Influence of atorvastatin and carboxymethylated glucan on the serum lipoprotein profile and MMP activity of mice with lipemia induced by poloxamer 407


Abstract: The effects of atorvastatin and carboxymethylated β-glucan (CMG) on the lipoprotein-cholesterol (LP-C) and lipoprotein-triglyceride (LP-TG) fractions and subfractions at the early stage of murine hyperlipidemia, and its pleiotropic anti-inflammatory effects, were studied. Atorvastatin and CMG were administered in ICR male mice with acute lipemia induced with a single injection of poloxamer 407 (P-407). A novel small-angle X-ray scattering method for the determination of fractional and subfractional composition of LP-C and LP-TG was used. In P-407-treated animals, there was a drastic increase of total cholesterol and especially TG. Atorvastatin decreased both the total cholesterol and TG, but not to control levels. CMG primarily decreased TG and was not as potent as atorvastatin. P-407 increased atherogenic LDL-C (IDL-C and LDL1,3-C subfractions) and very low-density lipoprotein-C (VLDL-C) (VLDL1,2-C and VLDL3,5-C subfractions) fractions, with an increase of the total anti-atherogenic HDL-C fraction (HDL2-C subfraction). Atorvastatin treatment of lipemia was followed by a decrease in the total LP-C, total LDL-C (LDL1,3-C subfraction), and the LDL1,3-TG subfraction. Additionally, atorvastatin treatment resulted in an increase in the serum matrix metalloproteases activity both in control and P-407-treated mice. In general, high-dose atorvastatin therapy exerts its lipid-lowering and pleiotropic effects in the early stages of acute lipemia induced in mice by treatment with P-407.

Key words: lipoprotein fractions and subfractions, lipemia, atorvastatin, carboxymethylated β-glucan, matrix metalloproteases.

Résumé : On a examiné les effets de l’atorvastatine et du carboxyméthyl β-glucane (CMG) sur les fractions et sous-fractions du cholestérol des lipoprotéines (C-LP) et des triglycérides des lipoprotéines (TG-LP) au stade précoce de l’hyperlipidémie murine, ainsi que leurs effets pléiotropes et anti-inflammatoires. On a administré l’atorvastatine et le CMG à des souris mâles ICR présentant une hyperlipidémie aiguë induite par l’injection de poloxamer 407 (P-407). On a utilisé une nouvelle méthode de diffusion des rayons-X aux petits angles pour déterminer la composition fractionnaire et sous-fractionnaire de C-LP et de TG-LP. Chez les souris traitées au P-407, le cholestérol total et en particulier les TG ont augmenté de manière significative. L’atorvastatine a diminué le cholestérol total et les TG, mais pas au niveau des valeurs témoin. Le CMG a diminué principalement les TG, mais avec une puissance inférieure à l’atorvastatine. Le P-407 a augmenté les fractions athérogènes du C-LDL (sous-fractions de CIDL et C-LDL1,3) et du cholestérol des lipoprotéines de très faible densité (C-VLDL) (sous-fractions de C-VLDL1,2 et C-VLDL3,5), en plus d’augmenter la fraction du C-DL antiathérogène total (sous-fraction de C-HDL2). Le traitement de l’hyperlipidémie à l’atorvastatine a entraîné une diminution du C-LDL total, du C-LDL1,3 total (sous-fraction de C-LDL1,3), et de la sous-fraction de TG-LDL1,3. Ce traitement a provoqué une augmentation de l’activité des MMP sériques tant chez les souris témoins que chez les souris traitées au P-407. En général, les effets pléiotropes et hypolipémiantes d’un traitement avec une forte dose d’atorvastatine se manifestent dans les premières phases de l’hyperlipidémie aiguë induite chez les souris mâles par l’injection de P-407.

Mots-clés : fractions et sous-fractions de lipoprotéines, hyperlipidémie, atorvastatine, carboxyméthyl β-glucane, métalloprotéinases matricielles.

[Traduit par la Rédaction]
Introduction

Statins are the mainstay of lipid-lowering therapy. Specifically, they lower low-density lipoprotein cholesterol (LDL-C) by inhibiting cholesterol neosynthesis and upregulating LDL receptors on the surface of hepatocytes. While their primary effect is on LDL, nevertheless, statins also slightly lower triglycerides and provide a moderate increase in high-density lipoprotein cholesterol (HDL-C). Multiple trials have demonstrated that lowering the concentration of LDL-C with statins substantially reduces the risk of future cardiovascular events in patients with hypercholesterolemia (Liao and Laufs 2005; Tentolouris et al. 2009). Recent studies suggest that statins may also have anti-inflammatory and pleiotropic properties (Luan et al. 2003; Visser et al. 2008). This supports a potential role of statins in plaque stabilization and thus in the management of acute coronary syndromes (Nicholls et al. 2005). However, the efficiency of “medium” and “high” doses of statins used in clinical medicine, and their local action on plaque formation (and plaque stabilization) independent of the LDL-C lowering in blood serum, are still under discussion (Nachtigal et al. 2007). Atorvastatin in high doses (up to 80 mg) is the most widely investigated lipid-lowering statin in clinical medicine for reducing high-sensitivity C-reactive protein (hs-CRP) in acute coronary syndrome (Gupta et al. 2008; Kleemann et al. 2011). It was suggested that high doses of statins were needed to exert their pleiotropic effects; however, high doses of statins have additional risk factors of increased adverse side effects and drug interactions (Roglans et al. 2002).

Another group of lipid lowering agents are the β-(1,3)-β-glucans. Water-soluble and water-insoluble β-glucans show significant differences in their biological activity (Rondanelli et al. 2009). Glucans of different origins (isolated from yeast, mushrooms, etc.) are made water soluble after chemical modification, and can be categorized as biological-response modifiers (Kogan et al. 2008). Some water-insoluble and, to a lesser extent, water-soluble glucans were introduced as a new approach to decreasing hypercholesterolemia and preventing atherosclerosis development (Johnson et al. 2009; Vetvicka and Vetvickova 2009). Water-insoluble glucans are known as effective macrophage stimulators and also exhibit protective action as antioxidants (Kogan et al. 2008; Goodridge et al. 2009). However, the lipid-lowering effects of water-soluble β-glucans are still unknown, especially their effects on triglyceride (TG) concentrations in vivo.

Recently, using a novel small-angle X-ray scattering (SAXS) method for the determination of fractional and subfractional composition of lipoproteins (LPs), we showed that a high dose of atorvastatin exerts its rapid lipid-lowering effect in lipemia induced in mice with 500 mg Triton WR 1339 (kg body mass)$^{-1}$ (Korolenko et al. 2011). Therefore, we wondered what effects atorvastatin would induce in another well-established mouse model of dyslipidemia and atherosclerosis developed by Johnston et al. (Johnston and Palmer 1997; Johnston 2004). The model developed by Johnston et al. uses a synthetic copolymer called poloxamer 407 (P-407) to induce dyslipidemia. With continued treatment of P-407, mice begin to form fibrofatty lesions in their aortas, beginning 1 month after the continuous administration of P-407 (Johnston et al. 1998; Johnston 2004). After 4 months of P-407 treatment, aortic atherosclerotic lesions are produced in the same size and density as those observed in classic diet-induced mouse models. The P-407 model has the distinct advantage of not inducing hemolysis, compared with the acute hyperlipidemic model induced with the administration of Triton WR 1339. Therefore, P-407, unlike Triton WR 1339, may be administered on a continuous basis to mice to induce atheroma formation (Johnston et al. 2001; Johnston 2004).

The effect of a single high dose (20–80 mg·kg$^{-1}$) of atorvastatin is not similar to repeated statin administration (up to 1–5 mg·kg$^{-1}$), which has been previously utilized with rabbits (Nicholls et al. 2007). In clinical medicine, intensive statin therapy has been shown to have significant immediate effects on the serum lipid profile (decrease in serum total cholesterol and LDL-C), with an occasional paradoxical increase in serum TG (Sundström and Vasan 2006). The elevation in TG with acute high-dose statin treatment suggests a different outcome when compared with long-term treatment of high-dose statin in these same patients (Vondrakova et al. 2010). Statins have been suggested to prevent plaque rupture in atherosclerosis by acting through matrix metalloproteases (MMPs), especially MMP-9, but early effects of both statins and β-glucans in acute lipemia are poorly understood and require further investigation. Therefore, the aim of this study was to investigate the early effects of high-dose atorvastatin and water-soluble β-(1,3)-glucan on the serum lipid profile in acute dyslipidemia induced by P-407 in mice and to evaluate their effects on MMP activity.

Materials and methods

Male ICR mice (breeding station of the Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Russia) having a body mass of 30–35 g were used. Poloxamer 407 (P-407) (Pluronic F-127, Sigma) was administered to mice, as a single intraperitoneal injection (i.p.), at a dose of 500 mg·kg$^{-1}$, following the methods of Johnston (2004). The animals were decapitated 24 h after a single dose of P-407, when significant cholesterolemia and triglyceridemia were noted. Atorvastatin (KORKA, Novo Mesto, Slovenia) was administered twice by oral gavage at a dose of 75 mg·kg$^{-1}$ at 3 and 24 h before P-407 administration, according to the scheme described earlier in the model of lipemia induced by Triton WR 1339 (Korolenko et al. 2011). Control mice received atorvastatin in the same dose. Carboxymethylated β-(1,3)-d-glucan (CMG, Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia) was administered twice at oral gavage at a dose of 25 mg·kg$^{-1}$ i.p., 72 and 24 h before P-407 administration. As was shown earlier, CMG at this dose induced macrophage stimulation both in rats and mice (Dergunova et al. 2009). Control mice received CMG at the same dose and frequency of administration. A separate group of control mice received an equivalent volume of the solvent (saline). Mice were deprived of food but had free access to water 15 h before euthanasia. All experiments followed the Canadian Council on Animal Care guidelines for ethical treatment of animals. The animals used in the present investigation were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care as stipulated in the Guide to the Care and Use of Experimental Animals.
**Animals** (Canadian Council on Animal Care 1984, 1993), and the use of animals was reviewed and approved by the Ethical Committee recommendations on working with laboratory animals at the Institute of Physiology, Siberian Branch of the Russian Academy of Medical Sciences (SB RAMS).

Serum was obtained after centrifugation of blood samples at 3000g for 20 min at 4°C (Eppendorf centrifuge 5415R, Hamburg, Germany) and stored at −70°C until analysis of total cholesterol and TG associated with LPs: LP-C, and LP-TG, as well as their fractions and subfractions. In serum, the total cholesterol and TG concentrations were determined using Triglycerides-Novo and Novochol kits (Vector-Best, Koltsovo, Novosibirsk Region, Russia). Photometry of the samples was performed on a 5010 semiautomatic photometer (Robert Rieke, Germany) with a temperature-controlled flow-through cuvette. Serum HDL-C was assayed with the help of a commercial kit (HDL-Cholesterol-Novo; Vector-Best), and non-HDL-C was calculated by subtracting HDL-C from the total serum cholesterol.

**Small-angle X-ray scattering**

According to Otvos (2002), LP fractions were divided into 4 main classes: high-density LP (HDL), low-density LP (LDL), very-low-density LP (VLDL), and chylomicrons (chylomicrons were not determined by this method in the present study); or into 7 subfractions: HDL1, HDL2, LDL, intermediate-density LP (IDL), VLDL3-5, VLDL1-2, and chylomicrons. Interval borders of fractions and subfractions (according to the scale of sizes, \( r_0 \)) were the same as those indicated by Otvos (2002).

A novel method for determination of fractional and subfractional composition of LPs using SAXS was employed (Tuzikov et al. 2002). This method is inexpensive, quick, and capable of determining the relative content of different LP fractions, both as a size distribution of various LP particles and as absolute units of the total concentration of lipid in LP fractions.

SAXS roentgenograms were obtained using a Siemens diffractometer (Germany) by step-by-step scanning with the use of a goniometer and X-ray scintillation detector. Small-angle roentgenograms were measured in the following angular range: \( h = 0.013-0.22 \, \text{Å}^{-1}, \) where \( h = 4 \pi \sin(\theta)/\lambda; \theta \) is a part of scattering angle (2θ). A special thermostatted (20°C) quartz capillary cuvette (0.6 mm in diameter) having a wall thickness of 0.01 mm was used. The radiation wavelength (λ) was 1.54 Å. The small-angle X-ray roentgenograms were corrected, taking into account background scattering, adsorption, and collimation, after which the X-ray data were smoothed. The first step of mathematical processing of the SAXS data and computation checks of functions for size distribution of spherical particles were executed using a special computer program and algorithms described earlier, and also by use of optimization programs (Tuzikov et al. 2002).

The results are reported as the mean ± SD of at least 3 different experiments for each sample analyzed. The differences between samples were analyzed by the Student’s \( t \) test, and results with \( p \leq 0.05 \) were considered statistically significant.

**Matrix metalloprotease activity assay**

The activity of MMP in serum was determined by a fluorescent method (Knight et al. 1992) against 1.6 μmol·L\(^{-1}\) MCA–Pro–Leu–Gly–Leu–DpA–Ala–Arg–NH\(_2\) (substrate; American Peptide Co., Sunnyvale, California, USA), at pH 7.5, which is cleaved by many MMPs (Knäuper et al. 1996; Will et al. 1996). According to a recent proteome analysis (Lombard et al. 2005; Butler et al. 2010), this assay determines several types of MMPs (MMP-2, MMP-3, MMP-7, MMP-10, and MMP-11). To exclude the impact of serine proteases in the cleavage of this substrate, MMP activity was measured in the presence of an inhibitor of serine protease, PMSP (phenylmethylsulphonyl fluoride; Sigma), at a final concentration of 1 mmol·L\(^{-1}\). Fluorescence measurements were recorded on a Shimadzu RF-530101 (PC) S spectrofluorometer (Japan) at 325 nm (extinction) and 393 nm (emission). Methylcoumarylamide (MCA, Sigma) served as a standard. The results were expressed as micromoles of MCA cleaved per litre per hour.

**Morphological study of liver**

For morphometrical study of liver tissue, samples were first fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol·L\(^{-1}\) phosphate buffer, postfixed in 1% osmium tetroxide solution, and then embedded in an Epon–Araldite mixture. Semithin, 1 μm tissue sections were obtained using the Ultramicrotome LKB-8800-V (Bromma, Vallingby, Sweden), stained with toluidine blue, and examined under a STAR Zeiss light microscope (Germany). The number of macrophage cells was counted per 1 mm\(^2\) of tissue at a final magnification of 640×. Not less than 50 fields of view were studied for every series of experiments. The cell measurements were made with the Motic Images 2000 program. Ultrastructural changes of liver cells were investigated with an electron microscope (JEM 1400, Jeol, Japan). Quantitative data were then processed using the statistical program STATISTICA 4.0, and the reliability of distinctions judged by the Student’s \( t \) test.

**Statistical analyses**

All values were reported as the mean ± SD. The results were analyzed for statistically significant differences using 1-way analysis of variance (ANOVA). Individual differences between the concentrations were then evaluated using Dunnett’s test. The difference between values was considered statistically significant at \( p < 0.05 \). Analysis of the differences in the values between the groups (LP-C and LP-TG fractions and subfractions) was calculated using the nonparametric Kruskal–Wallis test, and statistically significant differences (\( p < 0.05 \)) were determined using the statistical program STATISTICA 6.0.

**Results**

A single dose of P-407 (500 mg·kg\(^{-1}\)) administered to mice induced severe hypercholesterolemia and especially hypertriglyceridemia (Fig. 1), as was shown earlier. The increased cholesterol concentration was due mainly to elevation of atherogenic non-HDL-C (Fig. 1). Administration of atorvastatin to mice with hyperlipidemia induced by P-407 (500 mg·kg\(^{-1}\)) was accompanied by a significant (\( p < 0.001 \)) decrease in the serum concentrations of total cholesterol and, particularly, TG (Fig. 1). Atorvastatin decreased non-HDL-C (\( p < 0.001 \)), returning to the level found in control mice.
The influence of atorvastatin on P-407-induced lipemia in mice. Atorvastatin was administered twice by oral gavage at a dose of 75 mg·(kg body mass)$^{-1}$ (24 and 3 h before P-407). P-407 was administered as a single dose (500 mg·kg$^{-1}$) by intraperitoneal injection. The animals were used in the experiment 24 h after the single P-407 administration. The data are shown as the mean ± SD. Control group, n = 20; atorvastatin-treated and P-407-treated groups, n ≥ 15 at each point. In each case, the bars from left to right represent the following groups:

- total cholesterol; HDL-cholesterol; non-HDL-cholesterol; and TG.
- V, p < 0.01 and *, p < 0.001 compared with the control group and atorvastatin-treated group; #, p < 0.01 compared with the P-407-treated group.

(Fig. 1). However, both total cholesterol as well as HDL-C and TG in mice treated with P-407 plus atorvastatin were still significantly greater than corresponding concentrations in control mice. Preliminary (before P-407) treatment with CMG, administered twice at a dose of 25 mg·kg$^{-1}$, also demonstrated a hypolipidemic effect (decrease in TG level, p < 0.01, with only a tendency to decrease the total cholesterol) (Fig. 2). In general, the hypolipidemic effect of CMG was less potent than that observed with atorvastatin (Fig. 2). The elevation of serum cholesterol in the CMG-treated hyperlipemia group was related mainly to the increase of atherogenic non-HDL-C, which had only a slight tendency to decrease (statistically nonsignificant relative to the corresponding mean value in the P-407-only treatment group) (Fig. 2). The total cholesterol, non-HDL-C, HDL-C, and TG concentrations in P-407 + CMG treated mice were still significantly greater than corresponding concentrations in control mice (Fig. 2). There was no significant change in total serum HDL-C concentration in both treatment models evaluated as compared with P-407-induced lipemic mice (Figs. 1 and 2).

P-407 administration induced significant hyperlipemia, sharply increasing both the total LP-C (Table 1) and total LP-TG (Table 2) concentrations in the serum of mice. However, the increase in LP-TG was more dramatic (more than 7 times) than the elevation in LP-C (about 4 times) relative to levels in control mice (Tables 1 and 2). In the P-407-treated group of mice, we observed an increase in the serum concentration of the atherogenic LDL-C (up to 18 times) and VLDL-C (more than 10 times) fractions; there was also a slight elevation in the serum concentrations of the anti-atherogenic HDL-C fraction (p < 0.05) (Table 1). In the LDL-C fraction, the most prominent increase observed was that for the IDL-C subfraction (about 70 times), and, to a lesser extent, an elevation of the LDL$_{1,3}$-C subfractions (Table 1). In the VLDL-C fraction, an increase in the both VLDL$_{3,5}$-C and VLDL$_{1,2}$-C subfractions was observed (Table 1). In the HDL-C fraction, a slight increase in the HDL$_{2}$-C subfraction (p < 0.05) was noted (Table 1).

Atorvastatin administration to intact (control) mice did not induce any change in total LP-C (Table 1), decreasing total LP-TG (p < 0.05) concentrations (Table 2). Atorvastatin decreased the VLDL-C level (VLDL$_{3,5}$-C subfraction, p < 0.05) (Table 1) and the VLDL-TG fraction (VLDL$_{3,5}$-TG subfraction, p < 0.05) (Table 2).

In experimental hyperlipemia induced with P-407 (500 mg·kg$^{-1}$), atorvastatin decreased the total LP-C concentration (p < 0.05), mainly owing to the LDL$_{1,3}$-C subfraction (Table 1), but not the total LP-TG concentration (Table 2). However, both total LP-C and LP-TG serum concentrations were significantly greater when compared with these same indices in control mice (Tables 1 and 2). Simultaneously, atorvastatin induced a slight decrease in the HDL-TG (p < 0.05) and HDL-C (p < 0.05) fraction, which is mainly found in the HDL$_{2}$-C subfraction (Table 1).

In general, the changes in the LP-TG fractions and subfractions (Table 2) were similar to the trends observed in the changes in the LP-C fractions and subfractions described above (Table 1). In the P-407-treated group of mice, we observed an increase in the serum concentrations of the atherogenic LDL-TG (up to 30-fold) and VLDL-TG (about 10-fold) and antiatherogenic total HDL-TG fractions (Table 2). Among LDL-TG, the most prominent increase was that seen in the IDL-TG subfraction (more than 100 times), and a less...
Fig. 2. The influence of carboxymethylated β-glucan (CMG) on P-407-induced lipemia in mice. P-407 was administered by intraperitoneal injection (i.p.) as a single dose (500 mg·kg body mass)−1. Carboxymethylated β-glucan (CMG) at 25 mg·kg−1 was administered by i.p. 72 and 24 h before P-407. The animals were used in the experiment 24 h after the single P-407 administration. The data are shown as the mean ± SD. Control group, n = 21; CMG- and P-407-treated groups, n ≥ 15 at each point. In each case, the bars from left to right represent the following groups: total cholesterol; HDL-cholesterol; non-HDL-cholesterol; and TG. V, p < 0.05 and *, p < 0.01 compared with the control group; #, p < 0.05 compared with the P-407-treated group.

Table 1. Influence of atorvastatin on serum lipoprotein-cholesterol fractions and subfractions of mice with P-407-induced lipemia (500 mg·kg body mass)−1 (mean ± SD).

<table>
<thead>
<tr>
<th>Lipoproteins (LP)</th>
<th>Concentration (mmol·L−1)</th>
<th>1. Control (n = 7)</th>
<th>2. Atorvastatin (n = 8)</th>
<th>3. P-407 (n = 8)</th>
<th>4. Atorvastatin + P-407 (n = 7)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL3-C</td>
<td>1.31±0.44</td>
<td>1.10±0.18</td>
<td>1.52±0.70</td>
<td>1.35±0.364</td>
<td>p1&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>HDL2-C</td>
<td>0.61±0.31</td>
<td>0.69±0.18</td>
<td>1.59±0.672</td>
<td>1.13±0.234</td>
<td>p1&lt;0.05, p1,4&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.90±0.29</td>
<td>1.78±0.17</td>
<td>3.10±0.588</td>
<td>2.48±0.078</td>
<td>p1&lt;0.05, p1,3&lt;0.05, p1,4&lt;0.05, p3&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>LDL3-3-C</td>
<td>0.39±0.27</td>
<td>0.43±0.392</td>
<td>7.07±2.044</td>
<td>5.59±2.52</td>
<td>p3&lt;0.05, p1,4&lt;0.05, p3&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.01±0.0026</td>
<td>0.01±0.0028</td>
<td>0.74±0.56</td>
<td>0.88±0.468</td>
<td>p1&lt;0.05, p1&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.40±0.27</td>
<td>0.44±0.392</td>
<td>7.81±2.27</td>
<td>6.47±2.94</td>
<td>p1&lt;0.05, p1,3&lt;0.05, p3&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>VLDL3-5-C</td>
<td>0.06±0.026</td>
<td>0.02±0.014</td>
<td>0.39±0.196</td>
<td>0.28±0.18</td>
<td>p1&lt;0.05, p1,3&lt;0.05, p1,4&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>VLDL1-2-C</td>
<td>0.01±0.0052</td>
<td>0.01±0.011</td>
<td>0.27±0.14</td>
<td>0.21±0.078</td>
<td>p1&lt;0.05, p1,4&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.07±0.026</td>
<td>0.03±0.028</td>
<td>0.66±0.224</td>
<td>0.49±0.234</td>
<td>p1&lt;0.05, p1,4&lt;0.05, p3&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>LP-C (all)</td>
<td>2.39±0.442</td>
<td>2.24±0.448</td>
<td>11.57±2.91</td>
<td>9.45±3.35</td>
<td>p1&lt;0.05, p1,3&lt;0.05, p3&lt;0.05</td>
<td></td>
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</tbody>
</table>

Note: HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; LP-C, total lipoprotein cholesterol.

prominent elevation (about 20 times) was observed in the LDL1-3-TG subfraction (Table 2). There was an increase in the VLDL-TG fraction (VLDL1-2 ·TG subfraction, about 20-fold greater) and, to a lesser degree, the VLDL3-5-TG subfraction (Table 2). In the elevated LDL-TG fraction, we observed a slight increase in both the HDL3-TG and HDL2-TG subfractions (p < 0.05) (Table 2).

Atorvastatin treatment of lipemia had only a slight tendency (statistically nonsignificant) to lower the total atherogenic LDL-TG concentration (Table 2). Additionally, atorvastatin induced a decrease in the atherogenic LDL1-3-TG subfraction and HDL-TG fraction (p < 0.05) (Table 2).

Administration of atorvastatin to mice with hyperlipidemia (P-407, 500 mg·kg−1) was followed by an increase in the cleavage of the general MMP peptide substrate, suggesting an increase in the serum MMP activity (Fig. 3). Serum of mice made hyperlipemic with P-407 showed no increase in MMP activity relative to controls. The increase in MMP activity seen in mice treated with atorvastatin only, was greater than the increase in MMP activity in hyperlipemic mice treated with atorvastatin (Fig. 3). CMG had no effect on serum MMP activity, and in combination with P-407 induced a slight increase in serum MMP activity (p < 0.05) (Fig. 4).

In the P-407-induced hyperlipemia group, the following positive correlations were shown: between serum MMP activity and total serum cholesterol (r = 0.84) and between...
MMP activity and total serum TG ($r = 0.69$) concentrations. In control mice treated with atorvastatin, a negative correlation between serum MMP activity and HDL-C concentration ($r = 0.88$) was revealed; and in intact (control) mice, a positive correlation was observed between LDL-C and total serum TG ($r = 0.91$).

**Morphological study of liver cells**

Electron microscopic studies of liver in P-407-treated mice (500 mg·kg$^{-1}$) were similar relative to previously reported electron microscope results using a P-407 dose of 1000 mg·kg$^{-1}$ (Korolenko et al. 2010). With the present dose of 500 mg·kg$^{-1}$ of P-407, liver sinusoids were considerably extended 24 h after P-407 administration, and the numeric density of macrophages was significantly increased. The heterogeneity of liver macrophages was increased; dramatically enlarged macrophages were filled with granular material, giving their cytoplasm a foamy appearance (Fig. 5). The cytoplasm of hepatocytes was loose and mostly crumby, with several large vacuoles containing electron light material.

CMG administration to mice was followed by significant macrophage activation. The morphometric analysis demonstrated an increase in the number of liver macrophages (Fig. 6A) and the number of secondary lysosomes (and a reduction in the number of primary lysosomes) (Figs. 6B, and 6C). As compared with the control (Fig. 7A), the structure of liver macrophages revealed a high functional activity, specifically an increase in the size of the cells, many of which have invaginations formed from cytoplasmic membranes, an increased number of different-sized granules inside the cytoplasm, and developed endoplasmic reticulum and Golgi complex (Fig. 7B).

**Discussion**

We have successfully demonstrated that a high-dose of atorvastatin administered to hyperlipidemic mice significantly decreased non-HDL-C and therefore also decreased the total cholesterol concentrations. Serum levels of TG were also reduced. These changes in serum lipids were rapid, with levels of non-HDL-C returning to those observed in control animals 24 h following administration of atorvastatin. Similarly, we reported even more significant lipid-lowering effects with atorvastatin in mice that were made hyperlipidemic following administration of Triton WR 1339 (Korolenko et al. 2011). P-407, a nonionic detergent that is used in pharmaceutical formulations, causes significant hypercholesterolemia and hypertriglyceridemia in a dose-dependent manner when administered to rats or mice of either sex (Johnston 2004). The acute hyperlipidaemia induced by P-407 is an appropriate model to study the effects of lipid-lowering drugs. This model has been reported to effectively rank-order the therapeutic efficacy (potency) of currently marketed statin drugs in the United States (Johnston et al. 2001). The mechanism of action of P-407 includes the inhibition of lipoprotein lipase and endothelial lipase (Johnston 2004; Qiu and Hill 2007).

The findings from electron microscopy obtained in our work in the P-407-induced mouse model of lipemia were recently confirmed by others. It was shown that P-407 is taken up by endothelial and Kupffer cells in the liver, primarily through endocytosis. Additionally, the incorporation of P-407 causes loss of fenestrations in liver sinusoidal endothelial cells, which contributes to the pathogenesis of this experimental hyperlipidemia (Warren et al. 2011). Statin (atorvastatin) treatment significantly attenuated the lipid accumulation in isolated macrophages treated with oxidized LDL. Moreover, atorvastatin was suggested to reduce lipoprotein lipase and endothelial lipase expression by reducing the activation of LXRs and NF-$k$B, respectively (Qiu and Hill 2007).

In the present investigation, we attempted to establish the common features associated with 2 nonionic detergents, namely P-407 and Triton WR 1339, in mediating lipemia in mice. Previously, Millar et al. (2005) reported a 2-fold greater increase in serum triglycerides when mice were administered P-407 (1000 mg·kg$^{-1}$) compared with levels in mice treated with Triton WR 1339 (500 mg·kg$^{-1}$). However, if one compares the same doses of both detergents, namely 500 mg·kg$^{-1}$, a similar increase in the total serum cholesterol is observed, although serum TG levels are significantly greater in mice treated with Triton WR 1339 (Korolenko et al. 2011). Both nonionic detergents at the same dose of 500 mg·kg$^{-1}$ resulted in a similar (~5-fold) increase in the total LP-C. However, VLDL-C was about 2 times higher in mice treated with Triton WR 1339, and the LDL-C fraction

### Table 2. Influence of atorvastatin on serum lipoprotein-triglyceride fractions and subfractions of mice with P-407-induced lipemia (500 mg·kg$^{-1}$) (mean ± SD).

<table>
<thead>
<tr>
<th>Lipoproteins (LP)</th>
<th>Concentration (mmol·L$^{-1}$)</th>
<th>1. Control ($n = 7$)</th>
<th>2. Atorvastatin ($n = 8$)</th>
<th>3. P-407 ($n = 8$)</th>
<th>4. Atorvastatin + P-407 ($n = 7$)</th>
<th>$p$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL$\text{-}1$-TG</td>
<td>0.20 ± 0.078</td>
<td>0.17 ± 0.042</td>
<td>0.25 ± 0.117</td>
<td>0.22 ± 0.062</td>
<td>$p_{1-2} &lt; 0.05$, $p_{1-3} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>HDL$\text{-}2$-TG</td>
<td>0.10 ± 0.044</td>
<td>0.11 ± 0.036</td>
<td>0.26 ± 0.112</td>
<td>0.19 ± 0.036</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>HDL$\text{-}3$-TG</td>
<td>0.03 ± 0.047</td>
<td>0.28 ± 0.025</td>
<td>0.51 ± 0.112</td>
<td>0.41 ± 0.052</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$, $p_{3-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>LDL$\text{-}1$-TG</td>
<td>0.05 ± 0.031</td>
<td>0.05 ± 0.034</td>
<td>0.99 ± 0.23</td>
<td>0.80 ± 0.35</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$, $p_{3-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>IDL-TG</td>
<td>0.003 ± 0.0026</td>
<td>0.003 ± 0.0008</td>
<td>0.43 ± 0.336</td>
<td>0.51 ± 0.28</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>LDL-TG</td>
<td>0.05 ± 0.034</td>
<td>0.06 ± 0.047</td>
<td>1.43 ± 0.425</td>
<td>1.32 ± 0.603</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>VLDL$\text{-}1$-TG</td>
<td>0.11 ± 0.055</td>
<td>0.03 ± 0.0028</td>
<td>0.62 ± 0.03</td>
<td>0.48 ± 0.29</td>
<td>$p_{1-2} &lt; 0.05$, $p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>VLDL$\text{-}2$-TG</td>
<td>0.04 ± 0.018</td>
<td>0.03 ± 0.03</td>
<td>0.96 ± 0.43</td>
<td>0.73 ± 0.29</td>
<td>$p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>VLDL$\text{-}3$-TG</td>
<td>0.14 ± 0.059</td>
<td>0.06 ± 0.042</td>
<td>1.58 ± 0.30</td>
<td>1.19 ± 0.47</td>
<td>$p_{1-2} &lt; 0.05$, $p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
<tr>
<td>LP-TG (all)</td>
<td>0.49 ± 0.112</td>
<td>0.39 ± 0.064</td>
<td>3.52 ± 0.84</td>
<td>2.92 ± 1.06</td>
<td>$p_{1-2} &lt; 0.05$, $p_{1-3} &lt; 0.05$, $p_{1-4} &lt; 0.05$</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** HDL-TG, high-density lipoprotein triglyceride; IDL-TG, intermediate-density lipoprotein triglyceride; LDL-TG, low-density lipoprotein triglyceride; VLDL-TG, very-low-density lipoprotein triglyceride; LP-TG, total lipoprotein triglyceride.
was approximately 2 times greater in P-407-treated mice when each lipoprotein fraction was compared with the corresponding fraction in the controls.

In the present study, it is suggested that the difference between the 2 mouse models of lipemia are related not to the total serum cholesterol and TG levels, but primarily to the composition of the atherogenic LP fractions, specifically LDL-C and VLDL-C. Our data would seem to suggest that P-407-induced acute lipemia is more "pro-atherogenic", because it included elevation of LDL-C (recognized as the most important atherogenic lipoprotein), TG (independent risk factor in atherosclerosis), and VLDL-C (the most readily available measure of atherogenic remnant lipoproteins). Moreover, this mouse model of lipemia was treated by atorvastatin less effectively when compared with the Triton WR-1339 model. The lipid-lowering effect of atorvastatin was more significant in the model of lipemia induced by Triton WR-1339 when compared with the P-407-induced model and was related to the decrease in both the atherogenic fractions LDL-C and VLDL-C (and their subfractions). Recently, Burkhardt et al. demonstrated a role for Trib1 as a regulator of lipoprotein metabolism in mice (Burkhardt et al. 2010). Hepatic-specific overexpression of Trib1 reduced levels of plasma TG and cholesterol by reducing VLDL production; conversely, Trib1-knockout mice showed elevated levels of plasma TG and cholesterol owing to increased VLDL production.

Compared with atorvastatin, CMG did not elicit as potent a hypolipidemic effect in P-407-induced lipemia. Administration of CMG primarily caused a decrease in total serum TG. It was suggested that the positive TG-lowering effect of CMG was related to macrophage stimulation. Based on electron microscopic evidence, we found that CMG stimulated liver macrophages, increasing both their number and the number of secondary lysosomes. Previously, Dergunova et al. (2009) reported that CMG increased the secretion of TNF-α. The combined use of CMG with statins to modulate serum lipids has been suggested. However, according to a recent report by Eussen et al. (2011) it was shown that simultaneous intake of oat bran (enriched with β-glucan) and atorvastatin reduces the efficacy of atorvastatin to lower lipid levels and prevent atherosclerosis in LDLCR−/− mice. Owing to the increased significance of TG as an independent risk factor in atherosclerosis development (Harchaoui et al. 2009), β-glucans (especially water soluble) of different chemical structure may potentially hold promise as new agents with which to lower serum TG. In fact, it was previously reported that some of the β-glucans may also prove useful at reducing total serum cholesterol (Kogan et al. 2008; Vetvicka and Vetvickova 2009).

The effects from orally administered β-glucans hypocholesterolemic were related a reduction of the intestinal absorption of cholesterol by binding to β-glucans, and simultaneously, the oral statin effect in the combined treatment was
reduced. Consumption of a diet containing water-insoluble yeast-derived β-glucan was shown to stimulate macrophages and to lower plasma cholesterol and TG levels in a dose-dependent manner (Vetvicka and Vetvickova 2009); the biological effects of water-soluble β-glucans were not studied in sufficient detail. According to results obtained in the present work, water-soluble β-glucan parenteral administration demonstrated a TG-lowering effect and a less pronounced cholesterol-lowering effect. These data support the hypothesis about the involvement of macrophages in cholesterol metabolism. There is renewed interest in the potential usefulness of water-soluble β-glucans, not only as a treatment, but also as a prophylactic strategy in atherosclerosis.

It was suggested that synthesis of fat from carbohydrate may contribute to the decrease of plasma TG concentration after use of dietary fibers (containing β-glucan), hepatic de novo lipogenesis (Battilana et al. 2001; Rondanelli et al. 2009). β-Glucans have been shown to decrease the total plasma cholesterol and to inhibit lipogenic enzymes involved in cholesterol synthesis in liver cells (Battilana et al. 2001). Altered levels of circulating extracellular matrix markers, especially MMP-9, have frequently been observed in relation to manifestations of atherosclerotic disease and its risk factors (Sundström and Vasan 2006). In the present study, a correlation was shown between serum MMP activity and increased total cholesterol and TG concentrations in P-407-induced lipemia. This would seem to suggest the involvement of lipid-laden macrophages in secreting MMPs into the extracellular matrix and serum.

In general, in patients with atherosclerosis, circulating serum MMP activity was shown to decrease during statin therapy (Derosa et al. 2009), leading to a decreased rate of cardiovascular complications and cardiac events. Presently, intensive statin (atorvastatin) therapy has been recommended in patients with acute coronary syndrome, which effectively normalizes lipid parameters (Vondrakova et al. 2010). However, according to experimental results obtained in circulating serum. MMP activity increased in mice treated with a single high dose of atorvastatin, which in general had a positive effect in severe hypercholesterolemia and hypertriglyceridemia, but simultaneously increased serum MMP activity. The high-dose atorvastatin treatment exerted a rapid lipid-lowering effect in the early stages of acute lipemia, as well as a pleiotropic effect, i.e., increasing MMP activity (a potential negative side effect of atorvastatin). Similar results were obtained in another mouse model of acute lipemia induced by Triton WR 1339 (Korolenko et al. 2011). The high-dose atorvastatin treatment was shown to increase MMP activity in control mice, and mice with P-407-induced lipemia. This result was also observed in mice treated with the same dose of atorvastatin, in which hyperlipidemia had been achieved by administration of Triton WR 1339 (Korolenko et al. 2011). Therefore, it would appear
that increased serum MMP activity is associated with high-dose atorvastatin therapy. In patients with cardiovascular diseases, only C-reactive protein levels are significantly modulated by statins (Sukhova et al. 2002; Chow et al. 2007). These findings suggest that statin-mediated anti-inflammatory effects may contribute to the ability of statins to reduce the risk of cardiovascular disease.

In conclusion, we have shown that atorvastatin effectively lowered non-HDL-C in P-407-treated mice. This is significant because of the atherogenic potential of lipoprotein fractions comprising non-HDL-C (VLDL, LDL, and IDL). CMG, while still able to mediate a reduction in non-HDL-C, was less potent than atorvastatin. Atorvastatin was shown to increase serum MMP concentrations, while P-407 did not affect serum MMP levels when administered to control mice. Serum MMP levels were also significantly increased when P-407-treated hyperlipidemic mice were administered atorvastatin. Based on our electron microscopic studies of hepatic tissue, it would seem to suggest that lipid-laden macrophages potentially secrete pro-inflammatory cytokines and MMPs into both the extracellular matrix and the serum. According to the experimental data we obtained, the use of a lower dose (20 compared with 80 mg) of atorvastatin in patients with acute coronary syndrome (so as to induce a decrease of hs-CRP) can possibly offer an approach for early treatment or prevention of atherosclerosis. Future studies will involve identifying putative mechanisms associated with atorvastatin-induced elevation in serum MMPs.

**Acknowledgements**

Authors are grateful to Prof. C. Overall (University of British Columbia, Vancouver, Canada) for discussion of results on the MMP assays; engineer M. Tuzikov for help in small-angle X-ray scattering; Dr. I. Bruck and Dr. Y. Kisarova for help in providing the statistical analysis; and G.L. Brehneva for preparing the illustrations in the manuscript. This work was partially supported by an integrated grant of the Siberian Branch of the Russian Academy of Medical Sciences on natural immunomodulator study (2008–2009).

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**Fig. 5.** Influence of P-407 on the ultrastructure of liver cells. Electron micrograph of liver cells. In sinusoid lumen a large liver macrophage with foamy cytoplasm, filled by electron light granules. Magnification 20,000×. Mph, macrophage; Gr, granules; H, hepatocyte. P-407 was administered by intraperitoneal injection as a single dose of 500 mg·(kg body mass)−1. The study was performed 24 h after a single dose of P-407.
Fig. 6. Influence of carboxymethylated β-glucan (CMG) on morphometric data of liver macrophages. (A) Number of macrophages per 1 mm²; (B) number of primary lysosomes per 10 µm²; (C) number of secondary lysosomes per 10 µm². Increased number of liver macrophages (A) and increased number of their secondary lysosomes (C) are morphological signs of macrophage stimulation. CMG was administered twice to mice at a dose of 25 mg·(kg body mass)⁻¹, before the study was performed. The number of animals in each group was 7. *, p < 0.001 compared with the control group.
Fig. 7. Influence of carboxymethylated β-glucan (CMG) on ultrastructure of liver cells. Electronograms of liver macrophages. (A) Control mice; (B) CMG-treated mice. Electron micrograph of macrophage in sinusoid lumen containing granules of different size and density. The surface of macrophage with numerous invaginations (arrows, bold). Magnification 20 000×. Mph, macrophage; Gr, granules; H, hepatocyte. CMG was administered twice to mice at a dose of 25 mg·(kg body mass)\(^{-1}\), 72 and 24 h before the study was performed.


