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EFFECT OF THE LIPOSOMAL FORM OF DIATRIZOATE ON COMPOSITION OF BLOOD AND ORGAN LIPIDS IN EXPERIMENTAL ANIMALS

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In recent years there has been a steady increase in the use of liposomes as carriers of drugs for their targeted transport to organs of the reticuloendothelial system, both in the USSR and elsewhere [2, 6]. The use of liposomes in x-ray diagnosis has proved to be particularly promising. It has been shown that with the aid of the liposomal form of diatrizoate (Triombrast) the x-ray contrast of the spleen, liver, blood vessels, kidneys, urine, large intestine, and boundaries of tumors can be increased [1, 7, 8].

However, before liposomes can be used as a transport system for clinical purposes, their safety has to be guaranteed. In this connection it is important to know how the liposomal form of diatrizoate will affect the lipid composition of the organs and tissues, for its introduction into the blood stream in a dose of 2.5 ml of liposomal suspension/kg body weight for man will be accompanied by considerable lipid loading (for example, lecithin 75 mg/kg and cholesterol 35 mg/kg). The aim of the present investigation was accordingly to study the effect of the liposomal form of diatrizoate on the lipid composition of the blood and organs in laboratory animals, as judged by the hydrolysis and utilization of the administered lipids.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 120-150 g, kept on the standard animal house diet. Intact animals and those receiving the test preparation were deprived of food for 12-14 h before sacrifice.

The liposomal form of diatrizoate was obtained by the method described in [1].

The rats were given an intravenous injection of the liposomal form of diatrizoate (Triombrast, 76% solution, produced by the M. V. Lomonosov Kiev Pharmaceutical Chemical Factory) in the ordinary clinical dose of 1 ml/kg body weight (the doses are comparable for content of diatrizoate).

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TABLE 1. Effect of Liposomal Form of Diatrizoate (5 ml/kg) on Lipid Composition (mg%) of Blood and Organs of Male Albino Rats (M ± m)

Organ	Lipid	Control	2 h	12h	24h
Liver	PL	4,81±0,86	9,10±0,86*	10,86±2,18*	6,40±1,06
	ChS	3,13±0,25	7,44±0,87*	6,38±0,91*	4,51±0,54
	FA	2,67±0,39	6,66±0,65*	2,01±0,29	1,69±0,12
	TG	9,99±1,79	37,20±3,10*	10,98±3,20	9,86±0,54
	ECh	10,18±1,87	13,02±2,50	14,50±1,78	12,36±1,31
	PCh	1,58±0,13	3,46±0,22*	3,92±0,29*	2,59±0,17
Spleen	PL	6,51±0,54	11,39±0,98*	8,70±1,08	7,01±0,49
	ChS	6,99±0,59	20,25±2,80*	21,63±2,22*	10,55±1,08
	FA	1,95±0,36	9,67±1,24*	1,93±0,54	1,86±0,15
	TG	8,59±1,66	21,07±3,16*	7,55±1,89	7,19±0,96
	ECh	23,64±3,06	36,80±4,80	26,65±2,67	26,49±2,25
	PCh	2,52±0,08	4,21±0,09*	3,13±0,11*	2,77±0,21
Kidneys	PL	8,94±0,78	10,35±0,68	7,01±0,49	8,93±0,76
	ChS	8,92±1,53	12,23±1,39	10,01±0,65	11,07±1,65
	FA	2,16±0,86	3,72±0,44	1,66±0,09	2,01±0,47
	TG	11,82±2,00	13,50±1,70	4,76±1,09	9,02±2,01
	ECh	18,31±3,72	17,30±1,56	16,84±0,16	18,50±2,20
	PCh	2,58±0,18	2,95±0,14	2,78±0,18	2,62±0,44
Plasma	PL	28,90±2,59	73,46±5,26*	37,28±2,75*	33,73±3,12
	ChS	45,20±4,78	122,51±4,95*	64,72±4,18*	42,80±4,83
	FA	10,90±1,28	55,03±5,36*	12,70±0,95	12,24±1,35
	TG	101,33±12,33	323,00±16,20*	189,60±14,32*	74,00±14,89
	ECh	160,25±7,80	360,00±13,70*	195,20±10,48	154,50±5,30
	PCh	23,50±1,43	55,75±4,01	28,30±1,75	23,30±1,04
	PE	3,00±0,34	3,16±0,42	3,90±0,54	2,79±0,11
Erythrocytes	PL	14,26±1,63	44,86±4,37*	14,80±2,05	13,50±1,80
	ChS	20,10±2,57	79,96±2,57*	18,07±1,33	22,00±3,40
	PCh	8,52±1,08	21,45±1,44*	9,32±1,09	5,36±0,44
	PE	8,53±0,33	20,16±1,26*	5,32±0,53	5,35±0,58

Legend. PL) total phospholipid fraction, ChS) free cholesterol, FA) total fraction of fatty acids, TG) triglycerides, ECh) esterified cholesterol, PCh) phosphatidylcholine, PE) phosphatidylethanolamine. * indicates significant increase in level of lipids compared with control (P = 0.05).

The rats were decapitated under superficial ether anesthesia. Samples of 3 ml blood were taken from the heart into a syringe containing 0.3 ml of 3.8% sodium citrate solution. The blood thus obtained was centrifuged for 15 min at 3000 g, after which 0.2 ml of plasma and 0.2 ml of erythrocytes were sampled.

After laparotomy and thoracotomy the spleen, liver, and kidneys were removed (the liver and kidneys were washed free from blood with 20 ml of physiological saline by catheterization of the portal and renal veins respectively). A weighed sample of tissue of these organs was then homogenized in a Potter–Elvehjem homogenizer with Teflon pestle (ratio of tissue to physiological saline 1:40). Lipids were extracted from the resulting tissue homogenates by the method in [5]. Lipids extracted from the blood and organs were fractionated by thin-layer chromatography on Silufol plates (Czechoslovakia), using hexane: diethylether:glacial acetic acid (80:20:2) as the system for separating total lipids, and chloroform:methanol: water (65:25:4) for separating phospholipids.

The individual classes of lipids were detected by means of phosphomolybdic acid. Lipids were identified on the basis of the RF values for each class [2] and analysis of standards, which consisted of phosphatidylcholine, free cholesterol, cholesterol stearate, palmitin, and stearic and palmitic acids (from "Serva," Germany). The individual classes of lipids were determined quantitatively by analysis of chromatograms on an ECP-65 densitometer (Carl Zeiss, East Germany) at a wavelength of λ = 600 nm. Total lipids were determined by the sulfophosphovanillin method [9].

The results were subjected to statistical analysis with calculation of the mean value and error of the mean for samples of 9-11 experiments. The significance of differences observed between sampling means was determined by Student's test.

EXPERIMENTAL RESULTS

The results of determination of the lipid composition of the plasma, erythrocytes, liver, spleen, and kidneys in the control and 2, 12, and 24 h after injection of the liposomal form of diatrizoate are given in Table 1. Clearly 2 h after injection of the liposomal form of diatrizoate, the lipid composition of the organs, plasma, and cells showed changes, except

in the kidneys: the content of phosphatidylcholine, total phospholipids, and cholesterol was increased about 2-3 times (the greatest increase was shown by the erythrocytes), but the content of triglycerides and fatty acids was increased by 3-5 times. Meanwhile, non-liposomal diatrizoate, when injected in the equivalent dose, did not affect the lipid content in the organs, with the exception of an increase in the plasma triglyceride level by 1.9 times.

Consequently, it can be concluded that changes in the lipid composition of the blood, liver, and spleen observed 2 h after injection of the liposomal form of diatrizoate are associated mainly with the effect of the lipid vesicles themselves and not of the iodine-containing contrast material. Differences found in the effect of the liposomal form of diatrizoate in relation to different organs correlate well with data [4] according to which, 2 h after injection of liposomes containing ^{99m}Tc-labeled lipids into mice, 52.2 ± 7.9, 22.7 ± 4.5, 19.7 ± 3.2, and 5.2 ± 1.2% of ^{99m}Tc accumulate in the liver, spleen, blood, and kidneys, as percentages of the injected dose (40 mg/ml) respectively.

A tendency toward normalization of the lipid composition of the blood and organs was observed 12 h after injection of the liposomal form of diatrizoate, and by the 24th hour it no longer differed from the control (Table 1).

The results indicating a marked increase in the lipid content in the erythrocytes deserve special examination. The most likely explanation of this fact is nonspecific adsorption of liposomes on the erythrocytes. This explanation is supported both by the well known facts concerning the high nonspecific adsorption of liposomes on cells in vivo and in vitro [3] and the shorter time for normalization of the lipid comosition of the erythrocytes compared with the time needed for normalization of the lipid composition of the liver and spleen, where the intracellular mechanism of uptake and digestion of liposomes has been demonstrated [6]. A further argument in support of the adsorption mechanism of the increase in the lipid content in the erythrocyte fraction is coincidence of the time for normalization of the composition (12 h after injection) and the total elimination time of liposomes from the blood stream, estimated on the basis of excretion of ^{125}G-diatrizoate in these vesicles.

The investigation thus showed that although the liposomal form of diatriazoate causes elevation of lipid blood levels in the blood and in organs to which it has selected affinity, i.e., the liver and spleen, these changes are transient, they differ only a little from ordinary fluctuations of lipid composition connected with the taking of food, and they cannot be regarded as an obstacle in the way of the use of liposomal forms of x-ray contrast materials in clinical practice.

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