LITERATURE CITED

1. E. B. Burlakova, A. V. Alesenko, E. M. Molochkina, et al., Bioantioxidants in Radiation Damage and Malignant Growth [in Russian], Moscow (1975).
2. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biomembranes [in Russian], Moscow (1972).
3. Yu. P. Kozlov, V. S. Danilov, V. E. Kagan, and M. V. Sitkovskii, Free-Radical Lipid Oxidation in Biological Membranes [in Russian], Moscow (1972).
4. Yu. P. Kozlov, V. E. Kagan, and Yu. V. Arkhipenko, Mechanisms of Modification of Membranes of the Mouse Sarcoplasmic Reticulum by Molecular Oxygen [in Russian], Irkutsk (1983).
5. B. Chance and M. Nishimura, Methods Enzymol., 10, 65 (1967).
6. A. G. Golnall, C. I. Bardovill, and M. M. David, J. Biol. Chem., 177, 751 (1949).
7. D. Johnson and K. Lardy, Methods Enzymol., 10, 94 (1967).

PHOSPHOLIPID REPAIR OF LIVER MEMBRANES IN RATS POISONED WITH CARBON TETRACHLORIDE

O. V. Dobrynina, V. L. Migushina, S. Z. Shatinina, and N. B. Boldanova

UDC 616.36-091.8-099-0039.-02:615.451.234:547.953

KEY WORDS: carbon tetrachloride; cytochrome P-450; liposomes; phospholipids; liver microsomes.

Liposomes are being used increasingly more frequently in experimental and clinical medicine as microcapsules for administration of drugs, x-ray contrast compounds, enzyme preparations, and other biologically active substances.

This paper describes an attempt to use liposomes for the administration of phospholipid material in vivo for the repair of membranes of the endoplasmic reticulum (ER) of the liver, damaged by carbon tetrachloride (CCl_4). The criterion of repair was restoration of the phospholipid composition and enzyme activity of ER membranes.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 100-150 g. CCl_4 was injected by the intragastric route in a dose of 2.5 ml/kg in the form of a 50% solution in mineral oil.

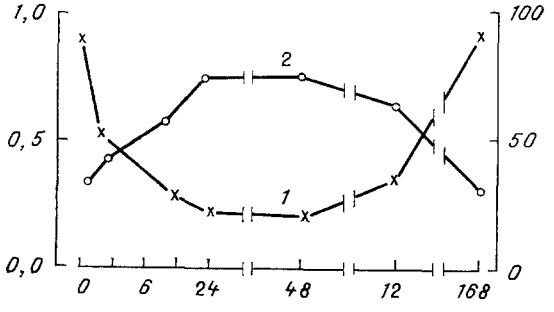

Fig. 1. Changes in cytochrome P-450 content and degree of its inactivation by the action of CCl_4. Abscissa, time after administration of CCl_4 (in h); ordinate: on left — cytochrome P-450 concentration (in mmoles/mg protein), on right — coefficient of inactivation (in %). 1) Cytochrome P-450 concentration; 2) coefficient of inactivation.

Department of Biochemistry, Medico-Biological Faculty, M. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 301-303, September, 1987. Original article submitted October 2, 1986.

 0007-4888/87/0009-1226$12.50

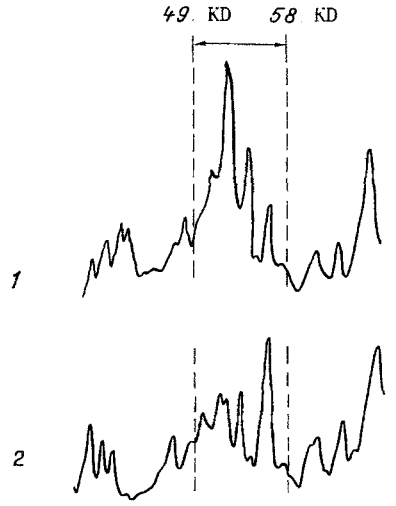

Fig. 2. Electrophoretic profile of protein composition of microsomes under normal conditions (1) and in CCl_4 poisoning (2).

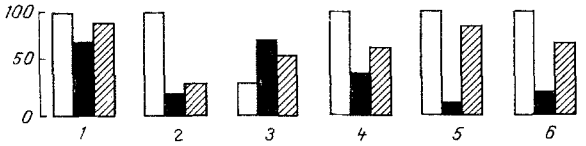

Fig. 3. Repair of damage to ER membranes with PL. 1) PL content; 2) cytochrome P-450 content; 3) degree of inactivation of cytochrome P-450; 4) G6P activity; 5) rate of demethylation of dimethylaniline; 6) rate of hydroxylation of aniline. Unshaded columns — control microsomes, black columns — microsomes damaged by CCl_4, obliquely shaded — microsomes after repair with phosphatidylcholine liposomes. 1, 2, 4, 5, 6) Percent of control level; 3) coefficient of inactivation.

Phospholipid therapy consisted of intraperitoneal injection of phospholipids (PL) in a dose of 100 mg/kg 2 h after injection of the poison. The microsomal fraction was isolated as described previously [4]. The concentration of cytochrome P-450 was measured spectrophotometrically [12]. The rate of inactivation of microsomal cytochrome P-450 was recorded for 30 min at 37°C in 0.1M Tris-HCl buffer, pH 7.4 [3]. The rate of N-demethylation of dimethylaniline and of para-hydroxylation of aniline was determined from the quantity of formaldehyde and para-aminophenol formed respectively [1]. Microsomal protein fractions were analyzed by electrophoresis in 10% polyacrylamide gel [10]. Glucose-6-phosphatase (G6P) activity in the microsomes was determined by the method in [2]. To prepare liposomes, egg phosphatidylcholine (PCh), obtained by the method in [9], was used. Multilayered liposomes were formed by the method in [6], and mono- and bilayered microsomes as in [7]. Positively charged PCh liposomes were formed by addition of cetyltriammonium bromide (CTAB) and negatively charged liposomes by addition of phosphoric acid (PA) in a concentration of 10 moles %. The PL concentration was determined as phosphorus [8] and the protein concentration by Lowry's method [11].

EXPERIMENTAL RESULTS

Damage to ER membranes was assessed as the rate of reconversion of the active form of microsomal cytochrome P-450 into its inactive form — cytochrome P-420 (coefficient of inactivation), by determining the change in G6P activity and hydroxylase activity of the microsomes and their protein and phospholipid composition. It was shown that in CCl_4 poisoning the concentration of cytochrome P-450 in ER membranes fell sharply (by 80%) and the rate of its inactivation increased. These two processes follow a parallel course to reach a maximum after 24 h, and are completely back to normal by the 7th day of the experiment (Fig. 1).

TABLE 1. Effect of Phospholipid Preparations on ER Membranes in CCl_4 Poisoning (M ± m)

Experimental conditions	Cytochrome P-450 concentration, nmoles/mg protein	Coefficient of inactivation of cytochrome P-450, %	G6P activity, %
Control	0,89±0,11	30,4±7,2	100
CCl_4	0,19±0,3	71,7±7,7	38±3,5
CCl_4+$PCh_{multilayered}$	0,26±0,09	57,4±7,7*	66,3±2,8*
CCl_4+PCh_{DOC}	0,26±0,06*	58,0±6,9*	45,0±3,0*
CCl_4+$PCh_{mono-and bilayered}$	0,13±0,04	89,0±11,2	—
CCl_4+PCh_{soy}	0,35±0,11*	59,0±6,2*	36,4±2,8
CCl_4+Azolectin	0,17±0,03	68,3±5,8	—
CCl_4+PCh_{PA}	0,32±0,04*	56,5±6,0*	44,0±2,5
CCl_4+PCh_{CTAB}	0,29±0,05*	54,5±5,2*	50,0±3,0
CCl_4+$PCh_{microsomal}$	0,28±0,07*	56,0±6,7*	—
CCl_4+PCh_{PI}	0,23±0,04	75,0±8,4	—
CCl_4+PCh_{SM}	0,21±0,05	70,0±5,7	—
CCl_4+PCh_{α}- tocopherol	0,17±0,04	65,0±12,6	—
CCl_4+$PCh_{carnosine}$	0,38±0,04	75,0±1,0	—
CCl_4+$PCh_{glutathione}$	0,17±0,02	57,0±6,0*	—
Lipostabil	0,16±0,4	64,6±7,4	31,8±2,6

Legend. Coefficient of inactivation of cytochrome P-450 was calculated by the formula $100-\frac{\Delta A^{30}_{450-420}}{\Delta A^{0}_{450-420}}\cdot 100$, where $\Delta A^{0}_{450-420}$ and $\Delta A^{30}_{450-420}$ denote the difference in absorption of the reduced cytochrome P-450 complex at 450 and 420 nm, respectively, at initial time and after incubation for 30 min. Samples were incubated in 100 mM Tris-HCl buffer; the protein concentration was 1-3 mg/ml. *$p < 0.05$ compared with CCl_4 samples. Mean values of 4-12 experiments shown, tissue from three animals being used in each of them.

At the peak of membrane damage, i.e., 24 h after administration of CCl_4, a reduction in their PL concentration by 30% was observed (from 0.275 ± 0.031 to 0.190 ± 0.024 mg PL/mg protein), which was accompanied by a change in the ratio between the protein component of the membranes, with a characteristic reduction of the contribution of fractions specific for cytochrome P-450, in the 49-58 kilodalton (kD) region (Fig. 2). Activity of microsomal G6P also was reduced by two-thirds compared with its level in native membranes. Thus the toxic action of CCl_4 after 24 h is characterized by marked changes in the protein–lipid composition and activity of marker enzyme systems of ER membranes.

To repair the injuries arising in the ER membranes we used PCh (egg) in the form of multilayered liposomes (Fig. 3). On their introduction into the poisoned animals the PL concentration in ER membranes was restored to normal, thereby reducing the loss of cytochrome P-450 by the membrane, although its absolute level remained low and normalization of the protein spectrum, according to the results of electrophoresis, was not observed. The rate of inactivation of the hemoprotein after injection of multilayered PCh liposomes also was partly restored to normal (by 20%). Meanwhile the functional parameters of the repaired membrane also recovered: hydroxylase activity of cytochrome P-450 were observed (the rate of oxidation of aniline and dimethylaniline increased to 70 and 80% of the control level respectively).

PCh, when injected in the form of micelles with 10% sodium deoxycholate (DOC), had an action similar to that of liposomes. PCh, injected in the form of mono- or bilayered liposomes, had no reparative effect (Table 1).

Testing lipostabil, which is used in practice for the treatment of diseases of the liver, on this model revealed no reparative action of this preparation with respect to any of the parameters studied (Table 1).

To make the reparative action of the PCh liposomes more effective in vivo, variation of the lipid composition of the liposomes was used. It was found that liposomes prepared from

soy PCh (with a high degree of unsaturation) had the same reparative action on the cytochrome P-450 system as liposomes from egg PCh, but they did not restore G6P activity (Table 1). The effectiveness of liposomes from egg PCh and a mixture of microsomal PL was the same, evidence that homologous PL have no tissue-specific effect. The addition of phosphatidylinositol (PI) or sphingomyelin (SM) to egg PCh made its action less effective (Table 1). Introduction of positively charged CTAB or negatively charged PA did not affect the reparative action of the original PCh liposomes.

To inhibit lipid peroxidation processes, which usually take place in CCl_4 poisoning, substances possessing antioxidative properties (α-tocopherol, carnosine) and a membrane stabilizer (SH-glutathione) were incorporated into the liposomes. On the addition of carnosine, the effectiveness of the reparative action of the preparation exceeded that of pure PCh, if assessed relative to the change in cytochrome P-450 concentration in the preparation of microsomes. Meanwhile, unlike PCh in the liposomes, this preparation had no protective action against the rate of inactivation of the hemoprotein. Introduction of α-tocopherol or of reduced glutathione into the liposomes did not facilitate repair of ER membranes.

The results of this investigation thus demonstrate that damaged ER membranes can be repaired in vivo with the aid of PCh liposomes. Multilayered liposomes of pure PCh had the most marked reparative properties. An increase in the degree of unsaturation of the fatty acid residues of PCh, and incorporation of antioxidants, SH-compounds, and other PL into the liposomes did not increase the reparative action of the original preparation.

LITERATURE CITED

1. A. I. Archakov, I. I. Karuzina, V. N. Tveritinov, and I. S. Kokoreva, Biokhimiya, 40, No. 1, 32 (1975).
2. V. S. Asatiani, Enzymatic Methods of Analysis [in Russian], Moscow (1969).
3. G. I. Bachmanova, O. K. Pogodina, I. I. Karuzina, and D. Mengazetdinov, Biokhimiya, 46, No. 2, 280 (1981).
4. I. I. Karuzina and A. I. Archakov, Biokhimiya, 44, 1149 (1979).
5. M. Kates, Techniques in Lipidology [Russian translation], Moscow (1975), pp. 72-77.
6. N. D. Bangham, I. de Gior, and G. D. Grellille, Chem. Phys. Lipids, 1, 225 (1967).
7. I. Barenholts, D. Cibbes, B. Litman, et al., Biochemistry, 16, 2806 (1977).
8. G. R. Bartlett, J. Biol. Chem., 234, 225 (1959).
9. R. M. C. Dawson, Biochem. J., 88, 414 (1963).
10. U. K. Laemmli, Nature, 227, 680 (1970).
11. O. H. Lowry, N. J. Rosebrough, A. G. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. T. Omura and R. Sato, J. Biol. Chem., 239, 2370 (1964).