METHODS

Comparative Characteristics of Lipemia Models Induced by Injections of Triton WR-1339 and Poloxamer 407 in Mice
V. M. Loginova, F. V. Tuzikov*, N. A. Tuzikova*, and T. A. Korolenko

Lipemia modeled by injections of Triton WR-1339 (500 mg/kg) and poloxamer 407 (500 mg/kg) to mice were compared. LP fraction and subfraction compositions were compared by small-angle X-ray scattering on a diffractometer. Both compounds in the same dose caused a sharp increase in serum concentrations of total cholesterol (CH) and triglycerides (TG), the increases in response to poloxamer 407 being more pronounced. The differences in the models consisted in the levels of atherogenic fractions: CH-VLDL (subfractions CH-VLDL\(_{1-2}\)) and CH-LDL, which were higher under the effect of poloxamer 407. Similar increases were observed for atherogenic fractions: TG-VLDL (subfractions TG-VLDL\(_{1,2}\)) and TG-LDL (subfractions TG-LDL\(_{1,3}\)). A specific feature of the model induced by poloxamer 407 was elevation of the concentrations of antiatherogenic CH-HDL and TG-HDL (subfractions CH-HDL\(_2\) and TG-HDL\(_2\)). Both models exhibited high similarity, but changes in atherogenic fractions were more pronounced under the effect of poloxamer 407.

Key Words: triton WR-1339; poloxamer 407; lipemia; lipoprotein fractions and subfractions

Lipemia is one of the most important risk factors for atherosclerosis and some cerebrovascular diseases [3]. Lipemia models reproduced in experimental animals by injections of Triton WR-1339 (WR1339) and poloxamer 407 (P407) are used for studies of the mechanism of atherosclerosis development [1,8,13]. The advantages of these models are simple reproduction, low toxicity of the polymers, dose-dependent effects of the compounds in reproduction of lipemia of different severity [1]. A detailed study of lipemia models induced by WR1339 and P407 is expected to provide data useful for testing of new hypolipidemic drugs (statins and fibrates) normalizing individual LP fractions. The mechanisms of experimental lipemia development, primarily changes in the serum LP fraction and subfraction composition, received insufficient attention. The proportions of various LP subfractions remain unclear, particularly of LDL, responsible for the negative pro-atherogenic effect, and HDL with the defense anti-atherogenic effect in experimental lipemia.

We have compared the lipemia models induced in mice by injections of WR1339 and P407.

MATERIALS AND METHODS

Lipemia models were created in animals by injection of WR1339 (Ruger Chemical Co.) and P407 (Sigma). Triton WR1339 is a nonionic detergent iso-octyl-polyoxyethylene phenol (molecular weight (280.40 n), with the common formula (C\(_{14}\)H\(_{29}\)O.C.H\(_{2}\)O.CH\(_{2}\)O)n; P407 (Pluronic F-127) is a polymer of nonionic origin, a copolymer block – polyoxyethylene polyoxy-
propylene (molecular weight 9840-14,600), with the common formula: $HO(C_2H_4O)a(C_3H_6O)b(C_2H_4O)aH$, where $a=100$, $b=65$. These substances are characterized by lysosomotropic effects and are used (P407) in pharmacology as emulsifiers, nanocarriers of various drugs, stimulating their therapeutic effect.

The study was carried out on ICR mice (vivarium of Institute of Cytology and Genetics). The animals received a single intraperitoneal injection of 500 mg/kg WR1339 or P407 [10,13] and after 24 h were sacrificed. Before sacrifice, the animals were fasting for 15 h with free access to water. Intact animals served as the control. The serum was separated by centrifugation of the samples at 3000g for 15 min at 4°C on an Eppendorf 5415 R centrifuge. The samples were stored at -70°C. The concentrations of total cholesterol (CH) and triglycerides (TG) were measured using Biokon kits. The composition of LP fractions and subfractions was studied by small-angle X-ray scattering on a Siemens diffractometer [14].

The results were presented as the proportion of the absolute values of each fraction in percent of control. The data were processed by Statistica 6.0 software. Depending on the type of distribution (evaluated by Kolmogorov–Smirnov’s method), the significance of differences between the means was evaluated by the parametric Student’s $t$ test and nonparametric Kruskal test. The differences were considered significant at $p<0.05$.

**RESULTS**

The inductors (P407 and WR1339) in the same dose (500 mg/kg) caused a drastic increase in serum concentrations of CH and TG, more pronounced in response to P407 (Table 1), with triglyceridemia more manifest than cholesterolemia. Cholesterolemia and triglyceridemia in response to P407 were more pronounced than after WR1339 injection ($p<0.01$; Table 1), which was in line with the previous data [1,5].

According to a previously suggested classification [12], LP are subdivided into four main classes: HDL, LDL, VLDL, and chylomicrons, or 7 subfractions (HDL$_{2e}$, HDL$_{3e}$, LDL, intermediate density LP (IDL), VLDL$_{3,5}$, VLDL$_{1,2}$, chylomicrons) [11,12].

Lipemia in mice induced by WR1339 and P407 was characterized by a significant elevation of CH-IDL and TG-IDL subfractions in the studied lipemia models reached the maximum levels in comparison with other subfractions (Figs. 1 and 2). No differences between CH-IDL and TG-IDL subfractions after injections of WR1339 and P407 were detected. The IDL were the most unstable LP particles forming from VLDL. Normally, some IDL are captured by liver LDL receptors, scavenger receptors, while others are hydrolyzed and transformed into LDL [14].

Of atherogenic fractions, the concentrations of CH-VLDL (subfractions CH-VLDL$_{1-2}$) and CH-LDL were higher ($p<0.05$) in response to P407 (Fig. 1). Injection of P407 also led to more pronounced increase in TG-VLDL (subfractions TG-VLDL$_{1-2}$, $p<0.01$) and TG-LDL (subfractions TG LDL$_{1-3}$, $p<0.05$; Fig. 2).

A specific feature of lipemia induced by P407 was an increase of the concentrations of antiatherogenic fractions CH-HDL (subfractions CH-HDL$_{2}$, $p<0.01$) and TG-HDL (subfractions TG-HDL$_{2}$, $p<0.01$; Figs. 1, 2). Injection of WR1339 to mice led to development

**TABLE 1.** Serum Concentrations of CH and TG (mg/dl) in Mice in Response to Injections of WR1339 and P407 (M±m; n=10)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>WR1339</th>
<th>P407</th>
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<tbody>
<tr>
<td>CH</td>
<td>108.40±11.61</td>
<td>189.60±11.61*</td>
<td>232.20±2.32**</td>
</tr>
<tr>
<td>TG</td>
<td>115.10±8.85</td>
<td>1610.70±155.76*</td>
<td>2283.3±88.5**</td>
</tr>
</tbody>
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Note. *$p<0.001$ in comparison with the control; $p<0.01$ in comparison with WR1339.