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Competition between chylomicrons and their remnants for plasma removal: a study with artificial emulsion models of chylomicrons

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In previous studies, protein-free emulsions of defined lipid composition were shown capable of simulating either the metabolism of chylomicrons (chylomicron-like emulsion) or their remnants (remnant-like emulsion), depending on the content of free, unesterified cholesterol. To validate further the assumption that remnant-like and chylomicron-like emulsion have metabolic pathways in common with their natural counterparts, studies of competition for plasma removal were undertaken: the remnant-like emulsion labeled with [^{3}H]triolein was injected sequentially twice in the carotid arteries of rats to compare the clearance of remnant-like emulsion of the second injection with the first (control). Prior to the second injection, a large bolus of the chylomicron-like emulsion or rat lymph chylomicron was injected, to check the hypothesis that remnant generated from chylomicron-like emulsion or natural chylomicrons could compete with and displace remnant-like emulsion particles from their tissue receptor sites. Experiments were also performed in rats treated with Triton WR-1339, to block the generation of remnants. Results showed that remnants derived from either natural chylomicrons or chylomicron-like emulsion both strongly competed with the remnant-like emulsion. In contrast, when transformation of remnants was prevented by Triton, the undegraded particles of chylomicron-like emulsion or natural chylomicron were unable to compete with or displace remnant-like emulsion from its sites of removal from the plasma. In agreement with plasma clearance data, the hepatic uptake of the remnant-like emulsion was inhibited by the surplus dose of natural chylomicrons. In contrast, the spleen uptake was unaffected by it.

Introduction

As recently stated [1], chylomicrons from lymph undergo continuous change while circulating in the bloodstream. Hydrolysis of their triacylglycerol moiety by lipoprotein lipase is the key event, accompanied by net transfer of phospholipid to HDL and changes in their apolipoprotein profile. The resulting chylomicron remnants, with depleted triacylglycerol but with preserved cholesteryl ester content are finally taken up by the tissues, mainly the liver.

Recently, we described two types of emulsions composed of triolein, cholesteryl oleate, cholesterol and phosphatidylcholine that were shown to reproduce the metabolism of chylomicrons

Abbreviations: PPO, 2,5-diphenyloxazole; dimethyl POPOP, 1,4-bis-(4-methyl-5-phenyl-2-oxazolyl)benzene.

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(chylomicron-like emulsion) and their remnants (remnant-like emulsion). Remnant-like emulsions had a much higher cholesterol/phospholipid molar ratio than chylomicron-like emulsions (1.70 and 0.40, respectively) [3].

When injected into carotid arteries of rats, the chylomicron-like emulsion had the two-step pattern characteristics of chylomicron metabolism, i.e., (a) triacylglycerol was removed faster than cholesteryl ester from the plasma compartment and (b) the particles trapped by the liver had most of the injected cholesteryl ester but only residual triacylglycerol [2]. On the other hand, as expected for remnants, remnant-like emulsion had the same plasma removal rates for triacylglycerol and cholesteryl ester, indicating that no lipolysis occurred, Also, as expected for remnants, remnant-like emulsion particles were trapped by the liver faster than the chylomicron-like emulsion.

In other work [4] we have demonstrated that Triton WR-1339 blocked triacylglycerol hydrolysis of the chylomicron-like emulsion, leading to impaired removal of these emulsion particles from the plasma. However, Triton had no effect on the remnant-like emulsion removal. These results were consistent with the behavior of natural chylomicrons and remnants, and also illustrate the usefulness of Triton as a device to inhibit lipolysis, without effect on the tissue uptake mechanism.

Remnants were shown to have higher affinity for liver receptors than native chylomicrons in hepatocyte monolayers [5–7] and liver plasma membrane assays [8]. In isolated perfused liver system, remnants were removed faster than their chylomicron precursor [9–14]. Nonetheless, some other studies show at least some intact chylomicrons were removed by the liver [15–18].

In the present work, we measured the rates of removal from plasma, to test for competition between the remnant-like emulsion and remnants from the chylomicron-like emulsion or natural chylomicrons. In another set of experiments Triton was injected to inhibit lipolysis; remnants generation from both chylomicron-like emulsion and natural chylomicrons was therefore prevented. Then, competition with remnant-like emulsion for removal was again tested with intact chylomicron particles, natural as well as artificial. The competition between chylomicrons and the remnant-like emulsion was also documented in tissue uptake experiments.

The aims of these studies were to show whether the artificial remnant-like emulsion leave the plasma compartment through the same tissue uptake mechanisms as the remnants generated from chylomicron-like emulsion or natural chylomicrons, and also to estimate the capacity of intact, non-metabolized particles to displace remnants from their receptor sites.

Materials and Methods

Preparation of emulsions

Triolein, cholesteryl oleate and cholesterol were purchased from Nu-Chek Prep. (Elysian, MN) and egg phosphatidylcholine from Lipid Products (Surrey, U.K.), each more than 99% pure by thin-layer chromatography.

Two different lipid mixtures (total mass, 100 mg) were prepared to obtain the chylomicron-like and remnant-like emulsions. Chylomicron-like emulsion mixture had 2% cholesterol, 23% phosphatidylcholine, 6% cholesteryl oleate and 69% triolein (% weight). Remnant-like emulsion mixture had 24.5% cholesterol, 24.5% phosphatidylcholine, 10.3% cholesteryl oleate and 40.7% triolein. The lipids were dispensed from stock solutions into vials. In the making of the remnant-like emulsion [^{3}H]triolein (Amersham) or [^{14}C]cholesteryl oleate was added. The specific activity of the triolein was $2.21 \cdot 10^6$ cpm/mg and of the cholesteryl oleate was $4.85 \cdot 10^6$ cpm/mg. After evaporation of solvents under nitrogen, the vials were placed overnight in a vaccum desiccator at 4° C to eliminate residual solvent.

Lipids were emulsified by sonication in 8 ml of 2.785 M NaCl solution (density, 1.101 g/ml) with a Branson Cell Disruptor (Danbury, CT) at approx. 55° C, using a 1 cm probe with continuous output of 70–80 W. Purification of crude emulsions was by ultracentrifugation in discontinuous gradients of NaCl solutions with densities of 1.065, 1.020 and 1.006 g/ml. The ultracentrifugation procedure was performed differently for the two types of emulsion. The chylomicron-like emulsion was inicially centrifuged at 12000 rpm for 15 min in an SW 41 Beckman rotor at 22° C. The remnant-like emulsion was centrifuged at 10000 rpm

for 20 min. After this first run, the coarse lipid that floated to the top of the 1.006 g/ml solution was removed by aspiration and replaced with its corresponding volume of fresh 1.006 g/ml solution. The chylomicron-like emulsion was again centrifuged, now at 36000 rpm for 25 min, and the remnant-like emulsion at 38000 rpm for 30 min at 22°C. The emulsion particles floating to the top of the gradient were aspirated and used for analysis and for competition studies. The lipid composition of the emulsion was determined by standard laboratory procedures [19–21]. Both emulsions had been previously characterized by negative staining electron microscopy [2,3].

Preparation of chylomicrons

Intestinal lymph was collected over ice with added EDTA (final concentration, 1 mM) for 24 h from male Wistar rats weighing 300–400 g, through a cannula implanted in the mesenteric lymph duct.

After surgery the rates were maintained in restriction cages and a constant infusion of cotton seed oil (0.035 ml/h) was delivered through a gastrostomy tube. Water drinking was ad libitum.

Saline (5 ml) (pH 7.0) $d = 1.006$ g/ml containing EDTA (1 mM) was layered on lymph (5 ml) into the Beckman SW 41 rotor tube and centrifuged at 24500 rpm for 20 min at 20°C. Chylomicrons were recovered from 1.5 ml of the creamy top layer aspirated from each tube and assayed as described above. They were used in the experiments within the ensuing 24 h.

Competition studies

The rats utilized in this study were non-fasted, non-anesthetized male Wistar rats weighing 250–300 g. Emulsions, lymph chylomicrons, saline solution and Triton were injected in a bolus through a polyethylene cannula (Intramedic PE 50) inserted under diethylether anesthesia into the right carotid artery, according to the protocols described below. Clotting was prevented by previous treatment of the cannula with silicon (Clay Adams, Parsippany, NJ). The animals were allowed to recover from anesthesia for 2–3 h in individual cages.

The remnant-like emulsion (0.3–0.5 mg of its triolein moiety) labeled with radioactive triolein was injected sequentially twice into the bloodstream of the rats to compare the plasma clearance rates of the second injection with the first one (control injection). The second injection was made 20 min after the first and 10 min after the intraarterial administration of isotonic saline solution (0.5 ml) (Experiment 1), chylomicron-like emulsion (30 mg of total lipids) (Experiment 2) or rat lymph chylomicrons (30 mg of total lipids (Experiment 4). The above experiments were repeated after the injection of Triton WR-1339 (600 mg/kg of body weight, [22]) 10 min before the first injection, to inhibit lipoprotein lipase activity (Experiments 3 and 5). In these experiments, only the triolein moiety was labeled, but from our previous work [4] it is known that for remnant-like emulsion the triolein and cholesteryl oleate plasma clearances are identical.

After the two injections of labeled remnant-like emulsion, 0.2-ml blood samples were drawn at 2 min intervals for 10 min. After the experiments, animals were killed by air embolization. 100 μl of blood plasma were extracted with chloroform/methanol (2 : 1, v/v) [23] and neutral lipid classes separated by TLC in the solvent system hexane/diethyl ether/acetic acid (70 : 30 : 1, by vol.) Bands corresponding to triacylglycerols were then scraped into counting vials for radioactivity measurement in 10 ml of scintillation solution (0.59 PPO/59 dimethyl POPOP/333 ml Triton X-100/667 ml toluene) [24] in a Beckman LS 100 C Spectrometer. Plasma clearance kinetics (fractional clearance rate) of [^{3}H]triolein were computed from monoexponential curves fitted by the least-square procedures.

In another set of competition experiments, the remnant-like emulsion labeled with [^{14}C]cholesteryl oleate was injected, as described above, in control and rats that had been given 30 mg of total lipids of lymph chylomicrons administered as a bolus 10 min before hand.

3 min after the labeled remnant-like mulsion injection, the animals were killed by air embolization and their livers, spleens, lungs, hearts, kidneys and adrenals as well as samples of their muscle and adipose tissue were quickly excised to determine the radioactive label uptake by these organs. Lipids from approx. 1 g of the tissues were extracted by chloroform/methanol (2 : 1, v.v) [23] and processed as described for plasma samples.

TABLE I

COMPOSITION OF THE EMULSIONS AND LYMPH CHYLOMICRONS

Results are given as mean ± S.E. The composition (%) of the initial sonicated lipid mixture is given in parentheses.

Constituents (% by weight)	Chylomicron emulsion (low-cholesterol) ($n = 8$)	Remnant emulsion (high-cholesterol) ($n = 5$)	Lymph chylomicrons ($n = 1$)
Cholesterol	1.9 ± 0.3 (2)	21.3 ± 2.7 (24.5)	0.7
Phospholipid	10.4 ± 1.3 (23)	18.7 ± 2.4 (24.5)	4.0
Cholesteryl ester	11.2 ± 3.0 (6)	18.7 ± 5.4 (10.3)	4.5
Triacylglycerols	76.5 ± 4.1 (69)	41.4 ± 3.4 (40.7)	90.8
Cholesterol/phospholipid (molar ratio)	0.39 ± 0.06	2.4 ± 0.5	
Cholesteryl ester/triacylglycerol (molar ratio)	0.13 ± 0.01	0.67 ± 0.2	

Results

Composition of emulsions prepared as described from their respective lipid mixtures and of the lymph chylomicron are shown on Table I.

Experiment 1 (Table II) was a control for the general design of the current experiment. Comparing the fractional clearance rates of the two injections of remnant emulsion, removal from the second injection was slowed by 1.4-fold. Nevertheless, this baseline difference can be considered as rather minor when compared to the results of the ensuing experiments, in which saline was replaced by the chylomicron-like emulsion or natural chylomicrons.

When injected after a load of chylomicron-like emulsion (30 mg of total lipids) in Experiment 2 (Table II), labeled remnant-like emulsion had its fractional clearance rate diminished 4.8-fold compared with the control first injection. This finding indicates that the remnant particles generated from chylomicron-like emulsion were capable of competing with the labeled remnant-like emulsion particles from their tissue receptor sites, thus preventing the plasma removal of remnant-like emulsion. In the ensuing Experiment 3, generation of re-

TABLE II

COMPETITION FOR PLASMA REMOVAL BETWEEN REMNANT-LIKE EMULSION AND CHYLOMICRON-LIKE EMULSION OR NATURAL CHYLOMICRONS

Fractional clearance rates of two sequential injections of [^{3}H]triolein labeled remnant-like emulsion are compared when the second injection was preceded by the administration of either saline solution, chylomicron-like emulsion (30 mg of total lipid) or natural chylomicrons (30 mg). The experiments were also performed with Triton WR-1339 (600 mg/kg of body weight, intraarterially 10 min, prior to first injection) to prevent remnant generation from chylomicron-like emulsion or natural chylomicrons. Results are mean ± S.E. Fractional clearance rate = $\ln 2/t_{1/2}$. $n = 7$ in every case, except for saline ($n = 4$). n.s., not significant.

Experiment	Fractional clearance rate of remnant-like emulsion (min^{-1})		P [a]
	first injection	second injection	
1. Saline	0.24 ± 0.01	0.17 ± 0.01	< 0.02
2. Chylomicron-like emulsion	0.29 ± 0.04	0.06 ± 0.01	< 0.001
3. Chylomicron-like emulsion, Triton treatment	0.28 ± 0.05	0.30 ± 0.03	n.s.
4. Natural chylomicrons	0.27 ± 0.02	0.07 ± 0.01	< 0.001
5. Natural chylomicrons, Triton treatment	0.25 ± 0.04	0.22 ± 0.03	n.s.

[a] Comparison between first and second injection (Student's t-test).

mnants from the chylomicron-like emulsion load was blocked by inhibition of the lipoprotein lipase action with Triton WR-1339. In this case, intact, non-metabolized chylomicron-like emulsion particles were not effective competitors with remnant-like emulsion for the receptor sites, as shown by the unaltered clearance of the chylomicron-like emulsion.

In experiments 4 and 5 (Table II) the name 30 mg lipid mass of natural chylomicrons obtained from lymph of donor rats was injected. The results were very similar to those following the injection of chylomicron-like emulsion: the fractional clearance rate of the remnant-like emulsion decreased approx. 4-fold when natural chylomicrons were administered alone. Also, like the chylomicron-like emulsion, when the natural chylomicrons were injected in association with Triton, the plasma removal of remnant-like emulsion was not altered.

Table III shows the uptake of the labeled remnant-like emulsion by several tissues (liver, spleen, lung, heart, kidney, adrenal, muscle and adipose tissue) measured 3 min after its injection. Whereas 42 and 6% of the injected cholesteryl ester moiety was taken up by the liver and the spleen, respectively, the uptake of the other tissues were 1% or less. When the remnant emulsion was injected after previous administration of the surplus mass of lymph chylomicrons, in the liver only the emulsion uptake was diminished by the competition with the remnants generated from the natural chylomicrons.

TABLE III

UPTAKE OF THE [^{14}C]CHOLESTERYL OLEATE MOIETY OF THE REMNANT-LIKE EMULSION BY DIFFERENT TISSUES IN CONTROL AND RATS SUPPLIED WITH A PREVIOUS SURPLUS DOSE OF NATURAL CHYLOMICRONS

30 mg of total lipids of the natural chylomicrons were administered intraarterially 10 min before the injection of the labeled emulsions. Organs were excised for lipid extraction and radioactivity determination 3 min after the injection of the emulsion. Results are mean ± S.E. The number of experiments is given in parentheses. The total organ mass of muscle and adipose tissue was calculated from Refs. 32 and 33.

Tissues	Organ uptake of [^{14}C]cholesteryl oleate (% of injected dose)	
	control	supplied with chylomicrons
Liver	42.54 ± 3.50 (11)	30.92 ± 1.5 (7) [a]
Spleen	5.97 ± 0.70 (10)	5.30 ± 0.70 (7)
Lung	1.07 ± 0.26 (5)	1.00 ± 0.20 (8)
Muscle	0.77 ± 0.13 (6)	0.84 ± 0.19 (7)
Heart	0.10 ± 0.03 (6)	0.14 ± 0.03 (8)
Kidney	0.13 ± 0.02 (6)	0.13 ± 0.03 (8)
Adipose tissue	0.07 ± 0.02 (6)	0.06 ± 0.01 (8)
Adrenal	0.01 ± 0.00 (6)	0.01 ± 0.00 (7)

[a] $P < 0.025$ by t-test.

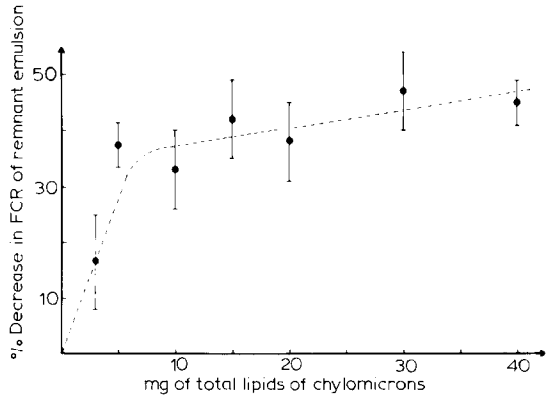

Fig. 1. Effects of increasing the injected dose of chylomicrons on the plasma clearance of the radioactive remnant-like emulsion. The chylomicron dose was supplied intraarterially 10 min before the injection of the remnant emulsion. Each point corresponds to 5–9 experiments of plasma clearance determination. Bars represent standard errors of the means. FCR, fractional clearance rate.

The effect of the previous injection of increasing chylomicron doses on the plasma disappearance kinetics of the remnant emulsion (dose-response curve) is shown in Fig. 1.

Discussion

A protein-free emulsion of defined lipid composition can model chylomicron metabolism. After injection, the apolipoprotein necessary for their metabolism is gained rapidly from apolipoprotein that is free in plasma or by exchange with other lipoproteins. Their surface lipid composition will determine the profile of adsorbed apolipoproteins, which in turn modulate the metabolic pathway of the emulsions. Accordingly, we showed in previous work [3] that cholesterol-enriched remnant-like emulsion bound less apolipoproteins A-I, A-IV and C and relatively more apolipoprotein E than

did the cholesterol-poor chylomicron-like emulsion and that this could be related to their different metabolic fates.

The hypothesis that remnant-like emulsion and also remnants of chylomicron-like emulsion have a common pathway with natural remnants is further strengthened by the demonstration that they share the same plasma removal mechanisms.

Remnants are primarily trapped by the liver parenchymal cells, although Kupffer and other non-parenchymal cell also participate [25–27]. In several studies [8,11,12,29,30], high-affinity, saturable receptors for remnants were found in rat liver membranes. Remnant particles appear to bind to the membrane as a unit [11,12,29,31]. In contrast, intact chylomicrons seem to be removed poorly by the liver [9–14].

The findings of the present work indicate direct competition between the artificial remnant-like emulsion and remnants derived from either natural chylomicrons or chylomicron-like emulsion. Sherrill and Dietschy [12], who found that the uptake of remnants by the liver was saturable, concluded that the sinusoidal membrane of the liver possessed a finite number of sites for the selective and rapid transport of chylomicron remnants.

When we injected a second small dose of remnant-like emulsion, 20 min after the first dose, clearance of the second dose was only minimally slowed compared with the first dose. Therefore, the hepatic receptor or transport mechanism was not saturated by the first dose. In contrast, a large dose of either chylomicron-like emulsion or natural chylomicrons inhibited the clearance of a subsequent small dose of remnant-like emulsion. In this case, sufficient remnants were formed from the injected triacylglycerol-rich particles to saturate the hepatic sinusoidal transport mechanism.

Consideration was given to the possibility that saturation of the hepatic transport mechanism for the remnant-like emulsion clearance was not by remnants, but by undegraded chylomicron-like emulsion or natural chylomicrons. However, this explanation seems unlikely for two reasons. First, undegraded chylomicrons are not taken up efficiently by the liver [9–14]. Second, after Triton WR-1339 was injected to inhibit lipolysis, and thence formation of remnants, the presence of injected undegraded chylomicron-like emulsion or natural chylomicrons did not complete with subsequent transport and clearance of the injected remnant-like emulsion.

The clearance of the second injection of remnant-like emulsion was not slowed even minimally when Triton was present to prevent lipolysis. This finding is probably explained by Triton blocking the formation of remnants, not only from the injected triacylglycerol-rich particles, but also from endogenous circulating very-low-density lipoprotein, which would also compete with remnant-like emulsion for clearance by the hepatic transport mechanism.

Apart from the liver and the spleen, trapping of the remnant emulsion cholesteryl ester by the other tissues was rather minimal, as expected for remnants of natural chylomicrons [1].

Interestingly, the chylomicron dose capable of inhibiting the remnant-like emulsion uptake by the liver at 3 min did nothing to the uptake of the extra-hepatic tissues. These results favor the hypothesis that the removal mechanisms operative in these organs are less specific, or at least of different nature, than the hepatic ones.

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