Original Article

The Effect of Rosuvastatin on the Liver Enzyme of NMRI Mouse

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Abstract

Background: Rosuvastatin is the newest statin family drug and acts as an HMG-CoA reductase inhibitor. Rosuvastatin can decrease the amount of cholesterol made by the liver and reduce the risk of heart disease. Since liver diseases are one of the significant causes of morbidity and mortality due to drugs toxicity, it is essential to check the liver's function during widely used drugs such as rosuvastatin. Therefore, this study aimed to investigate the effect of rosuvastatin on the liver in the mature female NMRI mouse strain.

Materials and Methods: In this experimental study, 30 adult female NMRI strains (mice) at a mean weight of 25-30 grams were divided into five groups control, sham, and treatment groups. The mice of treated groups, including 1, 2, and 3, received rosuvastatin in doses of 10, 20, and 40 mg/Kg of body mass by oral gavage for 21 days. The mice in all groups were dissected after completing the gavage, their hearts were examined, and blood samples were obtained to measure liver enzymes. Then, the mice were sacrificed, and the liver tissue was subjected to antioxidant enzymes. The ELISA test measured the concentrations of the antioxidant and liver enzymes.

Results: The results showed that rosuvastatin decreased GPX, MDA, and FRAP with an increase in SOD, AST, and ALT ($P<0.05$).

Conclusion: It was concluded that high doses of rosuvastatin could damage the liver.

Keywords: Rosuvastatin, Liver, Antioxidant, Statin

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Introduction

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), known as lipid-lowering agents[1]. Rosuvastatin, one of the members of the statin family, is the last and the only statin that comes to market after the removal of cerivastatin and one of the novel synthetic statins. The current category of HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors is an artificial hydrophilic lipid-lowering agent with sparing solubility in water and methanol, and a slight solubility in ethanol refers to rosuvastatin[2]. In addition, to be used in cholesterol-lowering therapy, rosuvastatin also demonstrates hepatoselectivtiy (high selectivity for the central place of cholesterol synthesis, which means liver cells), endures metabolism limitation through cytochrome P450 (CYP)[3]. Rosuvastatin, a prevalent prescribed form of statins, has a long half-life effect with particular efficacy of reducing plasma LDL (low-density lipoprotein)[4]. Rosuvastatin demonstrates many products on the lipid profile and has the potential

to decrease apolipoprotein B (the major protein of LDL) and triglycerides; it can increase high-density lipoprotein (HDL) cholesterol serum levels[5, 6]. Besides that, rosuvastatin exhibited high efficacy in decreasing low-density lipoprotein (LDL) cholesterol and, in metabolic syndrome, demonstrates some practical action on insulin sensitivity[7, 8].

Additionally, a hydrophilic property of rosuvastatin derives from the polar methyl sulfonamide moieties, and the existence of an opposing group on hydrophilic structure gives tissue affinity and solubility to the molecule. Compared with more hydrophobic statins, Rosuvastatin has high selectivity for liver cells (hepatocytes) with fewer drug-drug interactions and fast elimination. In other words, hydrophilic statins are more hepatoselective[5, 9, 10]. The up-taking process of rosuvastatin into hepatocytes is done through passive diffusion and active transport[11].

The elevation of the liver enzyme with rosuvastatin is comparatively low in the range of other statins—moreover, all therapies based on the lowering-lipid result in the temporary height of hepatic enzymes[12]. The elevation of liver enzymes related to statin is determined by dose- and drug-specific effect, which was reported as a sign of hepatic toxiciti[13]. Therefore, this paper aims to investigate the dose-dependent effect of rosuvastatin on the liver enzyme of NMRI mice.

Methods

Animal treatments: Adult female NMRI mice at a mean weight of 25-30 grams were used throughout this study. The mice were divided into five control, sham, and three treatment groups. In control groups, animals didn't receive any drugs. Animals in sham groups received normal saline (as a drug solution). The mice in three treatment groups received doses of 10, 20, and 40 (mg/kg of body mass) rosuvastatin by oral gavage. The dose selection of rosuvastatin was based on Famularo et al. (2007)[14] and Leite et al. (2014)[15] research. Twenty-one days after the final gavage, the mice in all groups were dissected, their hearts were dissected, and blood samples were obtained. Livers are rapidly removed and washed with saline solution (0.9%). 1 gram tissue is homogenized (liver 1:10 w/v) in a buffer containing 1% Triton X-100,150 mM NaCl, 20 mM sodium phosphate, pH 7.4. The livers are homogenized in a tissue homogenizer for 30 s on ice, followed by centrifugation at 10,000 × *g* for 10 min. After centrifugation, the supernatant is used to assay lipid peroxidation and antioxidant enzymes.

Biochemical assays: Serum alanine aminotransferase (ALT or SGPT) and aspartate aminotransferases (AST or SGOT) activities (Ziest Chem Diagnostics Co, Iran) were measured spectrophotometrically in serum according to the procedure described in the method of Reitman and Frankel (1957)[16]. The reagents AST and ALT were mixed (4:1), samples were added, and absorption was read at 505 nm. Values were expressed as units per liter. The activity of superoxide dismutase (SOD) was determined according to the pyrogallol method described by Maklund and Marklund (1974)[17]. 1 ml of tris HCL buffer with ten µl of pyrogallol and ten µl of diluted supernatant (1/ 10) were mixed in a cuvette. Then, absorbance was measured at 420 nm. The glutathione peroxidase (GPx) activity assay was determined by Paglia and Valentine (1967)[18]. The assay mixture contained 500 µL 0.1 M phosphate buffer, 100 µL of enzyme sample, 100 µL of glutathione reductase (0.24 U/mL), 100 µL of 10 mM GSH, 100 µL nicotinamide adenine dinucleotide phosphates and 100 µL of 12 mM t-butyl hydroperoxide. The mixture was monitored using a spectrophotometer at 340 nm for 5 min. The specific activities of SOD and GPx were expressed in Units/mg of protein. Lipid peroxidation (LP) assay was determined based on malondialdehyde (MDA) produced using the method of Buege and Aust (1978)[19]. Briefly, a weighed portion of liver tissues was homogenized in 100mM of phosphate buffer (pH 7.0). 1 ml of homogenate was added to 2 ml TBA (thiobarbituric acid) reagent and was shaken for 15 min. The mixture was incubated for 15 min and then centrifuged at 3000 *g*. The absorbance at 535 nm was measured. The activity of the LP enzyme was expressed as nmol/ mg protein. Ferric Reducing Antioxidant Power (FRAP) was measured by Koracevic (2001)[20]. According to the procedure, all solutions were mixed and pipetted into tubes (in µL) and incubated for 10 minutes at 100°C (in a boiling water bath); they were cooled in an ice bath. Absorbance was measured at 532 nm against deionized water. Values were expressed in µmol/mg protein.

Statistical analysis: The results were expressed as mean ± standard deviation (SD) of seven rats per group.

Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD using SPSS (22.0) statistical software. Values were considered statistically significant at P<0.05.

Animal experimentation was performed according to the ethical committee and the general ethical principles of the Declaration of Helsinki (World Medical Association 2001) (Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964) and local institutional animal care (approved by the rat protocol from Pasteur Institute, Iran) and ethical guidelines.

Results

Figure 1 showed that at the 40 mg/kg dose, rosuvastatin increased the AST and ALT levels compared to the control and sham groups (P<0.05). There are no significant changes in the liver enzymes at the dose of 10 and 20 mg/kg (Figure 1). Also, rosuvastatin at quantities of 20 and 40 mg/kg significantly decreased serum GPX, MDA, and FRAP levels compared to other groups (P<0.05). On the other hand, SOD activity was significantly increased at a 40 mg/kg dose of rosuvastatin than in the control and sham groups (P<0.05).

Discussion

Statins such as lovastatin, atorvastatin, and simvastatin are lipophilic and are metabolized by the cytochrome P-450 system. Some of them, like pravastatin and pitavastatin, are hydrophilic and undergo minimal hepatic metabolization, and rosuvastatin has an intermediate behavior[21]. The adverse side effects of statins may limit, but one study showed the negative effects include hepatic and renal dysfunction and a possible increased risk of diabetes [22]. Statin use may be associated with a slight increase in transaminases. De Denus et al. (2004) revealed that fluvastatin was the only drug associated with an increased risk of liver function abnormalities[23]. Liver toxicity is a class effect with the increased risk of elevated liver enzymes with increasing statin dose[24].

The liver enzymes results showed a significant increase in the level of AST at the dose of 40 mg/kg and ALT level at the same amount compared to the control and sham groups (Figure 1). In comparison, rosuvastatin in a dose of 10 and 20 mg/kg didn't affect the liver enzymes compared with 40 mg/kg (Figure 1). These results concordance with the study carried out by Mohamed et al. (2019)[25,] which described similar marks in using atorvastatin and simvastatin drugs. Also, a survey by Rasooli et al. (2016)[26] showed similar effects on liver enzymes in the use of paracetamol (Acetaminophen) as a widely used drug.

According to other studies, this elevation can be associated with hepatocellular injury, cholestatic liver injury, or mixed liver injury, i.e., combined hepatocellular and cholestatic liver damage [24, 27]. Alanine aminotransferase (ALT) and aspartate

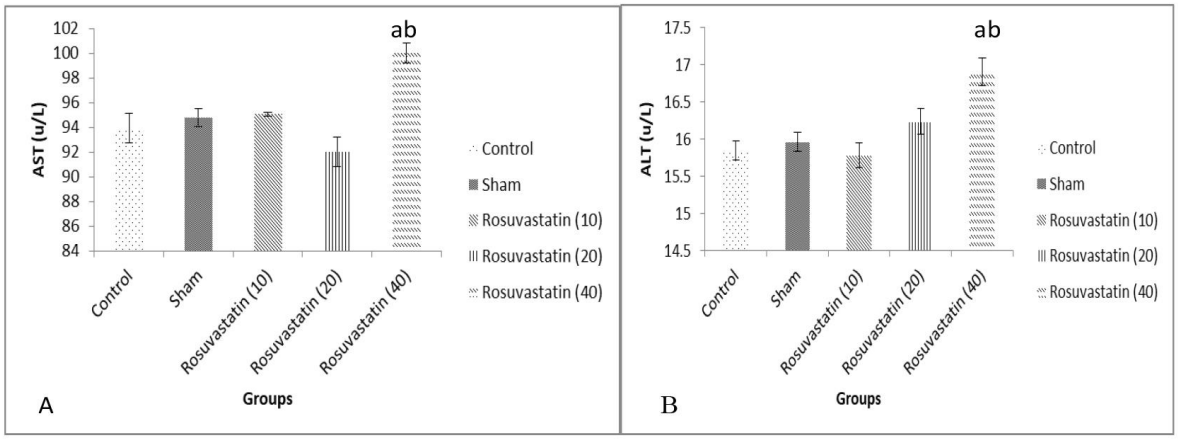

Figure 1. Mean values±SD of liver enzymes activity
Concentration of A) AST, B) ALT in different groups;
[a]P value <0.05 is considered significantly between control group and rosuvastatin groups.
[b] P value <0.05 is considered significantly between rosuvastatin groups (10, 20, 40 mg/kg of body mass)

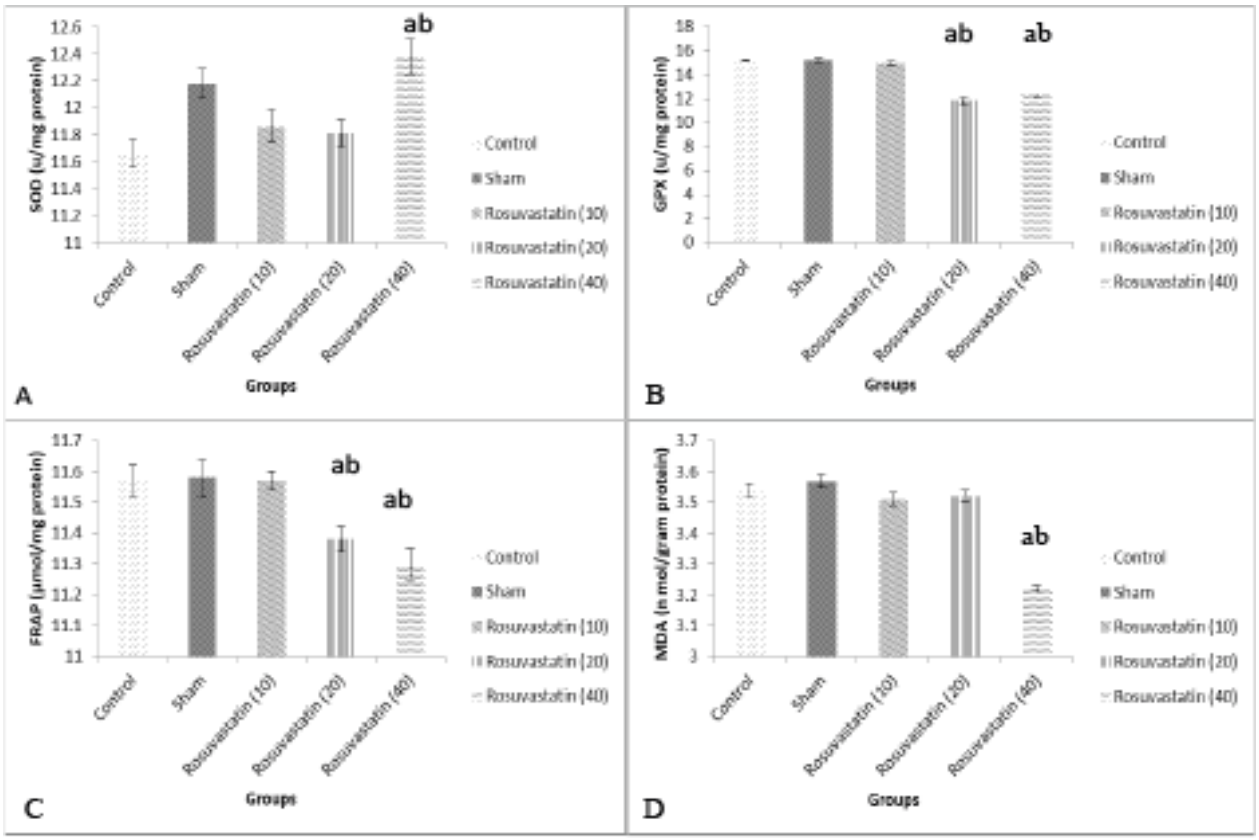

Figure 2. Mean values±SD of antioxidant enzymes activity
Concentration of A) SOD, B) GPX and C) FRAP D) MDA in different groups;
[a]P value <0.05 is considered significantly between control group and rosuvastatin groups.
[b] P value <0.05 is considered significantly between rosuvastatin groups (10, 20, 40 mg/kg of body mass)

aminotransferase (AST) are liver enzymes commonly used for liver function blood tests. Increased activities of liver enzymes, located in the cytosol naturally, in the bloodstream correlated with liver damage. So, their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage[28]. One study demonstrated that elevated serum hepatic transaminases induced by statin usually occur within the first three to twelve months after starting statin therapy[29].

Also, the results on the effects of rosuvastatin on serum SOD, GPX, FRAP, and MDA are presented in Figure 2. The levels of serum GPX, MDA, and FRAP were diminished markedly in the rosuvastatin group (at doses of 20 and 40 mg/kg) as compared to other groups ($P<0.05$). SOD activity was significantly ($P<0.05$) increased at a dose of 40 mg/kg in the rosuvastatin group compared to the control and sham groups. In comparison, no significant changes were found at a dose of 10 mg/kg in all groups compared to other groups. There is increasing evidence statin toxicity is closely linked to oxidative stress[30]. Oxidative stress is associated with several diseases and is defined as an imbalance between the synthesis of reactive oxygen species (ROS) and their elimination by antioxidant defense systems. Superoxide dismutase (SOD), malondialdehyde (MDA) as a marker of lipid peroxidation, and glutathione peroxidase (GPx) are the most critical antioxidant enzymes[31]. ROS is generated during the metabolism of statins, thus resulting in oxidative stress and hepatic tissue toxicity[32]. Lower doses of atorvastatin (2 or 5 mg/kg BW) showed significantly higher SOD activities and lower MDA levels[33].

Some studies demonstrated that one month of simvastatin or atorvastatin therapy reduced ROS production and systemic concentrations of MDA and increased extracellular SOD activity [34, 35]. Also, one study showed that MDA declined significantly in patients with atherosclerotic cerebrovascular disease and acute or chronic stroke who received rosuvastatin 20 mg/day for 1 month[36]. On the other hand, a decrease

of FRAP in plasma, as a factor in oxidative stress/antioxidant, may be due to enzymatic and non-enzymatic antioxidant activities causing an increased resistance and/or decreased susceptibility of the liver to free radical attack[37]. Shin et al. (2007) found a significant increase in antioxidant ability in their group of hypercholesterolemic patients treated with 40 mg simvastatin daily[38]. Statin therapy, with 20 mg of simvastatin daily, for at least three months, did not show any change in the total antioxidant capacity level done by Strzyżewski et al. (2013)[39]. Moreover, a study on diabetic rats showed that oral treatment with rosuvastatin at a dose of 10 mg/kg/day decreased the MDA levels and increased the glutathione and SOD activity of pancreas tissues in diabetic rats[40].

Conclusion

It was concluded from current results that rosuvastatin may elicit liver damage via alteration of the antioxidant defensive system. Also, our findings showed that rosuvastatin could induce oxidative damage in high doses by changing the antioxidant parameters and liver enzymes. However, more research needs to find the relation between animal and human toxicity of rosuvastatin on the liver.

Acknowledgment

None.

Conflict of interest

The authors further declare that they have no conflict of interest.

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