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Benign hepatic tumors in association with estrogenic therapy

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Summary

A long-term use of oral contraceptives may be responsible for an increased risk of benign hepatic tumors (hepatic adenoma, focal nodular hyperplasia (FNH), hemangioma). The purpose of this study was to determine the effect of estrogen administration on the liver function and morphology. Oestradiolum benzoicum was administered intramuscularly to female rats for 8 weeks. Liver sections were collected for histological and ultrastructural examination. The most common features were as follows: nodular regenerative hyperplasia, adenomas, peliosis hepatis and fatty changes. In animals which had received higher than therapeutic doses of estrogens the following ultrastructural changes were found: mitochondrial damage, megamitochondria, diffusely distributed round spaces with blood fluid inside, fatty changes, canalicular cholestasis and steatohepatitis with Ito cells. Data derived from this model should provide more accurate information useful in recognizing hepatotoxicity of a drug during initial toxicological studies and during initial evaluations in man before widespread clinical use, as well as in determining the risk of liver tumor development.

Keywords: estrogens, liver, adenoma, focal nodular hyperplasia, peliosis hepatis

Hepatic adenoma was first described as a complication of oral contraceptive therapy in 1973 (2). Since then a large number of cases of this tumor have been reported, over 90% of them in women taking contraceptive steroids (3). A benign proliferation of liver cells includes such changes as focal nodular hyperplasia (FNH), hepatocellular adenoma and macroregenerative nodule (1).

Regenerative hyperplasia denotes a hepatocellular lesion related to past or ongoing hepatocyte necrosis, along with other aspects of liver lesions, including hepatocyte hyperplasia. Macroscopically, the liver may or may not be enlarged, but the distortion of the shape of the lobes is possible. The nodules are clearly separated from adjacent parenchyma, but their color depends on the lipid and glycogen content of hepatocytes or the amount of blood present in the lesion (5). The available literature describes two characteristics essential for the diagnosis of regenerative hyperplasia. One is the presence of one or more discrete nodular damages of hepatocytes that lack the histologic features of neoplasia, but lobular architecture is distorted or missing in some area of the section. The other essential feature is the evidence of ongoing hepatic damage such as necrosis, degeneration, atrophy, fibrosis, inflammation and steatosis. Another type of damage described in the literature is the compression of adjacent hepatic parenchyma, which distorts the lobular architecture so that the central veins are partly collapsed, and portal areas may appear to be missing (9). Chronic inflammation, hepatic degeneration and oval cell proliferation may be present in sites of extensive hyperplasia located in portal areas and around the periphery of the nodules. Focal hyperplastic lesions are round in a two-dimensional view, within which there may be an increased number of mitoses, degenerating hepatocytes and microgranulomas. In some cases necrosis may not be present. This suggests that regenerating hepatocytes in areas of regenerative hyperplasia are relatively resistant to toxic effects of the chemical (13).

Among typical complications of estrogen use are hepatocellular adenomas (16). The pathogenesis of hepatocellular adenomas is unknown, but various

authors suggest that this lesion may be a response to a pre-existing vascular abnormality (19). These lesions are described as round or spherical, and often have an irregular border. Microscopically, these tumors are composed of nodules of parenchymal cells that are enlarged and eosinophilic, the lobular architecture is lost, and occasionally cellular atypia is observed. Adenomas may not be sharply demarcated from the normal liver parenchyma. There is a lack of continuity between the nodule and the surrounding unaffected liver (12). Steatosis and other degenerative changes may be present, but necrosis within adenoma is uncommon. Vascular disorders may also be observed (10).

The purpose of this study was to determine the causal link between estrogen preparations and hepatic cell adenoma or focal nodular hyperplasia.

Material and methods

The study was conducted in accordance with general principles for animal experimentation and under the guidelines of the Bioethical Committee of the Medical University of Lublin, Poland (8).

The experiment was conducted on outbred female Wista rats with an initial body weight of 180-300 g. The animals were housed in standard laboratory plastic cages (maximum 5 rats per cage) at a room temperature of 20 ± 3°C in a daylight cycle. Standard laboratory feed LSM® (Agropol; Motycz, Poland) and filtered municipal tap water were provided *ad libitum*. Food and water consumption were daily monitored. Animals were weighed on the first day of the experiment and at the end of the 2nd, 4th, 6th and 8th week. After a two-week acclimation period, the animals were randomly selected into 8 experimental groups. Oestradiolum benzoicum (Jelfa – Jelenia Góra, Poland) was administered intramuscularly for 8 weeks in six different doses: E1 – 0.00075 g/kg of the body weight (n = 15 number of rats) once a week; E1.1 – 0.00075 g/kg b.w. (n = 15), every three days; E2 – 0.0015 g/kg b.w. (n = 15), once a week; E2.1 – 0.0015 g/kg b.w. (n = 15), every three days; E3 – 0.003 g/kg b.w. (n = 15), once a week; E3.1 – 0.003 g/kg b.w. (n = 15), every three days. Two control groups were formed: K0 – untreated animals (n = 20), K1 – animals receiving an adequate quantity of oleum *pro injectione* (n = 20).

During autopsy immediately following decapitation, the whole liver was removed and weighed. A small section of the right liver lobe was taken. Fragments of the organ chosen for histological examination were fixed in 10% buffered formaldehyde solution and routinely processed into paraffin sections. Histological preparations were evaluated with a light microscope (Axiscop; Zeiss, Germany) with hematoxylin-eosin, azan and histochemical paS (periodic acid-Schiff) stains. Furthermore, an ultrastructural study was performed. Liver sections were fixed for 1 hour in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), followed by a phosphate buffer rinse overnight. The specimens were postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated in graded alcohol solutions and embedded in Epon resin. Semithin and ultrathin sections were cut with a Reichert Ultracut S ultramicrotone. Semithin sections stained with methylene blue were used to select blocks containing foci of periportal piecemeal necrosis. Ultrathin section were stained with uranyl acetate and lead citrate and examined in a Tesla BS-500 electron microscope.

Results and discussion

Adenomas usually appeared as solitary well-demarcated masses within a noncirrhotic liver in groups E2.1 and E3. Occasionally, more than one lesion was found. All tumors were round and usually had an irregular border (fig. 1). Upon sectioning, the tumors were fleshy but uncapsulated, brighter than the surrounding hepatic parenchyma, and usually exhibited zones of hemorrhage.

Histologically, the tumors were composed of hepatocytes arranged in plates. These noted plates were separated by compressed sinusoids. In the groups of animals which received the highest dose of estrogens (E3, E3.1), enlarged portal tracts were visible. In some histological samples the normal lobular architecture was completely destroyed (fig. 2). In ultrastructural examination, nuclei were generally small and irregular, and the nuclear-cytoplasmic ratio was normal. Multinucleated tumor cells were found in some cases. Nuclei with a degenerated hyperchromatic appearance were observed in groups E3 and E3.1. Estradiol also induced an increased incidence of glycogen and lipid

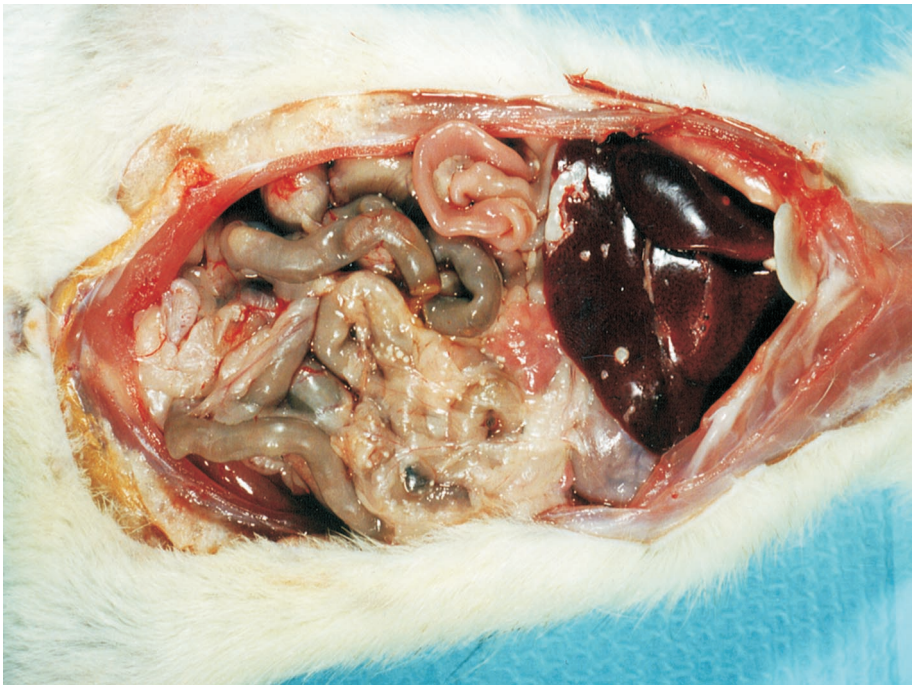

Fig. 1. Enlargement of the liver with multiple nodular lesions. E 3.1 (macroscopic view)

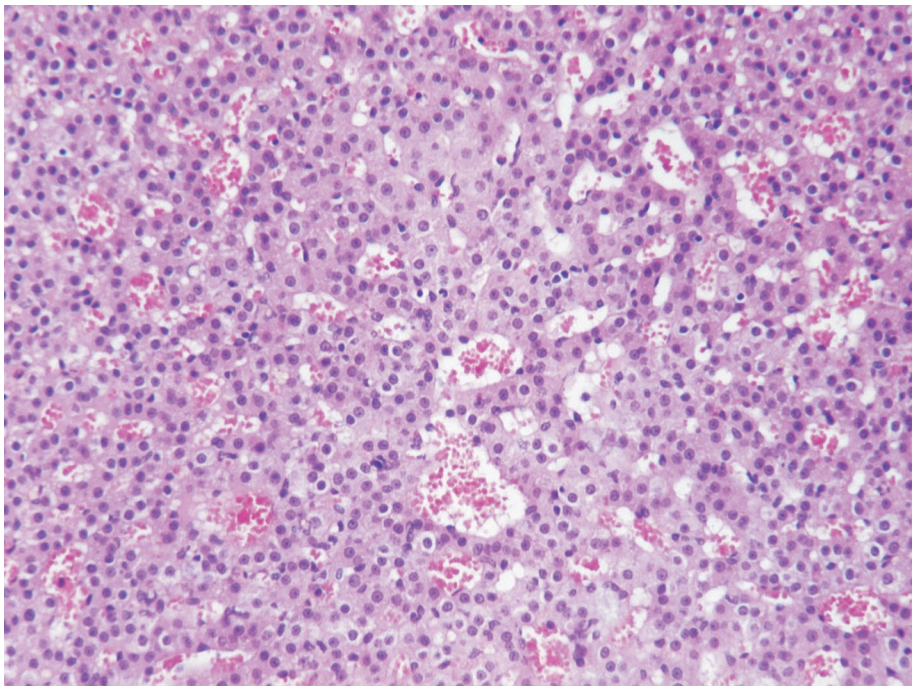

Fig. 2. Nodular hyperplasia composed of irregular cords of hepatocytes. (H+E. magn. approx. 100 ×)

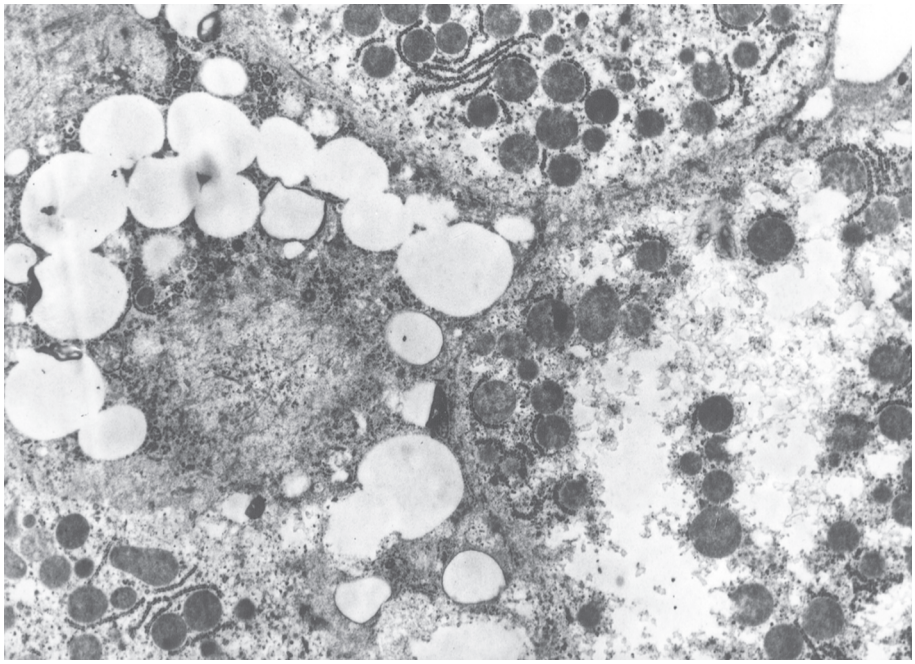

Fig. 3. Many lipid vacuoles and swelling of mitochondria together with decrease in glycogen granules in the cytoplasm of hepatocytes. (TME. magn. approx. 16 000 ×)

accumulation. Occasionally, fatty changes and canalicular cholestasis were revealed (E2.1, E3, E3.1). Focal fatty changes occurred without a specific lobular distribution. The cytoplasm of the cells in these foci contained clear spherical vacuoles ranging from small and multiple to large and solitary, filled with fat (fig. 3). Nuclei were centrally located or pushed to one side. In 12 cases, megamitochondria and changes of steatohepatitis with Ito cells were visible. Occasional bile canaliculi from the livers of estrogen-treated animals (E3, E3.1) showed a dilatation of the lumen, with simplified appearance due to the loss of microvilli. Ultrastructurally, cytoplasmic vacuoles were associated with a dilated and vascularized endoplasmic reticulum. Some mitochondria also appeared swollen. Clusters of erythrocytes inside dilated vessels and blood extravasation within the triads were found (E2, E3). In some cases large inflammatory infiltrations consisting of eosinophils and mononuclear cells were observed (E2, E3, E3.1). Numerous diffusely distributed round spaces with bloody fluid inside, similar to peliosis hepatis, were also seen (E3, E3.1).

The data obtained showed that estrogen supplementation could be responsible for the development of hepatocellular adenomas in the rat's liver. The intramuscular administration of Oestradiolum benzoicum led to lesions characterized by a well-circumscribed region of hyperplastic liver tissue with stellate fibrosis (E3, E3.1). Such changes could be diagnosed as adenomas consisting of nodules of parenchymal cells. Moreover, the lobular architecture was lost. In the groups of animals treated with the highest doses of estradiolum benzoicum fatty changes were observed. Furthermore, in these samples vascular disorders were clearly visible. Microscopically, in these groups hepatic angiostasis consisted of dilated vascular spaces filled with red blood cells. Adjacent parenchyma was compressed because of dilated spaces. This type of vascular disorder is often a component of other lesions such as foci of cellular alteration or hepatocellular neoplasms (5). Round blood-filled spaces were observed in different areas of the liver. These changes were compared with results obtained by Molleken (14), who also occasionally observed areas of organizing hemorrhage and the necrosis of hepatocytes. These findings could explain the pathogenesis of liver lesions. The increasing number of erythrocytes accumulated within hepatocytes and hepatocellular necrosis are responsible for the formation of blood space (14, 15). The available literature describes the association of peliosis hepatis with hepatic tumors (18). Furthermore, peliosis hepatis can be an important symptom of carcinogenesis.

Similar changes were also observed by other authors in samples of women's liver, especially in samples from women using oral contraceptives (20).

It is difficult to say if the results of our experimental studies apply to changes in humans. Diagnosis and interpretation of liver lesions in rodents became considerably easier after the issuing of Guides for Toxicologic Pathology by the Society of Toxicologic Pathology in collaboration with the American Registry of Pathology and the Armed Forces Institute of Pathology. In this experiment we used a rat model because the spontaneous incidence of liver tumors in most rat strains is low, on the order of 1-3%. In mice, for example, tumor incidence ranges from 5% to as high as 80% and varies depending on the animal's age and strain (6).

The pathogenesis of hepatocellular adenomas remains unclear in both human and animal models. Some investigations suggest that estrogen contained in the pill induces some vascular changes or induces cell hypertrophy (4). This hypothesis has been confirmed by our data. High doses of estrogens administered for a long time led to peliosis hepatis. Peliosis hepatis, large inflammatory infiltrations and vasculitis involving small caliber vascular channels were observed exclusively in groups E2.1, E3 and E3.1 in the neighbourhood of focal nodular hyperplasia. Similar changes accompanying FNH were observed in patients who had undergone a biopsy or resection of the liver. These changes, observed in our study and confirmed by other authors, show a relationship between adenomas, vascular disorders and estrogen therapy (7).

Other researchers made two important observations: 1. tumors did not develop unless the dose of the toxicant was sufficient to result in a significant necrosis of hepatocytes; and 2. intermittent exposure to the toxicant was more likely to produce tumors.

Data derived from this model should provide more accurate information useful in recognizing the hepatotoxicity of a drug during initial toxicological studies and during initial evaluations in man before widespread clinical use, as well as in determining the risk of liver tumor development.

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