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The relationship between estrogen and the development of liver vascular disorders

The liver is central to the metabolic disposition of all drugs and foreign substance. Drug-induced liver injury is potential complication of estrogen preparations. Thus, vascular disorders have been recorded as a rare consequence of taking oral contraceptive. This lesion is connected with vascular injury at any level of the hepatic venous drainage (10).

The primary estrogen-induced vascular disorders are peliosis hepatis and vasculitis. Peliosis hepatis has been described as a rare consequence of taking estrogens and contraceptive. This condition is characterized by the presence of blood-filled lacunar spaces and by areas ectatic sinusoids. The peliotic damage of the liver may rupture and lead to the hemoperitoneum (11). Both human and animal data supported such a link (13, 2). Vasculitis has been noted as necrotizing or non-necrotizing hypersensitivity and an inflammatory infiltrate involving all the walls of a vessel. Vasculitis is usually connected with the presence of increased numbers of eosinophils in either the blood or the tissues (6).

The aim of the study was to determine the influence of estrogen preparations on liver vascular disorders of rats during the long-time estrogens therapy.

MATERIAL AND METHODS

The whole experiment was based on an animal experimental model. The studies were designed according to the guidelines of Bioethical Committee, Medical University of Lublin. The experiment was conducted on female rats of Wistar strain with the initial body weight of 180-300g. The animals were subjected to reverse light cycling for 2 to 3 weeks before use. The middle dark point was set at 10 a.m. The rats were housed in standard laboratory cages (max. 6 pieces per cage). After acclimation period, the animals were gathered in 5 experimental groups of minimum 10 in a group. Oestradiolum benzoicum (Jelfa, Jelenia Góra, Poland) was used for the purpose of this study. Oestradiolum benzoicum was given i. m. one time per week for 8 weeks in three different doses: E1 – 0.00075g/kg of the body weight (n=15, number of rats); E2 – 0.0015g/kg b. w. (n=15); E3 – 0.003g/kg b. w. (n=15). Two control groups were designed: K0 – the untreated animals (n=15); K1 – the animals received the adequate quantity of *oleum pro injectione* (n=15). All the animals were killed by decapitation after 9

weeks of experiment and the livers were delivered by laparotomy. Fragments of organs assigned for histological examination were fixed in 10% buffered formaldehyde solution and transformed into paraffin sections, routinely. Histological preparations were evaluated in the light microscope (Axioscop of Zeiss make). The histological assays were determined using: hematoxylin-eosin, azan, and histochemical paS (periodic acid-Schiff) stains.

RESULTS

In the described experiment the quantity of dead rats in each group was subjected to statistical analysis. There were more dead rats in the groups of treated animals (32.3%) than in both control groups (19%). Histological evaluation revealed the presence of irregular staining of cells and the circular nuclear showed different stainability (K0, K1, E1, E2). The hepatic triad was clearly visible. Stainability of nuclears was the same in all cells. Similarly, the cytoplasm stained using the hematoxilin-eosin method did not show any significant changes (K0, K1, E1, E2). In all groups of animals, the places of regular lobulated structure were visible. The liver's cells were organized into clusters with not clearly visible borders. The lumen of vessel was dilated and the clusters of erythrocytes inside the vessel and blood extravasation within the triads were noticed (E1, E2).

In the cases of animals treated with higher doses of estrogens, large inflammatory infiltrations and vasculitis involving small caliber vascular channels were observed (E2, E3). The inflammatory infiltrate consisted of eosinophils and mononuclear cells (E2, E3, E1). Numerous, diffusely distributed rounded spaces with bloody fluid inside, which was occasionally clotted, were observed. In single cases the foci of parenchymal hemorrhage of some spaces were revealed (Fig. 1). Neighbouring hepatocytes were swollen and microvesicular fatty changes were clearly visible (Fig. 2).

DISCUSSION

The results show that long-term estrogen administration initiates vascular disorders, mainly vasculitis and peliosis hepatis. The vascular lesions were present in the groups of animals treated with higher doses of estrogens. Both venules and arterioles were spared, and aneurysms were not observed. According to T a x y, these vascular disorders can be used to characterize necrotizing hypersensitivity. In this kind of vascular lesions fibrinoid necrosis and inflammatory cells could be observed in all layers of the vessel (9).

In the present experiment, with the use of the light microscope no necrotic cells were observed. Most authors separate the hypersensitivity and panarteritis nodosa, but some changes suggesting vasculitis could be recognized like the last one (5). In addition to the vascular lesions associated with drug therapy, we have got accustomed to using the term of hypersensitivity angiitis. Some investigators indicated that hypersensitivity was related to the development of vascular damage but not to the development of panarteritis nodosa (3).

In our experiment, no necrotic cells were noted in any of the cases. It is compared with the results of other authors. They suggest that necrotizing vascular lesions occur in certain forms of hypersensitivity angiitis, but it is possible that necrotizing lesions are secondary phenomena (8, 9). The absence of necrotizing lesions can confirm the suggestion that vascular lesions can be non-necrotizing. It is important to note that the time of administration and the development of vasculitis vary. The similar changes of vasculitis character were observed in K1, E1, E2, and E3 groups. In the described study drug hypersensitivity is not dose or time dependent and is compared with results in the available

literature. These data can follow from the suggested theory that vasculitis is the result of antigen--antibody interactions (6).

According to Bierman in clinical practice, the patients with drug related vasculitis can be divided into two groups. The first group of patients developed only skin reaction. The other group developed systemic vasculitis, sometimes with skin reaction (7). Therefore, all the changes observed in the study seem to be of experimental character. However, it seems that this finding acknowledges clinical changes revealed in the second out of the above described groups.

The pathogenesis of peliosis for the liver remains unclear (8). Microscopically, rounded blood-filled spaces were seen to be involved in different areas of the liver. These areas were occasionally adjacent to areas ectatic sinusoids. The spaces containing erythrocytes were found (E2, E3). In 3 cases there were changes identified as a vascular hamartoma. These changes were compared with the results obtained by Molleken. This author, occasionally observed areas of organizing hemorrhage with parenchymal necrosis which we also noted, in single cases in light microscopy. These findings could explain the pathogenesis of liver lesions. The progressive numbers of erythrocytes accumulated within hepatocytes hepatocellular necrosis are responsible for the formation of blood space (6).

In the available literature, association of peliosis hepatis with hepatic tumors was described (1). It is difficult to refer the results of experimental studies to human peliosis hepatis. On the basis of the present study it can be said that this is possible, but the higher doses of estrogens and long-term treatment are required for the development of hepatic tumors. Peliosis hepatis can be the important symptom of carcinogenesis (4).The higher doses of sex steroids given through the long time led to peliosis hepatis. Early development of these changes allowed to avoid tumours, especially in patients of risk groups.

CONCLUSIONS

1. Estrogen can be responsible for the development of vascular disorders.
2. The observed changes suggest drug related vasculitis.
3. An increased awareness of pelosis hepatis may become an important symptom for pathologist, especially in patients at risk.

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EXPLANATION TO FIGURES

Fig. 1. The early stage of peliosis hepatis. The inflammatory infiltrate remains within the portal tract.

Fig. 2. Peliosis hepatis and microvesicular steatosis secondary to estrogen treatment.

SUMMARY

The liver is central to the metabolic disposition of all drugs and foreign substance. Drug-induced liver injury is a potential complication of estrogen preparations. The primary estrogen-induced vascular disorders are peliosis hepatis and vasculitis. Peliosis hepatis has been described as a rare consequence of taking estrogens and contraceptive. This condition is characterized by the presence of blood-filled spaces. Vasculitis has been noted as necrotizing or non-necrotizing hypersensitivity and an inflammatory infiltrate involving all the wall of a vessel. Vasculitis is usually connected with the presence of increased numbers of eosinophils either in blood or tissues

The aim of the study was to determine the influence of estrogen preparations on liver vascular disorders.

The experiment was conducted on female rats of Wistar strain with the initial body weight of 180-300g/kg of the body weight. After acclimation period, animals were gathered in 5 experimental groups of min. 10 in the group. Oestradiolum benzoicum was used for the purpose of this study. It was given i.m. once a week for 8 weeks in three different doses: E1 – 0.00075g/kg, E2 – 0.0015g/kg; E3 – 0.003g/kg of the body weight. Two control groups were designed: K0 – the untreated animals, K1 – the animals receiing the adequate quantity of oleum pro injectione. Fragments of organ assigned for histological examination were fixed in 10% buffered formaldehyde solution and transformed into paraffin sections. Histological preparations were evaluated in the light microscope. The histological assays were determined using: hematoxylin-eosin, azan and histochemical paS (periodic acid-Schiff) stains.

In the described experiment large inflammatory infiltrations and vasculitis (E2, E3) were observed. In the animals treated with higher doses of estrogens diffusely distributed infiltrations around spaces

with bloody fluid inside were revealed. The lumen of vessels was dilated. Estrogens can be responsible for the development of vascular disorders described as peliosis hepatis. The observed changes were suggestive of drug related vasculitis. An increased awareness of pelosis hepatis may become an important symptom for a pathologist, especially in patients at risk.

Wpływ estrogenów na rozwój zaburzeń naczyniowych wątroby

Wątroba stanowi centrum detoksykacji większości stosowanych leków. Toksyczne uszkodzenie tego narządu przez estrogeny jest zjawiskiem wielokrotnie podkreślanym w literaturze. Opisywano je jako ogniskową martwicę wątroby, rozrost guzkowy, zastój żółci w kanalikach żółciowych, a także jako gruczolaki i raki wątroby. Zmiany naczyniowe dotyczące zatok, większych naczyń żylnych i tętniczych określono jako *peliosis hepatis* i *vasculitis* (zapalenie naczyń). Peliozę definiujemy jako poszerzenie światła naczyń zatokowych z towarzyszącym uszkodzeniem ścian naczyniowych oraz z obecnością mikroskopowych jeziorek wypełnionych krwią. Wywołane lekami zapalenie naczyń może przebiegać w postaci martwicy z nadwrażliwości lub w postaci alergicznego zapalenia naczyń. Zmiany zapalne obejmują zwykle wszystkie warstwy ściany naczynia.

Celem pracy był zbadanie wpływu preparatów estrogenowych na zmiany naczyniowe wątroby szczura. Badania przeprowadzono na albinotycznych szczurach rasy Wistar. W czasie eksperymentu zwierzętom podawano Oestradiolum benzoicum. Zwierzęta podzielono na 5 grup eksperymentalnych: K0–grupa zwierząt zdrowych, które posłużyły jako wzorce, K1–grupa zwierząt, którym podawano preparat oleisty (*oleum pro injectione*) w dawce 1,2ml/100g masy szczura; E1, E2, E3–zwierzęta, którym podawano domięśniowo estrogeny długodziałające, raz w tygodniu przez 8 tygodni, w dawce odpowiednio 0,00075g/kg, 0,0015g/kg i 0,003g/kg masy szczura.

Preparaty histologiczne barwiono: metodą H+E, metodą PAS i metodą AZAN. Ocenę preparatów i dokumentację fotograficzną przeprowadzono w mikroskopie świetlnym. W preparatach mikroskopowych największe zmiany stwierdzono w grupach zwierząt leczonych większymi dawkami estrogenów. Obserwowano nacieki zapalne wokół triad i żył centralnych, poszerzenie światła naczyń zatokowych najczęściej w strefie centralnej, *peliosis hepatis* oraz przekrwienie (E2, E3).

W zastosowanym układzie doświadczalnym wykazano, że długotrwała terapia estrogenowa powoduje zaburzenia naczyniowe, których nasilenie zależało od użytej dawki leku. Dla patomorfologów stopień zaawansowania zmian o charakterze *peliosis hepatis* może być istotnym wskaźnikiem uszkodzenia wątroby.

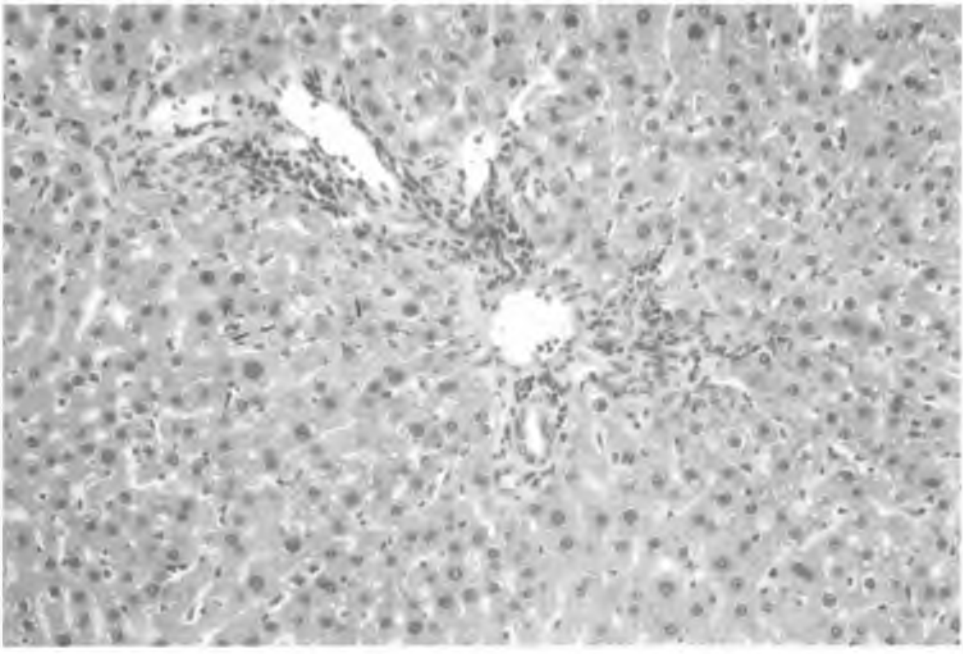

Fig. 1

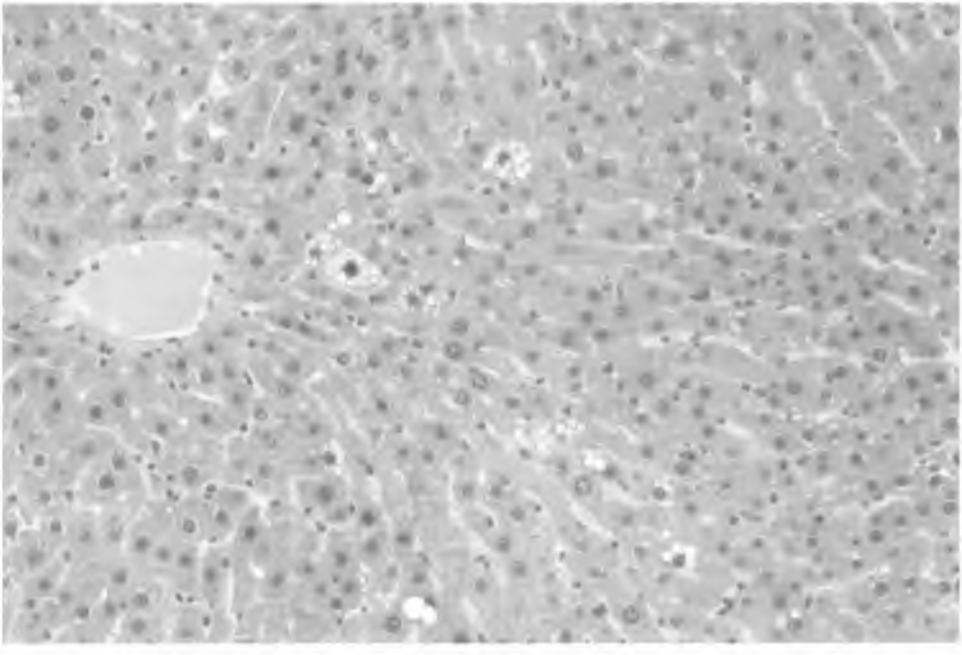

Fig. 2