Effect of Atorvastatin on Activities of Matrix Metalloproteinases and Chitotriosidase in Male and Female Mice with Experimental Hyperlipidemia

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Activities of matrix metalloproteinases and chitotriosidase were measured in blood serum from male and female ICR mice with moderate hyperlipidemia receiving atorvastatin (75 mg/kg). Hyperlipidemia in male and female mice was characterized by increased serum concentration of cholesterol and, especially, triglycerides. The observed changes were more pronounced in female mice. Administration of atorvastatin decreased cholesterol (but not triglyceride) level in intact males, but had no effect on these parameters in females; chitotriosidase activity increased in male and female mice, while activity of matrix metalloproteinases increased only in males. Administration of atorvastatin produced similar effects in male and female mice with moderate hyperlipidemia: decrease in the concentration of cholesterol and, particularly, of triglycerides. Activities of matrix metalloproteinases and chitotriosidase increased in males and females, this increase being more pronounced in males. The existence of a negative correlation between cholesterol and triglyceride concentrations and activities of matrix metalloproteinases and chitotriosidase in males suggests that these enzymes can serve as a therapeutic target during hyperlipidemia.

Key Words: *matrix metalloproteinases; chitotriosidase; hyperlipidemia; statins*

Hyperlipidemia is one of the most important risk factors for atherosclerosis, some cardiovascular diseases, and cerebrovascular disorders. Little is known about early effects of hyperlipidemia on various types of cells [13,14]. The development of hyperlipidemia is accompanied by lipid loading of macrophages, which results in activation of these cells and increase in secretory activity. These changes are followed by the formation of atherosclerotic plaques [6,9]. Previous experiments were performed to study the consequences of feeding atherogenic diet and plaque formation in animals. Lipid-loaded cells were shown to originate from blood monocytes that undergo local differentiation into macrophages [6]. The model of Triton WR 1339-induced hyperlipidemia in experimental animals is used to study the mechanisms for action of several hypolipidemic drugs [7,12]. This model is easily reproduced and relatively well studied. Triton is characterized by low toxicity [7,10]. Moreover, the detergent has a dose-dependent effect in inducing hyperlipidemia of different severity. Much recent attention was paid to a new enzyme of human macrophages, chitotriosidase (CT). Enhanced expression and high activity of this enzyme are observed at the site of plaque formation [2,4]. Matrix metalloproteinases (MMP) are involved in various stages of vascular wall remodeling and capsular formation or rupture in the atherosclerotic plaque. It is accompanied by migration and transformation of macrophages into foam cells [1,11,15]. The role of various types of MMP and macrophage activation in the development of hyperlipidemia at various stages of atherosclerosis remains unclear.

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Here we studied the early effect of hyperlipidemia on activities of MMP and CT in mice (criteria for lipid loading of macrophages) and evaluated the effect of hypolipidemic compounds (atorvastatin) on activities of these enzymes.

MATERIALS AND METHODS

Experiments were performed on male and female ICR mice weighing 25-30 g and obtained from the vivarium of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences). Triton WR 1339 (Ruger Chemical Co) in a single dose of 500 mg/kg was injected intraperitoneally to induce moderate hyperlipidemia in animals [2,7,10]. The suspension of atorvastatin (75 mg/kg; Atoris, KRKA) in 3% starch gel was administered intragastrically through a probe. Atorvastatin was given two times (24 and 3 h before Triton injection) according to the previously proposed scheme [4]. Previous studies showed that atorvastatin in this dose and regimen of treatment significantly decreased serum concentrations of cholesterol (CH) and triglycerides (TG), *i.e.* produced a hypolipidemic effect. The hypolipidemic effect was not observed when atorvastatin was administered in other doses or according to other schemes. The mice were euthanized 24 h after detergent administration. Intact animals received atorvastatin in the same dose; a special group of intact animals received an equivalent volume of starch gel (solvent).

Triton in a higher dose of 850 mg/kg was injected one time to induce severe hyperlipidemia [2,4,7,10]. Atorvastatin in a dose of 75 mg/kg was administered three times (24 and 3 h before Triton injection; and 24 h after detergent administration) [2,4]. The mice were deprived of food, but had free access to water for 15 h before euthanasia. Intact males and females served as the control. The serum was obtained by centrifugation on an Eppendorf 5415 R centrifuge at 3000*g* and 4°C for 20 min.

Activities of alanine transaminase (ALT) and aspartate transaminase (AST) in blood serum (markers of hepatocyte cytolysis) were measured colorimetrically with the corresponding kits (ALT-Novo and AST-Novo). The concentrations of TG and CH were measured using Triglycerides-Novo and Novochol kits (Vector-Best). Photometry of the samples was performed on a 5010 semiautomatic photometer with a Robert Riele temperature-controlled flow cuvette.

CT activity was evaluated by the fluorescent method using 4-methylumbelliferyl-β-D-N,N',N"-triacetylchitotrioside as the substrate (Sigma) at pH 5.2 [2,5]. Fluorescence was recorded at 360 nm (extinction) and 445 nm (emission). The results were expressed in nmol cleaved methylumbelliferone (MUF) per 1 ml over 1 h.

MMP activity was measured against the substrate MCA-Pro-Leu-Gly-Leu-DpA-Ala-Arg-NH_2 (American Peptide Co.) at pH 7.5 [11,15]. The use of this peptide substrate with a fluorescence quencher allowed us to evaluate primarily activities of MMP-2 (gelatinase A) and MMP-7 (stromelysin) [11,15]. Fluorescence was recorded on a Shimadzu RF-530101 (PC)S spectrofluorometer at 320 nm (extinction) and 390 nm (emission). Methylcoumarylamide (MCA, Sigma) served as the standard. The results were expressed in μmol MCA/liter/h.

The results were analyzed statistically by the parallel-series method (variational statistics) with SPSS 9.0 software and Student's *t* test. The differences between mean values were significant at $p<0.05$. The Spearman correlation coefficient was calculated.

The mean values for male and females of various experimental groups were compared to evaluate possible sex differences. The confidence interval was calculated by the Welch—Satter—Thwaite approximation using the effective number of degrees of freedom and effective mean-square deviation [5]. The symmetric confidence interval with a probability of 0.95 ($p=0.05$) was calculated to evaluate the significance of differences. The effect can be considered to be significant with a probability of more than 0.975 ($p\leq0.025$) when the confidence interval is above or below zero.

RESULTS

The concentrations of CH and TG in blood serum were similar in intact male and female mice (ICR; Fig. 1). It should be emphasized that TG level was higher in females than in males. By contrast, AST activity in males surpassed that in females (Table 1). The solvent (starch gel) had little effect on these parameters (Fig. 1).

Administration of atorvastatin to intact males decreased CH (but not TG) content (Fig. 1) and had no effect of these parameters in females (Fig. 1, Table 1). Activities of ALT and AST were elevated in male and female mice (Fig. 3). AST activity in females was higher than in males (Table 1). We revealed an increase in CT activity in male and female mice (Fig. 2). Activity of MMP was elevated in males, but decreased in females (Fig, 2, Table 1).

Administration of atorvastatin to male and female mice with moderate hyperlipidemia was accompanied by a decrease in the concentration of CH and, particularly, of TG (Fig. 1). Atorvastatin had no hypolipidemic effect in animals with severe hyperlipidemia: CH level increased to 7.80±0.45 and 9.40±0.50 mmol/liter and TG concentration increased to 31.30±1.13

and 34.20±1.01 mmol/liter in animals receiving Triton and Triton+atorvastatin, respectively (n=10). Therefore, further studies were performed to evaluate activities of MMP and CT only in animals with moderate hyperlipidemia that exhibited a strong response to atorvastatin.

Serum concentration of CH and, particularly, of TG increased significantly (Fig. 1). These changes were more pronounced in females (Table 1). It should be emphasized that the concentrations of CH and TG in these animals were much lower than in specimens with severe hyperlipidemia. Sharp increase in TG level suggests that administration of Triton serves as the model of hypertriglyceridemia (but not only as the model of hypercholesterolemia). This state is of considerable importance in studying the pathogenesis of atherosclerosis [7,10,12]. ALT activity was elevated in females and, particularly, in males (Fig. 3, Table 1).The observed changes reflect cytolysis of hepatocytes during lipid accumulation in liver cells (steatosis). CT activity was reduced (Fig. 2, Table 1), while MMP activity remained unchanged in males and females (Fig. 2).

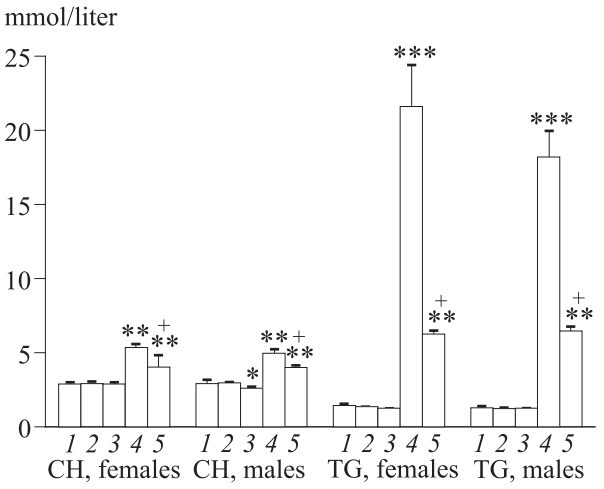

Fig. 1. Concentrations of CH and TG in blood serum from male and female mice after administration of atorvastatin and Triton WR 1339. Here and in Figs. 2 and 3: intact mice (*1*); administration of starch gel (n=10, *2*); administration of atorvastatin in starch gel (n=8, *3*); administration of Triton WR 1339 (500 mg/kg, *4*); administration of atorvastatin and Triton WR 1339 (500 mg/kg, *5*). *p<0.05, **p<0.01, and ***p<0.001 compared to intact animals of the same sex; ^{+}p<0.05 and ^{++}p<0.01 compared to animals receiving Triton WR 1339.

Administration of atorvastatin to male and female mice with moderate hyperlipidemia was accompanied by an increase in activities of MMP and CT (Fig. 2, Table 1). Similar changes were observed in intact mice receiving atorvastatin (except for the decrease in MMP activity in intact females). An increase in activities of ALT and AST was revealed in females and males (Fig. 3, Table 1), which reflects abnormality of liver functional tests. The increase in AST activity in females was similar to that in Triton-treated animals (Fig. 3). Increased cytolysis of hepatocyte was observed in males (Fig. 3). Comparative study of cytolysis in females and males showed that variations in ALT activity were more pronounced in males (Table 1).

Triton has an inhibitory effect on plasma lipoprotein lipase, which is followed by the development of hyperlipidemia and sharp increase in CH synthesis in liver cells of various animals (rats, mice, rabbits, and dogs) [10,12]. Accumulation of Triton in lysosomes of nonparenchymal and parenchymal liver cells occurs over a long period of time (more than 60 days). The detergent is slowly excreted by exocytosis (probably with the bile) [10]. Hyperlipidemia caused by injection of Triton in various doses is extensively used as the model to study the mechanism for hypolipidemic activity of statins, fibrates, and other drugs. Much attention is paid to studying the pleiotropic effect of statins [8]. The specific effects of statins were evaluated in male and female patients [3]. Our results indicate that ICR mice are characterized by sex-related variations in activities of CT and MMP. They are observed after administration of atorvastatin to intact animals

TABLE 1. Differences in the Parameters of Male and Female Mice with Moderate Hyperlipidemia after Administration of Atorvastatin

Groups (Δ males, females)	CH, mol/liter	TG, mol/liter	MMP, μmol MCA/liter/h	CT, nmol MUF/ml/h	ALT, U/liter	AST, U/liter
Intact	-0.03±0.30	+0.15±0.14*	96±111	17±77	1.2±2.2	-8.9±5.8*
Starch	-0.06±0.16	0.15±0.07*	116±156	5±51	-1.32±1.32*	-1.2±5.1
Atorvastatin	0.27±0.15*	0.0±0.02	-345±137*	41±68	11.0±7.7*	+24.0±14.0*
Triton WR 1339	0.39±0.36*	3.46±3.17*	57±107	-15±41	-7.8±1.7*	5.5±8.8
Atorvastatin+Triton	0.01±0.87	-0.2±0.30	-157±193	16±81	-2.0±6.6*	-12.0±16.0

Note. Sex differences were evaluated by the Welch—Satter—Thwaite approximation [5]. *p<0.05, differences between females and males.

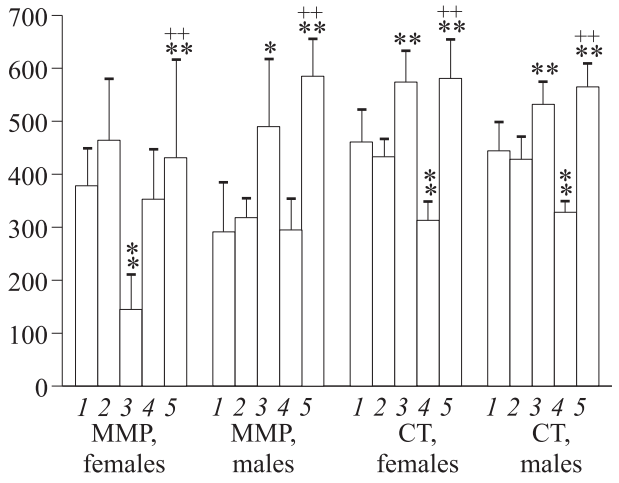

Fig. 2. Activities of MMP and CT in blood serum from male and female mice after administration of atorvastatin and Triton WR 1339. MMP activity, μmol MCA/liter/h; CT activity, nmol MUF/ml/h.

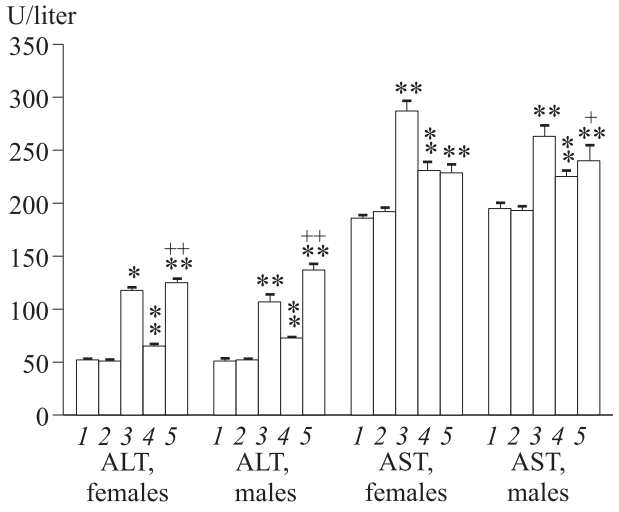

Fig. 3. Activities of ALT and AST in blood serum from male and female mice after administration of atorvastatin and Triton WR 1339.

and mice with moderate hyperlipidemia. A correlation was found between activities of CT and ALT in intact mice (positive correlation in males, r=0.877; and negative correlation in females, r=-0.694). These data illustrate the relationship between the increase in CT activity and liver dysfunction in males (but not in females). After administration of atorvastatin to males with moderated hyperlipidemia (but not to females), the concentration of CH correlated negatively with activities of MMP (r=-0.632) and CT (r=-0.597). A negative correlation was found between the concentration of TG and activities of MMP (r=-0.780) and CT in male animals (r= -0.818). A positive correlation was revealed between the concentrations of CH and TG and activity of ALT in females (r=0.820 and r=0.866, respectively).

We conclude that the early stage of hyperlipidemia in mice is accompanied by lipid loading of liver macrophages and increased secretion of proinflammatory agents by macrophages (various types of MMP, but not CT). These changes were particularly pronounced in males. Recent studies showed that CT of mice is located in cells of the gastrointestinal tract. This enzyme in humans is located in macrophages [2]. Enzyme functions in mice are poorly understood. Functional activity of this enzyme is believed to be associated with innate immunity. Decreased serum CT activity in mice with moderate hyperlipidemia reflects abnormality of liver functional tests during Triton-induced lipidosis [1].

It should be emphasized that administration of Triton was followed by a higher increase in the concentration of TG in blood plasma (compared to the level of CH). Treatment with the detergent may be used as a model of hypertriglyceridemia, which has an important role in the pathogenesis of atherosclerosis [12]. The concentration of TG increased more significantly than the level of CH in animals with moderate (Fig. 1) and severe hyperlipidemia. Atorvastatin had a stronger hypolipidemic effect on TG than on CH in specimens with moderate hyperlipidemia. This approach and experimental model hold much promise to study the mechanisms for correction of hypertriglyceridemia.

Statins act as inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. They not only produce the hypolipidemic effect, but also possess pleiotropic properties [8,15]. Much recent attention is paid to these properties of statins. They include inhibition of vascular wall inflammation, decrease in the degree of oxidative stress, recovery of endothelial function, and reduction of platelet aggregation and parietal thrombus formation [8]. We showed that administration of atorvastatin decreases serum MMP activity in intact females.

These changes reflect the anti-inflammatory effect of atorvastatin in female animals. The opposite effect of atorvastatin in intact males requires further investigations. Atherosclerosis is a chronic inflammatory process, which involves the immune system [15]. MMP and inflammation have an important role in the formation and resistance of atherosclerotic plaques [12,13]. Therefore, various classes of MMP may serve as a target in several cardiovascular diseases and, primarily, in atherosclerosis. The specific effects of atorvastatin in female and male mice should be taken into account in studying the sex differences in statin activity in humans.

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