Biochimica et Biophysica Acta 917 (1987) 344–346
Elsevier

BBA 50172

BBA Report

The effects of Triton WR-1339, protamine sulfate and heparin on the plasma removal of emulsion models of chylomicrons and remnants in rats

Mário H. Hirata [a], Helena C.F. Oliveira [a], Eder C.R. Quintão [a], Trevor G. Redgrave [b] and Raul C. Maranhão [a]

[a] *The Heart Institute (INCOR) and Lipid Laboratory of the São Paulo University Medical School Hospital and Faculty of Pharmaceutical Sciences, São Paulo University, São Paulo (Brazil) and* [b] *University of Western Australia, Nedlands (Australia)*

(Received 6 June 1986)
(Revised manuscript received 30 September 1986)

Key words: Chylomicron; Lipoprotein; Heparin; Protamine sulfate; Lipid emulsion; (Rat lymph, Plasma)

Protein-free lipid emulsions with compositions modelling chylomicrons (chylomicron-like emulsion) or chylomicron remnants (remnant-like emulsion) were injected intra-arterially into nonanesthetized rats. Compared with control untreated rats, treatment with Triton WR-1339, protamine sulfate or heparin strongly modified the plasma removal of triacylglycerols and cholesteryl ester moieties of chylomicron-like emulsions, but had little effect on removal rates of triacylglycerols or cholesteryl esters of remnant-like emulsions. The effects on chylomicron-like removal were similar to those on natural lymph chylomicrons. The relative lack of effects on remnant-like emulsion removal provides additional evidence that remnant-like emulsions are a metabolic model for natural chylomicron remnants.

In recent experiments [1,2], we showed that protein-free emulsions with lipid composition similar to lymph chylomicrons behaved metabolically like chylomicrons when injected into the bloodstream of rats: triacylglycerols were removed faster than cholesteryl esters from plasma and the particles trapped by the liver had the bulk of their cholesteryl ester but residual triacylglycerols only. In contrast, and as expected in chylomicron remnant metabolism, emulsions with a high content of unesterified cholesterol had identical plasma removal rates and liver uptakes of triacylglycerols and cholesteryl esters.

When in contact with plasma, emulsions promptly incorporate apolipoproteins, which thereafter modulate their metabolism. We found that the cholesterol-rich emulsion bound less apolipoproteins A-I, A-IV and C, and relatively more apolipoprotein-E than the low-cholesterol one [2]. This could have contributed to the differences in metabolic pathways of the two emulsion types.

Chylomicron transformation into remnants through lipoprotein lipase action is the key requirement for the particles to be efficiently trapped by the liver [3]: lipolysis impairment leads to the accumulation of chylomicron in the blood. On the other hand, remnants are ready for uptake by the liver without further requirement for lipoprotein lipase action.

To check further the usefulness of the two emulsions as probes for chylomicron metabolism studies, they were injected in rats whose lipoprotein lipase activity was sharply modified.

Glyceryl trioleate, cholesteryl oleate and cholesterol were from Nu Chek Prep. (Elysian,

Correspondence: R.C. Maranhão, São Paulo University Medical School, Lipid Laboratory, Av. Dr. Arnaldo 455, 3A, São Paulo 01246, Brazil.

MN). Egg phosphatidylcholine was from Lipid Products (Surrey, U.K.). All were confirmed to be 99% pure by TLC. Mixtures with 100 mg of lipids were prepared to obtain the two emulsion types as previously described [1,2]. The chylomicron-like emulsion mixtures had 2% cholesterol, 23% phosphatidylcholine, 6% cholesteryl oleate and 69% triolein (% weight). The remnant-like emulsion mixture had 24.5% cholesterol, 24.5% phosphatidylcholine, 10.3% cholesteryl oleate and 40.7% triolein. [^{14}C]Cholesteryl oleate and [^{3}H]glyceryl trioleate (Amersham International, U.K.) were added and the solvents were evaporated under an N_2 stream followed by overnight vacuum desiccation. They were sonicated in 8 ml of a 2.785 M NaCl solution, density 1.101 g/ml, with a Banson Cell Disruptor at 55°C, using a 1 cm probe with continuous output of 70–80 W. The crude emulsions were then centrifuged in discontinuous density gradients of NaCl solutions [4] in an SW 41 rotor (Beckman). Chylomicron-like emulsion was run at 12000 rpm for 15 min, and remnant-like emulsion at 10000 rpm for 20 min. The lipid layer that floated on top of the 1.006 g/ml density solution after this first centrifugation was aspirated and discarded and replaced with the corresponding volume of the 1.006 g/ml solution. Then they were centrifuged for the second time: chylomicron-like emulsion at 36000 rpm for 25 min, and remnant-like emulsion at 38000 rpm for 30 min. The emulsion particles that floated at the top of the gradients were recovered by aspiration and used in the experiments.

As assayed by standard laboratory procedures [5–7], chylomicron-like emulsion had $1.9 \pm 0.3\%$ cholesterol, $10.4 \pm 1.3\%$ phosphatidylcholine, $11.2 \pm 3.0\%$ cholesteryl oleate and $76.5 \pm 4\%$ triolein (% weight). Remnant-like emulsion had $21.3 \pm 2.7\%$ cholesterol, $18.7 \pm 2.4\%$ phosphatidylcholine, $18.7 \pm 5.4\%$ cholesteryl oleate and $41.4 \pm 3.4\%$ triolein. Both emulsions lacked proteins, and the chylomicron-like emulsion had a low content of cholesterol, whereas the remnant-like emulsion was saturated with cholesterol. As previously described [2], cholesterol saturation appears to be the determinant of remnant-like metabolic behaviour.

The emulsions were injected into the carotid arteries of nonanesthetized male Wistar rats weighing 250–350 g as described [1]. Rats were assigned to control and to Triton-treated (600 mg/kg of body weight), protamine-sulfate treated (2.0 mg by animal) and heparin-treated (250 IU/kg of body weight) groups. After the injection, blood samples were drawn into heparinized 0.5-ml plastic tubes at 2-min intervals during 10 min. Aliquots of 100 μl of the blood plasma were extracted with chloroform/methanol, 2:1 (v/v) and neutral lipid classes were separated by TLC in the solvent system hexane/diethyl ether/acetic acid (70:30:1, v/v). Bands corresponding to triacylglycerols and cholesteryl esters were scraped separately into vials for radioactivity measurement in the scintillation spectrometer (Beckman LS 100). Plasma clearance kinetics (FCR) were computed from the mono-exponential curves fitted by the least-squares procedure.

Results are shown in Table I. The fractional clearance rate for triacylglycerol removal from injected chylomicron-like emulsion was decreased by approximately 50% after treatment with protamine, and by approximately 85% after treatment with Triton WR-1339. In control rats the removal of cholesteryl ester from the same emulsion was slower than that of triacylglycerols, confirming our previous findings [1,2], and was further slowed by protamine or Triton treatment. Harwood et al. [8] reported similar findings for natural chylomicrons after the same dose of protamine. In contrast, heparin treatment considerably accelerated the removal of triacylglycerols and cholesteryl esters from chylomicron-like emulsion in the case of triacylglycerols to rates that were too rapid for accurate measurement.

The clearance rates for remnant-like emulsion were much less affected by Triton or heparin treatment. Small but significant changes in triacylglycerol removal were found, but cholesteryl ester removal was unchanged. The similarities for the removal rates of cholesteryl esters from remnant-like emulsion and chylomicron-like emulsion after heparin treatment were noteworthy, suggesting that remnant removal after heparin was at its maximum rate.

The calculated lipolysis index [1] in Table I gives an estimate of the removal by lipolytic mechanisms. As shown, it reflects the expected changes in lipoprotein lipase activity produced by the treatments.

TABLE I

EFFECTS OF TRITON WR-1339, PROTAMINE SULFATE AND HEPARIN ON PLASMA REMOVAL OF CHOLESTERYL [1-^{14}C]OLEATE (CE) AND GLYCEROL TRI[9,10(n)-^{3}H]OLEATE (TG) MOIETIES OF CHYLOMICRON-LIKE EMULSION AND REMNANT-LIKE EMULSION

Protamine sulfate, 200 mg/animal; Triton WR-1339, 600 mg/kg body weight; heparin, 250 IU/kg body weight.

Emulsion	Treatment groups	Removal from plasma (fractional clearance rate, FCR) (min^{-1})		Lipolysis index ($FCR_{TG} - FCR_{CE}$)
		[^{3}H]TG	[^{14}C]CE	
Chylomicron-like	control	0.19 ± 0.02 ($n = 9$)	0.11 ± 0.010 ($n = 8$)	0.09 ± 0.01 ($n = 8$)
	protamine sulfate	0.10 ± 0.01 [b] ($n = 5$)	0.05 ± 0.01 [b] ($n = 5$)	0.05 ± 0.001 [c] ($n = 5$)
	Triton WR-1339	0.03 ± 0.000 [b] ($n = 8$)	0.03 ± 0.00 [b] ($n = 8$)	zero [b] ($n = 8$)
	heparin	> 2 ($n = 10$)	0.25 ± 0.02 [b] ($n = 10$)	> 1 ($n = 10$)
Remnant-like	control	0.26 ± 0.03 ($n = 23$)	0.23 ± 0.03 ($n = 12$)	0.01 ± 0.01 ($n = 12$)
	Triton WR-1339	0.23 ± 0.02 [a] ($n = 13$)	0.22 ± 0.02 [a] ($n = 7$)	0.08 ± 0.02 [c] ($n = 7$)
	heparin	0.32 ± 0.04 [c] ($n = 7$)	0.24 ± 0.02 [a] ($n = 7$)	0.08 ± 0.02 [c] ($n = 7$)

[a] Not significant.
[b] $P < 0.001$.
[c] $P < 0.005$.

Our findings are consistent with the view that injected chylomicron-like emulsions were removed from plasma by mechanisms similar to those affecting natural lymph chylomicrons. On the other hand, injected remnant-like emulsions behaved as predicted for chylomicron remnants and were therefore relatively uninfluenced by treatments which altered the activity of lipoprotein lipase.

References

1 Redgrave, T.G. and Maranhão, R.C. (1985) Biochim. Biophys. Acta 835, 104–112
2 Maranhão, R.C., Tercyak, A.M. and Redgrave, T.G. (1986) Biochim. Biophys. Acta 875, 247–255
3 Redgrave, T.G. (1983) Int. Rev. Physiol. 28, 103–130
3 Redgrave, T.G., Roberts, D.C.K. and West, C.E. (1975) Anal. Biochem. 65, 42–49
5 Soloni, F.G. (1971) Clin. Chem. 17, 529–533
6 Zilversmit, D.B. and Davis, A.K. (1950) J. Lab. Clin. Med. 31, 155–160
7 Zlatkis, A. and Zak, B. (1969) Anal. Biochem. 29, 143–148
8 Harwood, J.L., Riley, S.E. and Robinson, D.S. (1974) Biochim. Biophys. Acta 337, 225–238