Fractional Composition of Blood Serum Lipoproteins in Mice and Rats with Triton WR 1339-Induced Lipemia

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We compared fractional composition of blood serum lipoproteins (LP) in female ICR mice and Wistar rats induced by single administration of a nonionic detergent Triton WR 1339 in doses of 300 and 500 mg/kg. Lipemia in animals of both species was characterized by a sharp increase in the concentration of cholesterol and, particularly, of triglycerides in blood serum lipoproteins by the 24th hour after administration of the detergent. We revealed a significant increase in the concentrations of atherogenic VLDL cholesterol (due to VLDL2), intermediate density lipoproteins, and LDL. These changes were more pronounced in rats. The model of lipemia can be used to study the role of fractional composition of lipoproteins and, particularly, of triglycerides in the pathogenesis of atherosclerosis. Moreover, this model holds much promise for evaluation of the efficiency of hypolipidemic drugs (statins and fibrates) in normalizing the increased level of atherogenic cholesterol of VLDL and LDL.

Key Words: *lipemia; lipoproteins; fractional composition; Triton WR 1339*

Lipemia is one of the major risk factors for atherosclerosis, cardiovascular diseases, and cerebrovascular disorders [1-4]. The model of lipemia in experimental animals induced by Triton WR 1339 (Triton) is used in studies of atherosclerosis pathogenesis and effect of hypolipidemic drugs (statins and fibrates) [5,11]. The advantages of this model are simplicity of the method and dose-dependent effect of Triton in the induction of lipemia of different severity [6-9]. This model was used for a long time. However, little is known about changes in the ratio between various fractions and subfractions of lipoproteins (LP), particularly of VLDL, LDL (adverse proatherogenic effect), and HDL (protective antiatherogenic effect) during experimental lipemia. Variations in the content of triglycerides (TG) are poorly understood. At the same time, the development of atherosclerosis is believed to be associated with changes in TG content [10]. Studying the model of Triton-induced lipemia is important for evaluation of the efficiency of new hypolipidemic drugs normalizing the content of some fractions of LP.

Here we studied fractional composition and subfractions of LP in blood serum of mice and rats with lipemia induced by single administration of Triton.

MATERIALS AND METHODS

Experiments were performed on Wistar rats (200-250 g) and female ICR mice (25-30 g). The animals were obtained from a vivarium of the Institute of Cytology and Genetics. To induce lipemia, the animals received an intraperitoneal injection of Triton WR 1339 (Ruger Chemical Co.) in single doses of 300 and 500 mg/kg [11]. The mice were euthanized 24 h after administra-

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tion of the detergent. Before euthanasia, the mice were deprived of food for 15 h, but had free access to water. Intact animals received an equivalent volume of the solvent (physiological saline).

Blood serum was obtained by centrifugation of blood samples on an Eppendorf 5415 R centrifuge at 3000*g* and 4°C for 20 min.

The fractional composition of blood serum LP was estimated by means of small-angle X-ray scattering [1-3]. This method allowed us to perform quantitative analysis for the total concentration of LP cholesterol (CH), LP TG, and fractions or subfractions of LP (HDL, LDL, and VLDL; except for chylomicrons) [1,2]. Previous studies showed that the parameters estimated by this method correspond to those evaluated by other techniques [2]. Small-angle X-ray scattering was measured on diffractometers (Siemens, Hecus) by the method of step-by-step scanning using a goniometer and X-ray scintillation detector.

Experimental data on the fractional and component composition of LP were processed using a general mathematical model for the structure of LP of various classes and subclasses. Mathematical treatment of small-angle X-ray patterns and calculation of size distribution of LP particles were performed by the algorithms and procedures of optimization [2].

In some experiments, the total concentrations of CH and TG in control samples of blood serum were measured with Triglitseridy-Novo and Novokhol kits (Vector-Best). Photometry of samples was conducted on a semiautomatic photometer 5010 equipped with a temperature-controlled flow cell (Robert Riele). In the presence of considerable amounts of chylomicrons in blood serum, the total concentration of CH and TG assayed by biochemical methods can exceed the total concentrations of LP CH and LP TG measured by small-angle X-ray scattering.

The results were analyzed statistically by the parallel-series method of variation statistics (Student's *t* test). The differences between the means were significant at $p<0.05$.

RESULTS

Injection of Triton in a single dose of 500 mg/kg was followed by a sharp increase in the concentrations of CH and TG in LP in rats and mice, which is typical of lipemia (Table 1). During this period (24 h), the concentration of LP TG increases more significantly than that of LP CH. Published data show that these changes are observed 1-8 h after administration of the detergent to rats (phase I of Triton action on LP; Table 1; Fig. 1) [6,10,11]. Administration of Triton in a dose of 300 mg/kg was also followed by a significant increase in the concentration of LP CH and, particularly, of LP TG (Table 1).

A significant increase in the concentration of atherogenic fractions of VLDL (by 40 times) under the influence of Triton in a dose of 500 mg/kg was related to changes in the content of subfractions 3-5 and, to a lesser extent, of fractions 1-2 (Fig. 1). We revealed a significant increase in the amount of intermediate density LP CH. In fraction HDL, only the content of subfraction HDL_3 tended to increase. Similar changes in CH content in fractions and subfractions of LP were observed 24 h after administration of the detergent in single doses of 300 and 500 mg/kg (Fig. 1).

The concentrations of LP CH and LP TG in mice were elevated after injection of Triton in a dose of 500 mg/kg. These changes in mice were less pronounced than in rats receiving Triton in the same dose (Table 1). An increase in the concentration of VLDL CH was mainly associated with variations in the content of $VLDL_{3-5}$ (similarly to rats; Fig. 2). The concentration of HDL CH (subfraction HDL_3) in mice increased more significantly than in rats (Fig. 2).

Our results indicate that Triton-induced acute lipemia in animals is accompanied by an increase in the concentration of atherogenic subfractions $VLDL_{1-2}$, LDL_{3-5}, and intermediate density LP. These data can be used to study the mechanism for action of hypolipidemic drugs. Comparative study of lipemia in rats and mice shows that Triton in doses of 300 and 500 mg/kg

TABLE 1. Concentrations of LP CH and LP TG in Blood Serum of Wistar Rats and ICR Mice 24 h after Single Administration of Triton (mmol/liter, *M*±*m*)

Animals	Group	LP CH	LP TG
Rats	Intact (*n*=6)	2.28±0.13	0.51±0.03
	Triton WR 1339, 300 mg/kg (*n*=6)	11.80±0.08*	8.95±0.76*
	Triton WR 1339, 500 mg/kg (*n*=10)	10.30±0.66*	8.03±0.76*
Mice	Intact (*n*=8)	2.14±0.18	0.71±0.10
	Triton WR 1339, 500 mg/kg (*n*=8)	6.13±0.49*	4.92±1.12*

Note. *$p<0.001$ compared to intact animals.

causes similar changes in the fractional composition and subfractions of LP. It should be emphasized that the degree of lipemia in rats was higher than in mice (500 mg/kg Triton). Moreover, the concentration of CH in subfraction HDL_3 was elevated in mice (Fig. 2).

Further increase in the single dose of Triton to 850 mg/kg was also followed by the development of severe lipemia in mice. The total TG concentration increased more significantly than the total content of CH in blood serum [11]. Our results indicate that administration of Triton can be used as a model of triglyceridemia (but not only of cholesterolemia) in mice. This state plays a role in the pathogenesis of atherosclerosis [12,13].

Triton WR 1339 (iso-octyl polyoxyethylene phenol) is a nonionic detergent that possesses the lysosomotropic properties. This substance is used for modeling lipemia in various animals (rats, mice, rabbits, dogs, and guinea pigs) [7,11]. The general enzymatic assays showed that administration of Triton to rats is accompanied by a sharp increase in the total concentrations of CH and TG in blood serum [11]. The development of lipemia is related to inhibition of serum lipoprotein lipase and abnormal clearance of VLDL [8]. Triton in doses of 850-1000 mg/kg is used for modeling lipemia and for isolation of Triton-loaded liver lysosomes. Some experiments (*e.g.*, studies with hypolipidemic drugs) are performed with the lower doses of Triton (200 and 300-500 mg/kg) [13-15].

Single intravenous injection of Triton in the low dose (200 mg/kg) was accompanied by a linear increase (by 20 times) in the concentration of TG in blood serum of rats. These changes were observed 1-8 h after administration of Triton. Serum CH concentration increased by the 24th hour after treatment and was 2-3 times higher than in intact rats (phase I of lipemia) [5-8,11]. Phase II of lipemia developed over the next 24 h (24-48 h after administration of the detergent). The concentration of CH progressively returned to normal during this phase. For evaluation of the hypolipidemic effect, the test agents were administered simultaneously or 1-3 day before injection of Triton [5-8]. Experiments with compounds modifying the synthesis TG were performed 2, 4, 6, and 8 h after administration of Triton (action on TG synthesis). A possible decrease in the synthesis of CH was evaluated by treatment with the test substances 6, 24, and 48 h after administration of Triton.

The inhibition of lipolysis is used to estimate the rate of VLDL secretion by the liver [11]. The rate of VLDL secretion can be evaluated from the increase in the concentrations of TG and CH over various periods after administration of the detergent. The test compounds modifying biosynthesis of TG and CH in the liver are active during phase I. By contrast, the

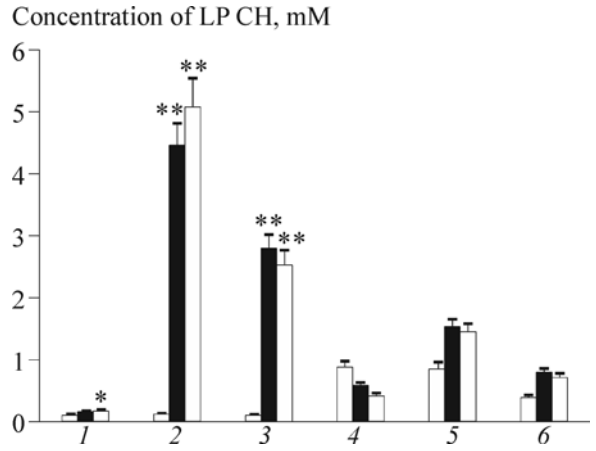

Fig. 1. Fractional composition of blood serum LP in rats after single administration of Triton WR 1339. Light bars, intact rats; dark bars, administration of Triton WR 1339 in a dose of 300 mg/kg; shaded bars, administration of Triton WR 1339 in a dose of 500 mg/kg. Here and in Fig. 2: VLDL, subfractions *1-2* (*1*); VLDL, subfractions *3-5* (*2*); intermediate density LP (*3*); LDL, subfractions *1-3* (*4*); HDL, subfraction *2* (*5*); HDL, subfraction *3* (*6*). **p*<0.05, ***p*<0.001 compared to intact animals.

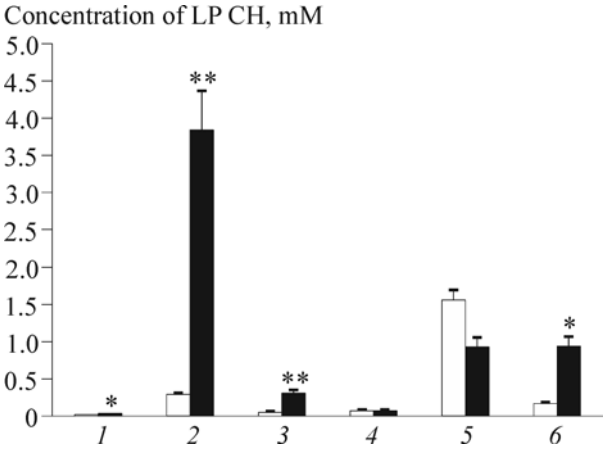

Fig. 2. Effect of Triton WR 1339 on the fractional composition of blood serum LP in mice. Light bars, intact mice; dark bars, administration of Triton WR 1339 in a dose of 500 mg/kg.

compounds affecting the excretion and metabolism of CH are active during phase II. The model of Triton-induced lipemia has several advantages. However, some authors reported that this detergent can cause damage to cell membranes. This effect is observed after repeated treatment with Triton in high doses. Published data show that lipemia can be induced by poloxamer (Pluronic F-127), which does not damage the cell membrane [5-8].

A Triton-induced increase in the concentration of blood serum lipids (particularly of CH) can be related to high absorption of this agent from the intestine, increased synthesis of endogenous CH, or impaired release (clearance) of CH from the serum. Administration of Triton to rats is followed by an increase in

the synthesis of CH by liver cells and change in LP clearance [11]. To reduce the concentration of blood serum lipids, it is necessary to inhibit the synthesis of endogenous CH (elevated after treatment with Triton), decrease the absorption, or stimulate the excretion of LP lipids from blood serum. Fibrates (PPAR-α agonists), inhibitors of CH absorption (Ezetimibe), fatty acid sequestrants, and statins (HMG-CoA reductase inhibitors) hold much promise in this respect.

Changes in LP during experimental lipemia induced by Triton (300 and 500 mg/kg) are similar to those observed in patients with type 2b dyslipoproteinemia (increase in the concentration of highly atherogenic LDL and VLDL) and type 3 dyslipoproteinemia (increase in the concentration of TG and intermediate density LP). The changes induced by single administration of Triton in high dose (850 mg/kg) are similar to those revealed during type 1 hyperlipoproteinemia. This state is characterized by an increase in the contents of CH, TG, low atherogenic fractions of LP, chylomicrons, and VLDL.

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