Morphologic Effects of Oral Contraceptives (Norinyle) on the Guinea Pig Liver

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In view of continuous clinical reports of liver damage following oral contraceptive use, morphologic changes of the liver following the administration of a combined type of oral contraceptive (Norinyle) were studied in guinea pigs by light and electron microscopic examinations. Two interesting changes, one in Kupffer cells and another in the hepatocytes were observed. The Kupffer cells were hyperplastic and enlarged due to the phagocytosis of a large amount of fibrin which were apparently formed intravascularly and were being cleared by Kupffer cells. It is thought that enlarged Kupffer cells and excessive fibrin formation beyond the clearing capacity of Kupffer cells may lead to blockage of sinusoids resulting in a Budd-Chiari like syndrome. The nucleus of the hepatocytes showed mild enlargement and a clear nucleolar segregation which is regarded as one of the ultrastructural evidences of chemical carcinogenesis. Therefore, it is speculated that this change may be related to tumorigenesis in the liver following oral contraceptive administration. Other changes consisted of ischemic necrosis and fatty changes in occasional animals, alteration of mitochondrial configuration and hyperplasia of smooth endoplasmic reticulum in hepatocytes.

Oral contraceptives are used by millions for the purpose of birth control. Although their merit is overwhelming, some adverse effects are continuously being reported.

The liver is known to be affected both functionally and morphologically by oral contraceptive use. Functionally impairment of excretion of sulfobromophthalein (Fallagher et al., 1966; Kappas, 1967; Ockner and Davidson, 1967), cholestasis (Orellana-Alcalde and Dominiquez, 1966; Schaffner, 1966; Stoll et al., 1966; Ockner and Davidson, 1967), increase of serum alkaline phosphatase (Orellana-Alcalde and Dominiquez, 1966; Thulin and Nermark, 1966), and elevation of serum transaminases (Schaffner, 1966; Stoll et al., 1966; Thulin and Nermark, 1966; Ockner and Davidson, 1967) were reported. Morphologically hepatic cell necrosis (Eisalo et al., 1964; Borglin, 1965; Orellana-Alcalde and Dominiquez, 1966; Stoll et al., 1966; Thulin and Nermark, 1966; Ockner and Davidson, 1967), Budd-Chiari like syndrome (Ecker et al., 1966; Sterup and Mosbech, 1967; Grayson and Reilly, 1968; Rothwell-Jackson, 1968; Somayaji et al., 1968; Jick et al., 1978) and tumorigenesis (Howard et al., 1977; Kent et al., 1977;
Keifer and Scott, 1977; Kinch and Lough, 1977; Rosenfield, 1978; Trias et al., 1978) were reported.

However, most of these reports are clinical observations, and there exists quite a controversy in regard to the causal relationship between oral contraceptives and hepatic lesions. The present study was aimed at investigating by light and electron microscopic observations the morphologic changes in the liver of guinea pigs during the administration of a combined type of oral contraceptive (Norinylle), to clarify some of clinical controversy.

MATERIALS AND METHODS

Forty guinea pigs, 10 untreated and 30 Norinyle treated, weighing 300 to 500 gms, were used for the experiment. For each treated animal, 1 tablet of Norinyle which contained 1mg of norethindrone (Progestogens; 17-α-ethynyl-17-hydroxy-4-estren) and 0.05 mg of mestranol (estrogens; ethyl-estradiol-3 methyl ether) was given daily in saline solution by mouth for 3 to 38 days. At 3, 6, 9, 12, 16, 20, 25, 32, and 38 day intervals, 1 from the untreated controls and 3 from the Norinyle treated animals were killed, and specimens of the liver were taken immediately.

For light microscopic examinations, a piece of the liver was fixed in 4% neutral formalin and then embedded in paraffin. About 5 μ thick sections were prepared from paraffin blocks, and stained with hematoxylin-eosin, PAS, D-PAS, and PTAH methods. For electron microscopic examinations, a small piece of the liver was chopped under glutaraldehyde solution into 1 cm mm sizes and fixed in 1% osmium tetroxide solution in phosphate buffer (pH 7.4) for 1 hour, then embedded in Epon following dehydration through graded alcohol.

Ultrathin sections were prepared with a glass knife and stained with uranyl acetate and lead citrate. Ultrastructural observations were made with a Hitachi H-500 model electron microscope.

RESULTS

Light microscopic findings:

Light microscopic changes of the hepatocytes were rather mild and only slight cloudy swelling, occasional fatty changes, and a few incidences of ischemic necrosis were found. Mild to moderate degree of fatty changes was noted in animals receiving Norinyle for 9 to 20 days, and it was most marked at the 12th and 20th days (Fig. 1). Ischemic necrosis was noted in only two animals, one focal at the 9th day, and another at the 20th day. The necrosis was of coagulative type but no definite thrombotic occlusion was noted at the adjacent vascular lumen although sinusoids and central veins were markedly dilated. Around necrotic areas, small numbers of polymononuclear cell infiltrations were noted, and the surrounding hepatocytes showed fatty changes(Fig. 2).

Kupffer cells showed various degree of hyperplasia and some Kupffer cells contained a large globular cytoplasmic hyaline body(Fig. 2) from the 3rd day and the number of Kupffer cells containing hyaline inclusions increased as the days passed. The most numerous number of Kupffer cells containing this inclusion was noted at the 38th day. These inclusion bodies reacted strongly positive to PAS, resistant to diastase, and strongly positive with PTAH, indicating that they are most likely fibrin in nature(Fig. 3, 4). No evidence of intracellular or canalicular cholestasis was noted.
Electron microscopic findings:

Electronmicroscopically, characteristic changes were observed in the nucleus of hepatocytes and cytoplasm of Kupffer cells. The nucleus of hepatocytes showed mild enlargement during the 9th to 20th day. The chromatin became aggregated around the nucleolus at the 9th day (Fig. 5), became increased and peripherally condensed along nuclear membranes later on (Fig. 6). The nucleolus was prominent and showed gradual segregation of amorphous and fibrilar parts (Fig. 6) starting from the 6th day. In the cytoplasm, SER was hyperplastic from the 6th day on while RER was rather decreased in amount, and mitochondria showed irregular configuration, loss of cristae, and decrease of size and numbers (Fig. 7, 9). Mild cytoplasmic vacuole formation and a small amount of lipid droplets as well as increase of secondary lysosomes were also noted (Fig. 8). Kupffer cells were hypertrophic and contained a large amount of fibrin from the 9th day on, and cytoplasmic organelles were hyperplastic (Fig. 9, 10). Red blood cells and other cell debris were also phagocytized (Fig. 9, 10). Some fibrin strands were also noted within sinusoids.

DISCUSSION

The most interesting findings were clear evidence of intravascular fibrin formation and its clearance by Kupffer cells, and nuclear enlargement with nucleolar segregation of hepatocytes. Hepatic cell necrosis was rather unusual and noted only in two instances, at the 9th and 20th day of drug administration. Kupffer cells were hyperplastic and frequently contained globular cytoplasmic inclusions which reacted strongly positive to PAS, resistant to diastase, and strongly positive to PTAH. These characteristics are consistent with fibrin, and it was proved to be fibrin definitely by electron microscopic observation. The numbers of fibrin containing Kupffer cells and the amount of fibrin phagocytized increased as the duration of Norinyle administration increased. It is thought that this finding is the evidence of intravascular fibrin formation during norinyle administration, and that Kupffer cells are continuously clearing fibrin from the blood stream by phagacytosis. Poller et al. (1979) studied effects of manufacturing oral contraceptives on blood clotting of the workers, and reported acceleration of platelet aggregation, acceleration or increase of prothrombin time, factor VII and X. Enhancement of coagulability of blood is attributed to the action of estrogenic agent, but a combination of progestins accelerate it further. Two instances of ischemic hepatic cell necrosis are comparable to the clinically observed Budd-Chiari syndrome during oral contraceptive administration reported by several authors (Ecker et al., 1966; Sterup and Mosebech, 1967; Grayson and Reilly, 1968; Rothwell-Jackson, 1968; Somayaji et al., 1968; Jick et al., 1978). In spite of continuous intravascular fibrin formation, a low incidence of occlusive thrombotic phenomena in this study can probably be attributed to rapid clearance of newly formed fibrin by Kupffer cells and possibly by the RES systems in other organs as well. Deykin (1966) reported rapid clearance of activated coagulation factors (IX and XI) by the liver in serum induced hypercoagulability. However, no report of fibrin clearance by Kupffer cells during oral contraceptive administration is noted in the literature. It is interesting to speculate that enlarged Kupffer cells may block sinusoidal blood flow,
and if intravascular fibrin formation exceeds the clearing capacity of the RES system, thrombosis may ensue to result in veno-occlusive phenomena which will produce hepatocellular necrosis and a Budd-Chiari like syndrome. Irey et al. (1970) reported structural changes of vascular walls following oral contraceptive administration, and this certainly will further facilitate vasoocclusive mechanism.

As regard to nuclear changes, it is interesting to relate nucleolar segregation with carcinogenesis. Wogan and Newberne (1967) and others (Bernhard et al., 1965; Tulpule et al., 1964) reported that the early ultrastructural change observed during chemical carcinogenesis, particularly after aflatoxin administration, is segregation of the nucleolus. Considering the recent reports of hyperplasia, adenoma, and carcinoma in the liver following oral contraceptive administration by several authors (Keifer and Scott, 1977; Kinch and Lough, 1978; Rosenfield, 1978; Trias et al., 1978), the nucleolar segregation in this experiment may provide some evidence on the tumorogenic action of oral contraceptives. The nuclear changes in this experiment suggest that oral contraceptives affect DNA metabolism in some way, as reported by Blackburn et al. (1976), and eventually may induce tumors.

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Fig. 1. Guinea pig liver at 12 days after Norinyle administration, showing a moderate degree of fatty changes and hyperplasia of Kupffer cells. H&E, ×400

Fig. 2. Guinea pig liver at 20 days after norinyle administration, showing focal ischemic necrosis associated with polys and mononuclear cell infiltration at periphery, fatty changes in surrounding hepatocytes, and two Kupffer cells containing globular bodies with cytoplasm at the center. H&E, ×400

Fig. 3 and 4. Guinea pig liver at 38 days after Norinyle administration, showing many Kupffer cells containing large globules which stain strongly positive to PAS (Fig. 3) and PTAH staining (Fig. 4). ×400
Fig. 5. Electronmicrophotograph of a hepatocyte nucleus at 9 days after Norinyle administration, showing two distinct nucleoli (ncl), and aggregation of chromatin around the nucleolus. ×22,000

Fig. 6. Electronmicrophotograph of a nucleus of hepatocyte at 20 days after the Norinyle administration, showing peripheral condensation of nuclear chromatin and segregation of fibrilar and amorphous parts of nucleolus (ncl). ×30,000
Fig. 7. Electronmicrophotograph of hepatocyte cytoplasm at 12 days after Norinyle administration, showing irregular configuration of mitochondria (m) with loss of cristae, and a few fibrin strands in Kupffer cells (f). ×16,000

Fig. 8. Electronmicrophotograph of hepatocyte cytoplasm at 12 days after Norinyle administration, showing hyperplasia of SER (ser), lipid droplet (li) and appearance of secondary lysosome (ly). ×22,500
Fig. 9. Electronmicrophotograph of a Kupffer cell at 16 days after Norinyle administration, showing a phagocytized red blood cell (rbc) and many fibrin strands (f). ×22,500

Fig. 10. Electronmicrophotograph of a Kupffer cell at 25 days after Norinyle administration, showing a large amount of more coarse fibrin strands (f) and phagocytized cell debris. ×16,000