes with the normal processing of lipids and other materials for secretion from the liver parenchyma; lipids thus accumulate. Focal fatty change occurs in rat liver without a specific lobular distribution. The fatty changes described in literature were also noted in our experiment in the E2.1, E3, E3.1 groups.

In the previous study regarding the hepatotoxicity effect of estrogen administration, performed on the same group of rats, vascular lesions were reported [28, 29]. The vascular lesions were present in the groups of animals treated with higher doses of estrogens. Both venules and arterioles were spared, and no aneurysms were observed. Large inflammatory infiltrations and vasculitis involving small caliber vascular channels were visible (E2, E3). The inflammatory infiltrate consisted of eosinophils and mononuclear cells (E2, E3, E3.1). According to Taxy [30], these vascular disorders can be used to characterize necrotizing hypersensitivity. In our experiment, single necrotic hepatocytes were noted (E3.1). This is compatible with the results of other authors who suggest that necrotizing vascular lesions occur in certain forms of hypersensitivity angiitis, but it is possible that necrotizing lesions are a secondary phenomena [25]. It is important to note that the time of administration and

the development of vasculitis varies. Similar changes of a vasculitis character were observed in the K1, E1, E2 and E3.1 groups. In the described study, drug hypersensitivity is not dose- or time-dependent, and these results are comparable with the results described in the available literature. These data can follow from the suggested theory that vasculitis is the result of antigen-antibody interactions [31].

The pathogenesis of peliosis of the liver remains unclear [32]. In our experiment, rounded, blood-filled spaces were seen to be involved in different areas of the liver. These areas were occasionally adjacent to areas of ecstasic sinusoids. These spaces containing erythrocytes were found in groups E2, E3 and E3.1. Such changes were compared with the results obtained by [33]. Those authors occasionally observed areas of organizing hemorrhage with parenchymal necrosis, which we also noted, in single cases in light and electron microscopy. The progressive numbers of erythrocytes accumulated within hepatocytes and hepatocellular necrosis are responsible for the formation of the blood-filled spaces.

The association of peliosis hepatitis with hepatic tumors has been described in the available literature [34]; although it is difficult to refer the results of experimental studies to human peliosis hepatitis. On the basis of the present study, however, it can be stated that this is possible, but higher doses of estrogens and long-term drug-administration are required. In group of animals administered with the highest doses of estrogen, peliosis hepatitis was observed in the neighbourhood of focal nodular hyperplasia (E3, E3.1). Thus, peliosis hepatitis could be an important symptom of carcinogenesis [4]. Early development of these changes allowed avoidance of tumors, especially in patients of risk groups.

Obtained data and review of literature suggest that estrogen preparations could be responsible for toxic damage of rat liver, described as fatty change, vascular disorders, peliosis hepatic, and focal nodular hiperplasia. Data derived from such a model should provide more accurate information regarding the relationships between dose and toxic damage risk of liver in women. Further study, not only morphological but also biochemical and metabolic, are needed.

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