EXPERIMENTAL-MORPHOLOGICAL STUDY OF THE EFFECT OF LIPOSOMES IN CCl_4 POISONING

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Considerable attention is now being paid to the use of liposomes as microcapsules for the incorporation of drugs and biologically active substances. The use of liposomes in hepatology is particularly interesting, because up to 80% of liposomes are assimilated by the liver cells [2]. We know that the toxic action of CCl_4 is due to destructive and functional changes in the liver. Through the formation of the free CCl_3^- radical, CCl_4 activates a chain reaction of lipid peroxidation, which gives rise to structural and functional changes in the intracellular membranes [5]. In addition the toxic action of CCl_4 is due to its inhibitory influence on activity of certain enzymes: cytochrome P-450, succinate dehydrogenase (SDH), glucose-6-phosphatase (G6Pase), etc.

The aim of this investigation was to study whether liposomes can be used as a toxinotropic antidote in CCl_4 poisoning.

EXPERIMENTAL METHOD

Preparations of crystalline cholesterol and dicetyl phosphate (from Serva, West Germany) and stearylamine (from Koch-Light, England) were used to obtain liposomes. The liposomes were prepared from ovolecithin, obtained by the method in [3]. The purity of the final product was verified by thin-layer chromatography on silica-gel in a system of chloroform–methanol–water (65:35:4). The concentration of phospholipids was determined by the method in [4]. Lipids (50 mg/kg) were dissolved in chloroform and dried in a rotary evaporator until a thin film was obtained. To this film was added 1.0 ml of a buffered solution containing 140 mM NaCl, 40 mM Tris-HCl, pH 7.4, and the sample was shaken until the lipid was completely emulsified. The suspension of liposomes was then treated with ultrasound in the tubular emitter of a UZND-1 disperser at 44 kHz and 4°C for 10 min.

Experiments were carried out on noninbred albino mice kept on the ordinary animal house diet. A model of experimental toxic liver damage was created by giving a single subcutaneous injection of a 50% oily solution of CCl_4 to the animal in a dose of 0.2 ml/100 g body weight [1]. Liposomes were slowly injected into the caudal vein in a concentration of 0.8 mmole lipid/kg body weight immediately after the injection of CCl_4.

The liver tissue was fixed in 10% neutral formalin and Carnoy's fluid, and paraffin and frozen sections were prepared. The paraffin sections were stained with hematoxylin and eosin, and by the PAS reaction with corresponding control to determine the glycogen content. Frozen sections were stained with Sudan Black to determine phospholipids. Enzyme activity was demonstrated in native tissue: SDH activity by Nachlas' method, adenosine triphosphatase (ATPase) by the method of Wachstein and Meisel, G6Pase by the same method, cytochrome oxidase (CCO) by Nachlas' method, and acid phosphatase (AP) by Gomor's method [7]. For electron-microscopic investigation the liver was fixed in 2.5% glutaraldehyde solution and postfixed in 1% osmic acid solution, processed by the usual method, and embedded in Epon-812. Ultrathin sections were cut on the UMPT-3 ultramicrotome and stained with uranyl acetate and lead citrate by the method in [8]. The sections were studied in the UMV-100L electron microscope. Material for investigation was taken 24 h after application of CCl_4.

EXPERIMENTAL RESULTS

Microscopic investigation showed necrosis of liver cells in the zones of injury (chiefly centrilobular; Fig. 1a), together with hyperchromatosis and pycnosis of the Kupffer cell nuclei.

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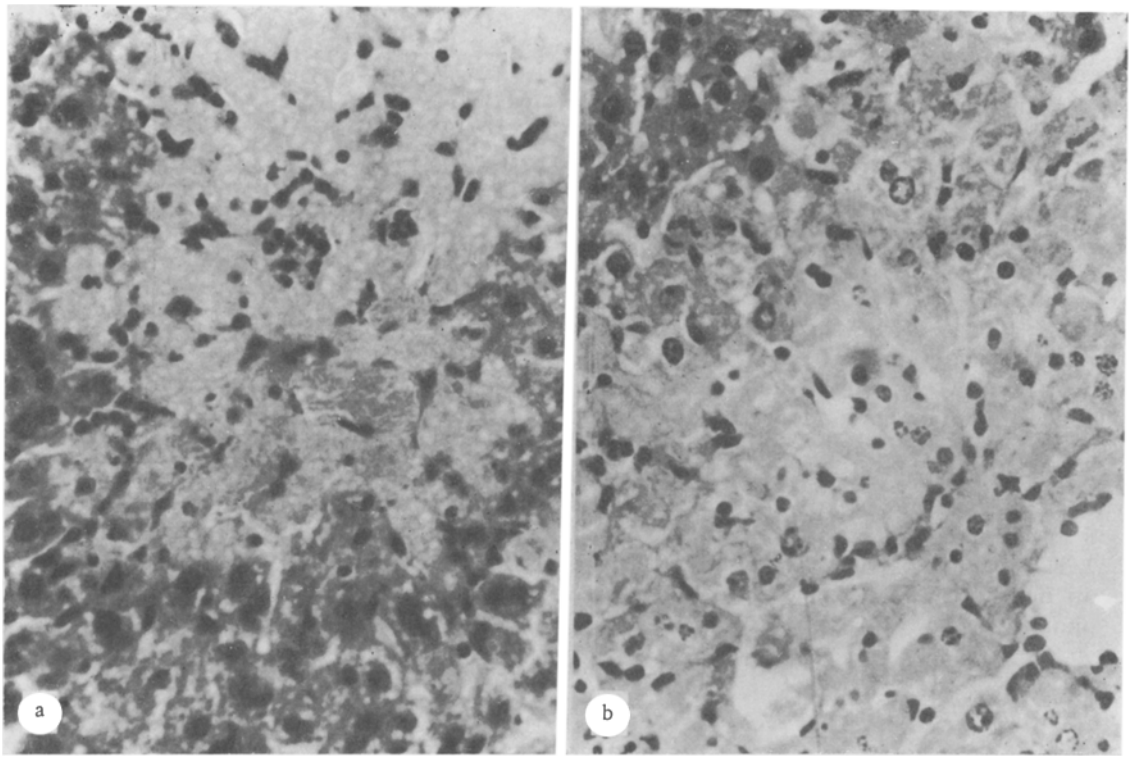

Fig. 1. Alternative changes in the mouse liver: a) centrilobular necrosis of hepatocytes after injection of CCl_4; b) dystrophic changes in hepatocytes after injection of CCl_4 and liposomes silultaneously. Hematoxylin and eosin. 460×.

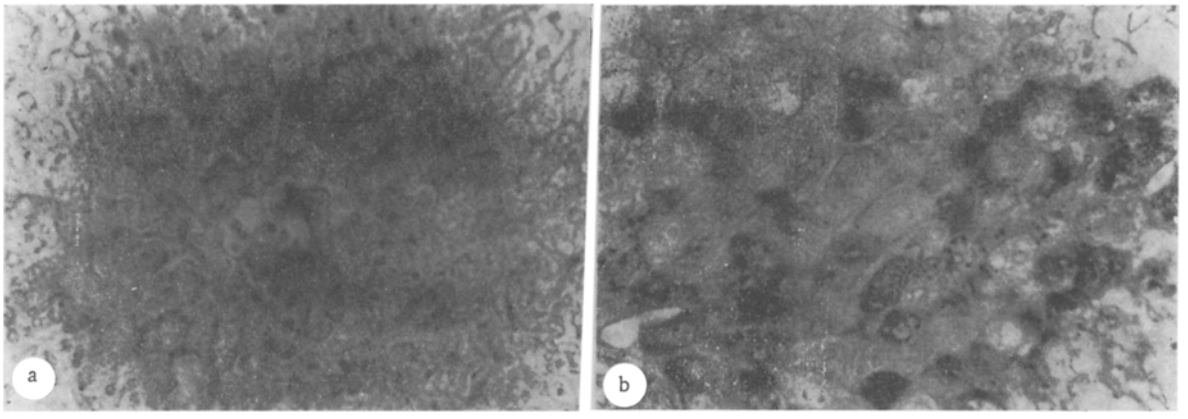

Fig. 2. Mouse liver: glycogen concentration: a) sharp decrease in glycogen concentration after injection of CCl_4; b) focal increase in glycogen concentration after injection of CCl_4 simultaneously with liposomes. PAS reaction. 460×.

Regenerative changes were more intensive in hepatocytes located in the immediate vicinity of the focus of injury, as shown by an increase in size of the nuclei and the presence of large chromatin granules in them. Moderate congestion of the central veins and also of vessels of the portal system also was observed. Increased vascular permeability could be seen, in the form of an enhanced perivascular lympho-leukocytic response. Electron-microscopic examination revealed considerable injury to subcellular structures, as shown by changes in the endoplasmic reticulum, which was reduced and fragmented, and also swelling of the mitochondria and disorganization of the cristae. Osmiophilic inclusions could be seen in the matrix of the mitochondria. In addition, the lipid content in the cytoplasm was increased. The changes discovered may be evidence of depression of protein synthesis and a disturbance of the bioenergetics of the hepatocytes. The structural changes described above agreed with the results of the histochemical investigations: a sharp decrease in the glycogen concentration (Fig. 2a)

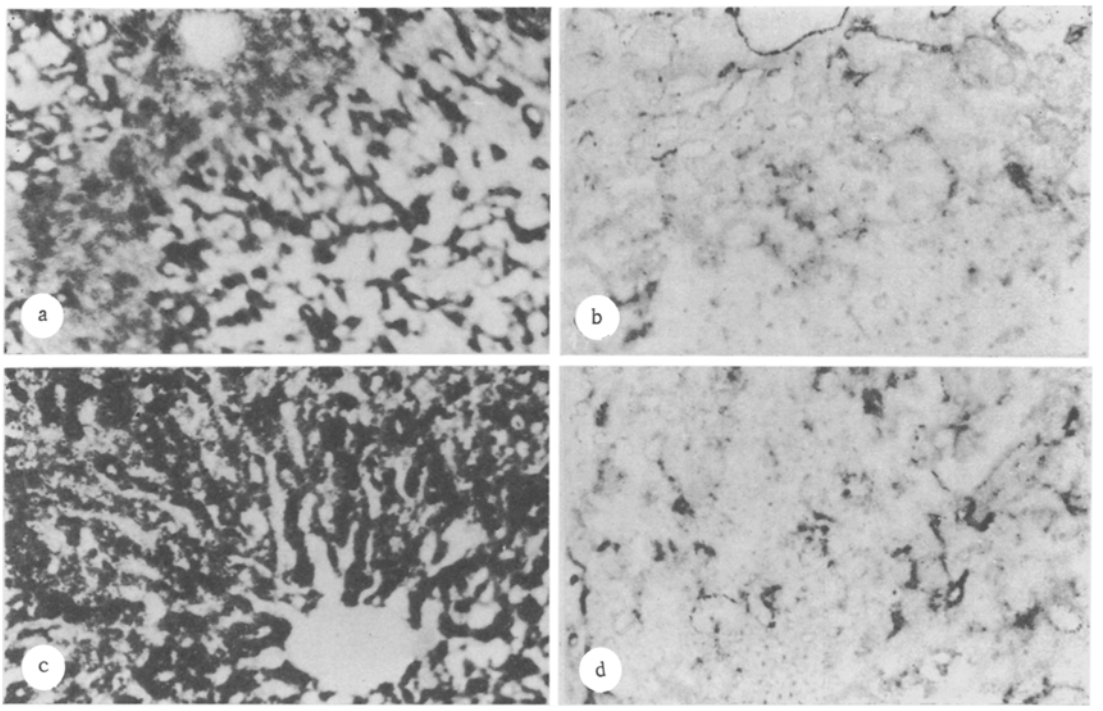

Fig. 3. Mouse liver: activity of enzymes of energy metabolism (180×): a) marked decrease in SDH activity after injection of CCl_4. Nachlas' reaction; b) sharp decrease in ATPase activity after injection of CCl_4. Wachstein and Meisel's reaction; c) preservation of considerable SDH activity after simultaneous injection of CCl_4 and liposomes. Nachlas' reaction; d) focal decrease in ATPase activity after simultaneous injection of CCl_4 and liposomes. Wachstein and Meisel's reaction.

and an increase in the lipid concentration could be seen in the center of the hepatic lobules and, to a rather lesser degree, around their periphery. Activity of enzymes of aerobic (G6Pase) metabolism was considerably depressed, and, to a lesser extent, so also was that of the key enzyme of the mitochondrial respiratory chain — CCO, evidence of severe liver damage. Together with the results of electron microscopy, these data revealed marked injury to structural components of the endoplasmic reticulum, for which G6Pase is the marker enzyme, and to a lesser degree, the disturbance of permeability of the lysosomal membranes (a decrease in AP activity was observed in the center of the lobules, and to a rather lesser degree, at the periphery).

Injection of liposomes in CCl_4 poisoning causes a marked decrease in the toxic action of the poison. All changes were less marked both quantitavely and qualitatively. By contrast with the previous group, in the pathological focus total necrosis of the liver cells was not observed. Changes in the hepatocytes were mainly of the type of cloudy-swelling degeneration, with varied degrees of damage to the nuclei (Fig. 1b). Some hepatocytes were in a state of degeneration, evidence that the changes discovered are reversible. Incidentally, injection of liposomes also helped to ensure better preservation of the Kupffer cells, evidently on account of the active uptake of liposomes from the blood stream by these cells.

Less marked disturbances of the intracellular membranes were observed electron-microscopically: only partial degranulation of membranes of the endoplasmic reticulum, together with vesicular fragmentation of the rough endoplasmic reticulum. The matrix of the mitrochondria was moderately dense, with single cristae.

The enzyme-histochemical tests revealed a smaller decrease in activity of the enzyme studied. especially SDH (Fig. 3c) and ATPase (Fig. 3d). Parallel with this, the normal glycogen concentration was restored in the cytoplasm of the hepatocytes (Fig. 2b) but the lipid concentration was reduced.

These changes are evidence that injection of liposomes weakens to some degree the lytic action of the poison and contributes to restoration of bioenergetic processes and also normalization of liver function.

The results suggest that the protective effect of liposomes, by virtue of their large surface area (2×10^3 cm^2/ μmole of lipid [6]) is due to binding both of CCl_4 and CCl_3- and of products of CCl_4 metabolism and peroxidation. Lipids contained in liposomes, which can be incorporated into damaged zones of lipid structure of cytoplasmic and intracellular membranes, may perhaps also play a replacement function.

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