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Research Paper

Influence of atorvastatin on fractional and subfractional composition of serum lipoproteins and MMP activity in mice with Triton WR 1339-induced lipaemia

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Abstract

Objectives The effects of atorvastatin on the atherogenic and anti-atherogenic lipoprotein-cholesterol (C-LP) and lipoprotein-triglyceride (TG-LP) fractions and subfractions at the early stage of murine acute hyperlipidaemia, and its pleiotropic anti-inflammatory effects via the activity of matrix metalloproteinases (MMPs) were studied.

Methods Atorvastatin (75 mg/kg) was administered to ICR mice with acute lipaemia induced by a single injection of Triton WR 1339 (500 mg/kg). A novel small-angle X-ray scattering (SAXS) method was used for the determination of the fractional and subfractional composition of C-LP and TG-LP.

Key finding In Triton WR 1339-treated mice, there was a drastic increase in the atherogenic low-density C-LP (C-LDL) fraction, intermediate density lipoprotein-cholesterol (C-IDL) subfraction, and very low-density C-LP (C-VLDL) fractions ($C\text{-}VLDL_{3\text{–}5}$ subfraction). Additionally, there was an increase in the $C\text{-}HDL_3$ subfraction. Treatment of lipaemia with atorvastatin resulted in the normalization of the atherogenic C-LDL fraction and the C-IDL subfraction. A decrease in C-VLDL ($C\text{-}VLDL_{3\text{–}5}$ subfraction), total cholesterol and, especially, triglyceride (TG) concentrations was also demonstrated. Similar results were obtained with the TG-LP fractions and subfractions. Additionally, atorvastatin treatment resulted in an increase in the serum and liver MMP activity.

Conclusion High-dose atorvastatin therapy exerts its rapid lipid-lowering and pleiotropic effect(s) in the early stages of acute lipaemia induced with Triton WR-1339.

Keywords atorvastatin; lipaemia; lipoprotein fractions and subfractions; matrix metalloproteinases

Introduction

The mechanism for the pleiotropic biological effects of statins is still being investigated. Statins are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, and are widely used for lowering plasma low-density-lipoprotein cholesterol (C-LDL) levels. As such, they decrease the complications of cardiovascular disease.[1–3] The primary effects of statins are related to blocking cholesterol biosynthesis and reducing C-LDL and intermediate-density-lipoprotein cholesterol (C-IDL) levels by up-regulation of LDL receptors in the liver.[4–7] Statins are somewhat effective in the treatment of hypertriglyceridaemia.[8–11] In this context, it is important to investigate more precisely the changes of atherogenic and anti-atherogenic fractions and subfractions of lipoproteins (LP) enriched by cholesterol (C) or triglycerides (TG) involved in hyperlipidaemia.

It is known that atorvastatin, and other statins, exert anti-inflammatory and immunomodulating activity, improve endothelial function, decrease vascular inflammation and oxidative stress, and inhibit expression of the MHC class proteins.[3,10] The impact of statins on each of these processes is not fully understood in humans with cardiovascular disease. However, it has been shown that statins decrease both serum C-reactive protein (CRP) and

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the levels of inflammatory biomarkers (tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, soluble intracellular adhesion molecule (sICAM)) and restore impaired balance of matrix metalloproteinase (MMP)/tissue inhibitor of metalloproteinase (TIMP).[12,13] In the liver, statins have been shown to block activated hepatic stellate cells (HSC) and induce their apoptosis *in vivo*.[14] Long-term statin use is not without some adverse side effects, such as liver damage (atorvastatin) and raised risk of haemorrhage.[1,3] Statins have also been suggested to prevent plaque rupture in atherosclerosis by acting through MMPs, especially MMP-9,[14–17] but the early effects of statins on different types of MMPs in acute lipaemia is poorly understood and requires further investigation. Recently, we reported the use of a novel small-angle X-ray scattering (SAXS) method for the determination of the fractional and subfractional composition of lipoproteins (LPs). Using a well-documented mouse model of chemically-induced (poloxamer 407) atherogenic dyslipidaemia, we recently reported the changes observed in serum lipoprotein fractions and subfractions using SAXS.[18] We suggest that our SAXS method could also be used in other chemically-induced mouse models of acute lipaemia (e.g. the Triton WR-1339-induced mouse model of hyperlipidaemia). It was concluded from our previous work with the poloxamer 407-induced mouse model of hyperlipidaemia that the use of SAXS may be extremely beneficial to more completely quantify the changes observed in serum lipoprotein fractions and subfractions of cholesterol and triglyceride.[18] Therefore, it appears that SAXS would be very useful to evaluate the role of hypolipidaemic drugs in mouse models of acute lipaemia.

The mechanism of the protective effects of statins in the early stages of acute lipaemia is still not well understood. We suggest that acute lipaemia induced in mice by either Triton WR-1339, or poloxamer 407 (two well-accepted hyperlipidaemic agents), is much more effective in increasing atherogenic LP, as compared with atherogenic dyslipidaemia observed in humans. Thus, our novel SAXS method may serve as a convenient tool to assess the effects of a typical statin drug (atorvastatin), or other hypolipidaemic drugs, in modulating the serum concentrations of specific LP fractions and subfractions in these models of acute hyperlipidaemia.

Therefore, the aim of this study was to evaluate the effects of atorvastatin on the atherogenic and anti-atherogenic C-LP and TG-LP fractions and subfractions in the initial stage of murine hyperlipidaemia, with special attention to atorvastatin's pleiotropic anti-inflammatory effects via the biological activity of MMPs.

Materials and Methods

Induction of lipaemia and atorvastatin administration

Male ICR mice (Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia), 25–30 g, were used. All animal procedures were carried out in accordance with approved protocol and recommendations for proper use and care of laboratory animals (European Communities Council Directive 86/609/CEE). Triton WR 1339 (Triton) (Ruger Chemical Co., Irvington, NJ, USA) was administered to mice as a single, intraperitoneal injection at a dose of 500 mg/kg[19] (the number of intact mice – 17, Triton WR 1339-treated, 15, atorvastatin-treated, 15, Atorvastatin + Triton WR 1339–15). To induce severe hyperlipidaemia; a separate group of mice were injected intraperitoneally with a single dose (850 mg/kg) of Triton,[15,16] (9 mice per group). Atorvastatin (KRKA, Novo Mesto, Slovenia) was administered twice by oral gavage at a dose of 75 mg/kg; specifically, at 3 and 24 h before Triton administration. Previous studies showed that atorvastatin, used at this dose and dosing frequency, significantly decreased serum concentrations of cholesterol and TG (i.e. it produced a hypolipidaemic effect) when Triton was used at a dose of 500 mg/kg.[20] The hypolipidaemic effect was not observed when atorvastatin was administered at other doses, or according to other dosage regimens, nor in severe lipaemia when using a dose of 850 mg/kg of Triton. The mice were euthanized by decapitation 24 h after Triton administration. Intact (control) mice received atorvastatin at the same dose, while a separate group of intact mice received an equivalent volume of the solvent (saline). The mice were deprived of food, but had free access to water for 15 h before euthanasia.

Analysis of lipoprotein fractions and subfractions using small-angle X-ray scattering

Serum was obtained after centrifugation of blood samples at 3000*g* for 15 min at 4°C (Eppendorf Centrifuge 5415R; Eppendorf, Hamburg, Germany) and stored at −70°C until analysis of the total cholesterol and TG of LP; namely, C-LP and TG-LP, respectively, and their fractions and subfractions. In serum, the total cholesterol and TG concentrations were determined using Triglycerides-Novo and Novochol kits (Vector-Best, Novosibirsk Region, Russia). Photometry of the samples was performed on a 5010 semiautomatic photometer (Robert Riele, Berlin, Germany) with a temperature-controlled flow-through cuvette.

According to Otvos,[21] LP fractions were divided into the following four main classes: high-density LP (HDL), low-density LP (LDL), very-low-density LP (VLDL) and chylomicrons (chylomicrons were not determined by SAXS) or seven subfractions: HDL_3, HDL_2, $LDL_{1–3}$, IDL, $VLDL_{3–5}$, $VLDL_{1–2}$ and chylomicrons. Interval borders of the fractions and subfractions were the same as described and utilized by Otvos.[21] Determination of the fractional composition of LPs was accomplished using the SAXS approach (Siemens diffractometer, Siemens, München, Germany).[18,22] This method is inexpensive, quick, does not require special preparation of the serum before the assay and is capable of determining the relative content of different LP fractions according to both their size distribution and absolute units of total lipid concentration. The first step in processing of the SAXS data determines the mathematical function associated with the size distribution of spherical particles using special optimization computer programs and algorithms described earlier.[22]

Matrix metalloproteinase activity assay

Homogenates of liver were prepared in 0.25 M sucrose (pH 7.2) at 4°C, as was previously described,[23] and were kept at −70°C before the determination of enzyme (MMP)

activity. The activity of MMP was measured against the fluorogenic substrate MCA-Pro-Leu-Gly~Leu-DpA-Ala-Arg-NH_2 (American Peptide Co., Sunnyvale, CA, USA), 1.6 μM, at pH 7.5, using a fluorescent method described by Knight *et al*.[24] According to the manufacturer's product information the use of this peptide substrate with a fluorescence quencher allowed us to evaluate the activity of MMP-2 (gelatinase A) and MMP-7 (matrilysin 1).[24] Fluorescence quencher Dpa (*N*-3-(2,4-dinitrophenyl)-L-α,β-diaminopropyonyl) is responsible for the 'stability' of the substrate molecule and, after cleavage of the Gly~Leu bond by MMPs, increased fluorescence was determined as a measure of MMP activity. 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, phenylmethylsulphonylfluoride (PMSF; Boehringer Mannheim GmbH, Mannheim, Germany), at a final concentration of 0.5 mM. The reaction was stopped by addition of 50 μM EDTA, an inhibitor of MMPs. Fluorescence measurements were recorded on a Shimadzu RF-5301 (PC) S spectrofluorometer (Japan) at 325 nm (excitation) and 393 nm (emission). Methylcoumarylamide (MCA; Sigma) served as a standard. The results were expressed as μmol of MCA cleaved per litre per hour for serum, and μmol MCA cleaved per gramme of protein per hour for liver homogenates. Protein concentration was measured as was described earlier.[16,23]

Statistical analysis

The results were analysed for statistically significant differences using one-way analysis of variance. Individual differences between the concentrations were then evaluated using Dunnett's test. All values were reported as the mean ± the standard deviation (SD). The difference between values was considered statistically significant at $P < 0.05$. The Spearman correlation coefficient was also calculated.

Results

A single dose of Triton (500 or 850 mg/kg) administered to mice induced significant hypercholesterolaemia and, especially, hypertriglyceridaemia, in a dose-dependent manner (Figure 1). Administration of atorvastatin to mice with moderate hyperlipidaemia induced by administration of 500 mg/kg of Triton was accompanied by a decrease in the serum concentrations of total cholesterol and, particularly, TG (see Figure 1). Atorvastatin administered at the same dose and dosing frequency had no hypolipidaemic effect in mice with more severe hyperlipidaemia (Triton, 850 mg/kg). In fact, with the higher dose of Triton, the serum concentrations of total cholesterol and TG following atorvastatin treatment were similar relative to the data obtained in mice receiving high-dose Triton, but no atorvastatin (Figure 1).

Triton administration (500 mg/kg) induced significant lipaemia, sharply increasing both the total C-LP (see Table 1) and total TG-LP (see Table 2) concentrations in the serum of mice. However, the increase in TG-LP was much more dramatic (more than 10 times) than the elevation in C-LP (about 5 times), relative to control mice (Tables 1 and 2).

Atorvastatin administration to intact (control) mice did not induce any change in the total C-LP and TG-LP

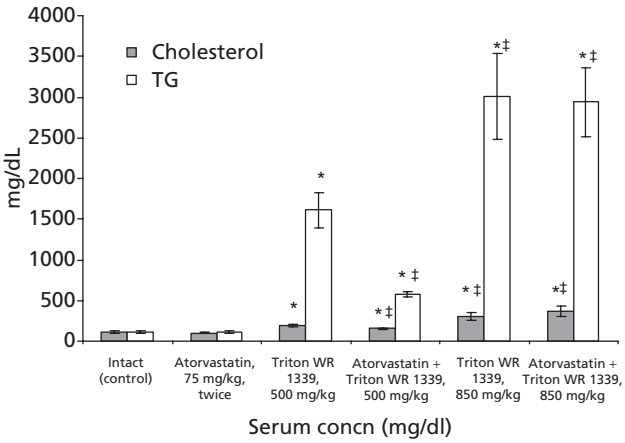

Figure 1 The effect of atorvastatin administration on serum total cholesterol and triglyceride (TG) concentrations during Triton WR 1339-induced lipaemia in mice. Data are presented as mean ± SD, $n = 17$ for control and 15 for experimental group. $*P < 0.001$ versus control. $‡P < 0.001$ versus Triton WR 1339, 500 mg/kg.

concentrations but with mild lipaemia (500 mg/kg), it decreased the total serum C-LP and TG-LP concentration (Tables 1 and 2). However, both serum TG-LP and C-LP concentrations were significantly greater when compared with these same indices in control mice.

In the Triton-treated group of mice (500 mg/kg), we observed an increase in the serum concentration of the atherogenic C-LDL (up to 8 times) and C-VLDL (up to 25 times) fractions, but there was no change in the serum concentrations of the anti-atherogenic C-HDL fractions (Table 1). Among C-LDL, the most prominent increase was observed for the C-IDL subfraction (about 19 times) and, to a lesser extent, a slight elevation (statistically non-significant) of the $C\text{-}LDL_{1–3}$ subfractions (Table 1). An increase in the $C\text{-}VLDL_{3–5}$ subfractions (up to 26 times), and a minor elevation of the $C\text{-}VLDL_{1–2}$ subfractions, was also shown. Among C-HDL, an increase in $C\text{-}HDL_3$ subfraction was noted (Table 1).

Except for a significant reduction in the concentration of atherogenic C-LDL and C-IDL to a value observed in controls (Table 1), treatment of lipaemia with atorvastatin did not reduce the other C-LP fractions to the control values. However, atorvastatin did induce a decrease in the atherogenic C-VLDL fraction, primarily due to a decrease in the $C\text{-}VLDL_{3–5}$ and in less degree $C\text{-}VLDL_{1–2}$ subfractions (Table 1). In general, the changes in the TG-LP fractions and subfractions (Table 2) were similar to the trends observed with the changes in the C-LP fractions and subfractions described above (Table 1). Atorvastatin treatment of lipaemia significantly lowered atherogenic TG-LDL and TG-IDL concentrations to normal values (Table 2), and reduced the total TG-LP fraction, but not to normal baseline values determined for control mice.

In this study, a positive correlation was found between the total C-VLDL and total TG-VLDL concentrations ($r = 0.943$, $P < 0.05$) and the total C-LDL and TG-LDL ($r = 0.942$, $P < 0.05$). The concentration of the total TG-HDL fraction was not changed, but the concentration of the $TG\text{-}HDL_2$ subfraction was slightly decreased (Table 2).

Table 1 The effect of atorvastatin on the concentration of C-LP fractions and subfractions (mg/dl) in murine serum during Triton WR 1339-induced lipaemia

LP fractions, subfractions	One-way analysis of variance			Control, intact	Atorvastatin, 75 mg/kg, twice	Triton WR 1339	Atorvastatin + Triton WR 1339
	df	*F*	*P*	1	2	3	4
C-LP (All)	3.26	40.59	<0.001	83.0 ± 19.51	103.8 ± 33.96	430.6 ± 125.22***	237.0 ± 54.12*** $P_{3-4} < 0.001$
C-VLDL	3.25	52.8	<0.001	11.9 ± 5.31	4.8 ± 2.25	299.4 ± 100.37***	169.4 ± 46.97*** $P_{3-4} < 0.01$
C-$VLDL_{1-2}$	3.28	5.94	0.003	0.54 ± 0.37	0.4 ± 0.18	3.5 ± 3.15**	1.4 ± 0.712 $P_{3-4} < 0.05$
C-$VLDL_{3-5}$	3.25	52.064	<0.001	11.3 ± 5.05	4.4 ± 2.11	296.0 ± 100.11***	168.0 ± 47.23*** $P_{3-4} < 0.001$
C-LDL	3.24	7.05	0.0015	4.4 ± 4.03	3.8 ± 2.79	38.5 ± 32.66**	4.1 ± 2.20 $P_{3-4} < 0.01$
C-IDL	3.24	7.69	0.0009	1.8 ± 1.76	1.9 ± 1.28	35.7 ± 32.18**	1.9 ± 1.97 $P_{3-4} < 0.01$
C-LDL_{1-3}	3.24	1.63	0.20	2.6 ± 2.54	2.8 ± 2.23	4.9 ± 2.36	2.8 ± 1.40
C-HDL	3.27	3.09	0.43	66.9 ± 20.25	96.9 ± 25.33*	90.8 ± 21.19	72.2 ± 23.65
C-HDL_2	3.26	6.42	0.002	60.2 ± 17.94	87.6 ± 23.06	45.6 ± 19.51	39.8 ± 28.95
C-HDL_3	3.27	14.99	<0.001	6.7 ± 3.66	8.6 ± 8.12	46.2 ± 24.24***	39.9 ± 13.54***

Mice were used 24 h after Triton WR 1339 administration (500 mg/kg). Data are presented as mean ± SD (Dunnett's test), $n = 8$ mice per group. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$ versus control

Table 2 The effect of atorvastatin on the concentration of TG-LP fractions and subfractions (mg/dl) in murine serum during Triton WR 1339-induced lipaemia

LP fractions, subfractions	One-way analysis of variance			Control, intact	Atorvastatin, 75 mg/kg, twice	Triton WR 1339	Atorvastatin + Triton WR 1339
	df	*F*	*P*	1	2	3	4
TG-LP (All)	3.26	34.34	<0.001	62.9 ± 14.29	57.3 ± 20.70	761.9 ± 312.58***	475.9 ± 108.22*** $P_{3-4} < 0.01$
TG-VLDL	3.26	32.34	<0.001	35.2 ± 13.93	16.6 ± 6.32	680.2 ± 304.68***	439.5 ± 105.34 *** $P_{3-4} < 0.05$
TG-$VLDL_{1-2}$	3.26	.8.00	<0.0.01	4.8 ± 3.60	4.4 ± 2.16	32.3 ± 24.88**	14.8 ± 6.31 $P_{3-4} < 0.05$
TG-$VLDL_{3-5}$	3.26	29.99	<0.001	30.5 ± 11.31	12.2 ± 4.54	651.3 ± 305.57***	426.3 ± 106.62 *** $P_{3-4} < 0.05$
TG-LDL	3.23	19.82	<0.001	3.4 ± 2.89	4.7 ± 3.73	36.9 ± 18.88**	3.6 ± 2.97 $P_{3-4} < 0.001$
TG-IDL	3.23	7.25	<0.01	2.4 ± 2.36	3.0 ± 1.46	47.6 ± 42.86**	2.5 ± 2.63 $P_{3-4} < 0.01$
TG-LDL_{1-3}	3.27	1.15	0.345	1.0 ± 0.66	2.3 ± 2.77	2.3 ± 1.63	1.5 ± 0.96
TG-HDL	3.28	1.99	0.138	24.4 ± 7.58	32.7 ± 12.45	34.7 ± 8.19	27.4 ± 8.80
TG-HDL_2	3.25	12.53	<0.001	21.9 ± 6.65	32.3 ± 8.93*	19.0 ± 6.57	10.3 ± 4.18** $P_{3-4} < 0.05$
TG-HDL_3	3.26	18.86	<0.001	2.5 ± 1.44	3.2 ± 2.97	18.5 ± 8.70***	14.8 ± 4.93***

(Data are presented as mean ± SD, $n = 8$ mice per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0001$ versus control (Dunnett's test)

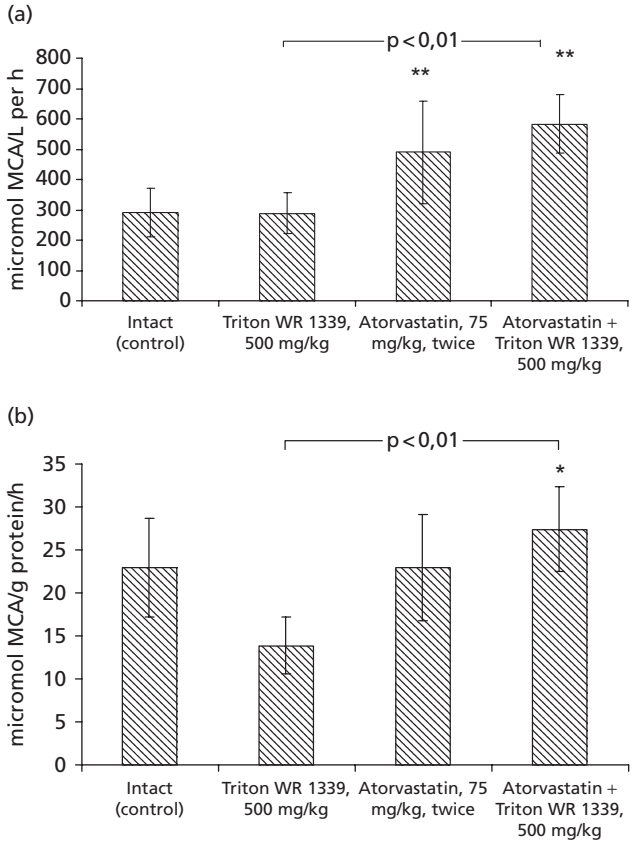

Figure 2 Effect of atorvastatin administration to mice on serum (a) and liver (b) MMP activity during lipaemia. The mice were used in the experiment 24 h after a single Triton WR 1339 administration (500 mg/kg). Data are presented as mean ± SD, $n = 9$. $^{*}P < 0.05$ versus control, $^{**}P < 0.01$.

With regard to MMP activity, administration of atorvastatin to mildly hyperlipidaemic mice (Triton, 500 mg/kg) resulted in an increase in the serum MMP activity (Figure 2a). A similar trend was observed in control mice treated with atorvastatin (Figure 2a). When compared with controls, mildly-lipaemic mice showed a tendency to reduction in MMP activity in liver tissue (Figure 2b). Administration of atorvastatin restored MMP activity in the livers of mice pretreated with Triton, with a mean value that was slightly greater than the corresponding value determined for control mice (Figure 2b). In the experiment that induced mild lipaemia, followed by atorvastatin administration, we determined an inverse correlation between both the serum cholesterol concentration and MMP activity ($r = -0.632$, $P < 0.01$), and the serum TG concentration and MMP activity ($r = -0.780$, $P < 0.01$).

Discussion

Atherosclerosis is a complex disorder of lipid metabolism and a chronic inflammatory disease.[25] Changes in lipoprotein metabolism and hyperlipidaemia play an important role in the progression of atherosclerosis and are significant in predicting the development of cardiovascular events as risk factors.[11,26] However, the initial period of atherosclerosis development, and the interaction of lipoproteins with cells involved in the dyslipidaemic state, merits further investigation.

Mouse models of hyperlipidaemia and atherosclerosis are useful tools with which to study the role of individual lipoproteins of different classes in hyperlipidaemia and atherosclerosis development.[6] With the assistance of a new physicochemical SAXS method recently used by our laboratory,[18] it was possible to simultaneously investigate all three major classes of lipoproteins (VLDL, LDL, HDL) and seven subfractions (HDL_3, HDL_2, $LDL_{1–3}$, IDL, $VLDL_{3–5}$, $VLDL_{1–2}$ except chylomicrons).

Using the SAXS method,[22] we have shown some similarities between the Triton and poloxamer 407 models of lipaemia in mice, which was described earlier.[18] In this study, it was shown that high-dose atorvastatin therapy produced normalization of the atherogenic C-LDL fraction and C-IDL subfraction, as well as the TG-LDL and TG-IDL subfractions, compared with control mice. These changes in the LP-C and LP-TG fractions and subfractions following atorvastatin treatment have been described previously in humans, although it should be mentioned that there are some rather interesting differences between species after atorvastatin administration.

Our results indicate that the lipid-lowering effect of high-dose atorvastatin in mice is mediated through an increased conversion of VLDL to LDL, with total normalization of the LDL and IDL fractions, incomplete restoration of the VLDL fraction, and formation of non-atherogenic HDL subfraction.

The Triton hyperlipidaemic mouse model is a widely used model for inhibition of lysosomal lipolysis and evaluation of the effects of different hypolipidaemic drugs. Triton WR-1339 is a known inhibitor of hepatic and lipoprotein lipase and induces a dose-dependent acute hyperlipidaemia in mice similar to the non-ionic detergent, poloxamer 407.[6,15] In this study, Triton WR-1339, at a dose of 500 mg/kg, induced a mild lipaemic state that was effectively treated by high-dose atorvastatin, but atorvastatin's hypolipidaemic effects were not observed when we induced a severe lipaemic state with Triton.

Triton is a lysosomotropic agent that is taken up inside lysosomes of liver cells (especially in liver macrophages – Kupffer cells).[16] Macrophages are key players in the pathogenesis of atherosclerosis, not only during the onset of the early symptoms of atherosclerosis but also in the final stages when they are associated with plaque formation and disruption.[2] It was shown that lipid loading of primary human macrophages by VLDL (enriched by TG) resulted in stimulation of basal pro-inflammatory cytokine secretion from cells (IL-1β); thereafter, it was suggested that this process is important in atherosclerosis.[27] Close interaction between macrophages and the extracellular matrix (ECM) has been shown to be modified in atherosclerosis during formation of lipid-laden macrophages, and those functions are changed during lipid overloading. Free cholesterol enrichment of either plasma or endosomal membranes in macrophages leads to activation of signaling via various Toll-like receptors (TLRs), prolonged p38 mitogen-activated protein kinase activation and induction of MMPs.[28–31]

There are some notable differences in the atherogenic plasma lipid profile between humans and mouse models.

Treatment with high doses of statins (atorvastatin, up to 20 mg/kg) has been shown to have some species-specific effects in rats.[30–32] Atorvastatin, at high doses, induces key enzymes controlling rat liver lipid synthesis and VLDL assembly, probably as a result of the overexpression of sterol-regulatory element binding proteins (SREBP-2). Despite the induction of key enzymes involved in VLDL production, statins reduce plasma TG levels, suggesting that different mechanisms may be involved in the hypotriglyceridaemic effect of statins at high and low doses.[30]

We have also demonstrated that an inverse correlation exists between the cholesterol concentration and serum MMP activity and between the TG concentration and serum MMP activity in the experiment that induced mild lipaemia. MMPs play a critical role in the development of coronary artery disease and atherosclerosis by remodelling ECM components, and they are also known to be involved in inflammation and immune system dysfunctions.[17] It has been hypothesized that macrophages play a key role in inducing plaque rupture by secreting proteases that destroy the ECM that provides physical strength to the fibrous cap.[1,17] Despite reports detailing the expression of multiple proteases by macrophages in rupture-prone regions, there is some scientific evidence that MMPs (e.g. MMP-9, MMP-2) are related to macrophage-mediated ECM degradation and can induce plaque rupture.[32–34] It has been shown that endothelial lipase is increased by inflammation and promotes LDL uptake into macrophages.[31–33]

To the best of our knowledge, the effects of statins in acute lipaemia have not been investigated to date. We demonstrated that acute lipaemia did not induce any change in serum MMP activity, but that high-dose atorvastatin administration resulted in an increase in serum MMP activity during acute lipaemia. This may have resulted from macrophage activation and increased secretion of MMPs during treatment by atorvastatin and reflects a change in the ratio between MMP and tissue inhibitors of MMP (TIMP) activity.[17] The existence of a negative correlation between cholesterol and TG concentrations and serum MMP activity in the present study suggests that these enzymes are involved in the therapeutic effect of atorvastatin at high doses. In humans with atherosclerosis, increased expression and elevated concentrations of circulating MMPs have been detected: MMP-9 (related to formation of plaque), which is secreted by plaque macrophages, and also MMP-9, which originates from PMNs; MMP-2 (from macrophages); and other types of MMPs.[17]

The total serum MMP activity that we measured in this study using an enzymatic assay provided information on several MMP types, including predominantly gelatinase A (MMP-2) and matrilysin 1 (MMP-7).[17,24] However, according to more recent proteome analysis, this assay may include other types of MMPs, like stromelysins (MMP-3, MMP-10, MMP-11), all of which, play a role in plaque instability.[29,34,35] The advantage of the MMP activity assay we used is that it identifies only the active forms of enzymes, and not enzymatically inactive pro-enzymes or MMP complexes with either endogenous TIMPs or α_2-macroglobulin.

In this study, we also demonstrated that MMP activity in liver tissue slightly decreased in the mild lipaemic state we induced using Triton, but that subsequent atorvastatin treatment increased liver MMP activity significantly. These results would seem to suggest that atorvastatin may potentially exert its lipid-lowering and pleiotropic effects in the early stages of lipaemia, when apoptosis of stellate cells can be induced by lipaemia and liver macrophages accumulate excessive amounts of lipids. In fact, these results may suggest that atorvastatin treatment affects the MMP/TIMP ratio, and may contribute to pleiotropic anti-inflammatory and immunoregulatory properties of statins.[36,37] Lastly, these data also suggest that MMPs (MMP-2, MMP-7 and possibly other MMPs such as MMP-9) may possibly play a role in the early stages of atherogenesis (i.e. when lipaemia is just beginning to develop).

Conclusion

This study, using atorvastatin-treated mice with acute Triton-induced lipaemia, demonstrated that high-dose atorvastatin effectively normalized atherogenic total C-LDL and TG-LDL fractions, C-IDL and TG-IDL subfractions, and resulted in a simultaneous increase in circulating serum MMP activity, possibly due to changes which occurred in liver cells (e.g. macrophage activation and MMP secretion). Although atorvastatin treatment did not change the total anti-atherogenic fractions of HDL, it did decrease the TG-HDL_2 subfraction. These data indicate that high-dose atorvastatin exerts a hypolipidaemic effect in the Triton-induced acute lipaemic mouse model, but does not significantly prevent the formation of the pro-atherogenic precursors of LDL, namely C-VLDL and TG-VLDL, which have been shown to be independent risk factors in human atherogenesis.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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