Rosuvastatin: Morphological and Respiratory Effects of High Doses on Liver Mitochondria from Hypercholesterolemic Mice

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Abstract

Background: Statins are the cornerstone of therapy in patients with hyperlipidemia. In high risk patients statins are employed for aggressive therapy, however part of the users are intolerant to these drugs. The aim of this study was to analyze the undesirable effects of moderate, median and high doses of rosuvastatin in CD-1 male mice that received a cholesterol-rich diet, focusing in the morphological and functional changes on hepatocyte mitochondria.

Methods: We studied in a mouse model the combined administration of a cholesterol-rich diet (HD) along with a moderate high dose of rosuvastatin (Ro): 1, 2.5 or 5 mg/Kg/day during several days. Animals (n=6) were sacrificed, the liver mitochondria were isolated for analysis of respiratory function and microscopic studies. The respiratory control (state 3/state 4) and the O_2 expenditure (nanoatoms/min/mg proteins) were evaluated.

Results: Rosuvastatin doses higher than 20 mg/Kg/day induced premature death in hypercholesterolemic mice but not in mice with a cholesterol-free diet. Doses from 2.5 to 5 mg/Kg/day also induced morphological and functional alterations in mitochondria but the hypercholesterolemic animals survived longer. A dose of 1 mg/Kg/day, which is close to the maximal therapeutic dose employed in humans, did not affect mitochondrial architecture or respiratory function after two months of treatment. We analyzed the effect of rosuvastatin on the hepatic tissue where statins are most retained after their administration, and the main site of endogenous cholesterol synthesis.

Conclusions: Our results contribute to understand the undesirable side effects of rosuvastatin in hypercholesterolemic mice, effects that can also be present in human being intolerant to statins.

Introduction

Statins are the drugs of choice for first and second prevention of atherosclerosis cardiovascular diseases (ASCVD). They were introduced in the clinical practice since 1987, after the isolation of compactin from *Penicillium citrinum* cultures [1]. The statins introduction to clinical medicine was followed by an extensive use in patients with high serum cholesterol levels to control the β-containing lipoproteins recognized as ASCVD risk factors [2, 3]. Rosuvastatin is a compound most used in clinical practice, it is a relative hydrophilic statin whose structure is shown in figure A; the higher dose employed in humans is 40 mg/day, which corresponds for an individual with 60 Kg body weight to 0.66 mg/Kg/day.

Some medical societies have recommended an aggressive use of statins to treat patients with high ASCVD risk [4, 5], at least in the subgroup identified as candidate for high-intensity statin therapy [6, 7]. All statins available in the market have the same effect on cholesterol biosynthesis, inhibiting in a competitive way the 3-hydroxy-3-methylglutaryl coenzyme A reductase [8, 9], the enzyme that catalyzes the mevalonate formation. This action affects predominantly the liver cholesterol synthesis, responsible for 80% of its endogenous production in the human body [10].

Statins also reduce oxidative stress by modulating the redox systems. This is probably a mechanism by which they exert beneficial effects on the cardiovascular system, but the oxidative stress may also be responsible for statin-induced adverse effects [11].

As any pharmaceutical compound, statins are not exempted of undesirable side effects specially after high therapeutic doses for prolonged periods of time or in patients that present intolerance to this type of drugs. Intolerance to statins in general is associated to muscle symptoms, with a prevalence of 7–29% of the patients employing standard statin doses [12, 13].

There are two types of statins, the natural compounds that were isolated from fungi cultures and the synthetic compounds produced in the laboratory. Synthetic statins are more active compounds and potentially produce more undesirable effects [14]. The solubility of statins also influences their potential accumulation in tissues, as could occur with the lipid-soluble compounds [15].

Animal models can be used to evaluate and compare the side effects of statins [16 3]. One of the first adverse effects reported was that of lovastatin on rabbits [17], which was aggravated when a cholesterol-rich diet was added. Some studies have used rodent models to analyze the statins side effects in hypercholesterolemic animals. Previous reports included lovastatin [18] and other statins [19]. This study includes data from mice with a cholesterol rich diet that received moderate or high doses of rosuvastatin looking for the adverse effects on liver mitochondria that are not present when the same rosuvastatin doses were administered to mice with a cholesterol-free diet.

Material And Methods

CD-1 male mice, 30 g body weight, were maintained in light-dark cycles of 12 hours. They received a laboratory chow diet (CD) containing 18% protein, 5% fat and 5% fiber. The food was powdered, and the statin was added in the amounts mentioned ahead and administered orally by different periods of time as indicated forward. The hypercholesterolemic diet (HD) contained 2% cholesterol and 0.6% sodium deoxycholate. Rosuvastatin 20 mg tablets were obtained from Medimart, Slovenia. All other compounds were purchased from Merck-Sigma Mexico.

Each part of the experiment included animals with CD and HD alone (n = 6). The experimental procedures were managed according to the Official Mexican Standard NOM-062-ZOO-1999 and the guidelines established by the Research Committee for the Care and the Use of Laboratory Animals of the Universidad Nacional Autónoma de México.

Experimental design

A) The animals received a continuous treatment with rosuvastatin (Ro) in order to evaluate the survival rate:

CD + Ro (rosuvastatin 0, 20, 50, 100, 200, 400 mg/Kg/day)

HD + Ro (rosuvastatin 0, 20, 50, 100, 200, 400 mg/Kg/day

B) The animals received a continuous treatment with moderate high statin doses to evaluate hepatocyte mitochondrial respiration. The microscopic observations were performed in representative samples of each group after different treatment lengths.

CD + Ro (rosuvastatin 0, 20 mg/Kg/day)

HD + Ro (rosuvastatin 0, 20 mg/Kg/day)

C) The animals received a continuous treatment with median statin doses to evaluate hepatocytes mitochondrial respiration and to perform microscopic observations in representative samples of each group after different treatment lengths:

HD + Ro (rosuvastatin 0, 5 mg/Kg/day)

HD + Ro (rosuvastatin 0, 2.5 mg/Kg/day)

HD + Ro (rosuvastatin 0, 1 mg/Kg/day)

Animals sacrifice

The animals were sacrificed by decapitation. Blood and other tissues were immediately obtained for biochemical analysis or microscopic studies.

Mitochondria isolation and incubation

Liver was homogenized in 250 mM sucrose, 0.5 mM HEPES, 0.5 mM EGTA (SHE). pH 7.2, using a Thomas pestle tissue grinder (piston-type Teflon pestle) and mitochondria were isolated employing a refrigerated centrifuge MPW-353R Med Instruments, Varsovia, following the method described by Frezza et al., [20]. The mitochondria obtained by centrifugation were incubated for 10 min with 0.5% albumin to eliminate fatty acids and resuspended in SHE solution. Protein content in mitochondria was evaluated by the Bradford method [21]. The incubation media contained: KCl 240 mM, HEPES 60 mM, H_3PO_4 4 mM, EGTA 4 mM, Succinate 10 mM, $MgCl_2$ 4 mM, mitochondrial protein 4 mg, pH 7.2, final volume 3.2 ml. An YSI oxygen meter 5300 model was employed to measure the oxygen consumption. The mitochondrial respiration was stimulated by the addition of 10 µL of 200 mM ADP and the respiratory control was evaluated.

Biochemical parameters

Serum determinations included total cholesterol, triacylglycerols, HDL-C (high density lipoprotein cholesterol), glucose, urea, creatinine, AST (aspartate aminotransferase) and ALT (alanine aminotransferase), employing a semi-automatic equipment of clinical chemistry from Random Access Diagnostics.

Microscopy studies

The liver was studied both by light and electronic microscopy. The tissue slices dyed with hematoxylin-eosin were observed in a Nikol Eclipse 180 microscope. For electronic microscopy the mitochondrial pellet and the liver tissue were fixed with 3% glutaraldehyde in cacodylate buffer. The samples were processed in the microscopy unit of the Neurobiology Institute, Universidad Nacional Autónoma de México, employing a Jeole transmission electronic microscope model JEM-1010.

Statistical analysis

The mice survival was expressed in percent. One-way analysis of variance (ANOVA), followed by Student–Newman–Keuls test and differences were considered to statistical significance when $p < 0.05$. For morphologic analysis we used representative samples from each of the animal groups.

Results

Effect of high rosuvastatin doses on hypercholesterolemic mice survival

Table 1 shows the survival rate for different doses of Ro. The combined administration of the statin plus a cholesterol-rich diet produced a premature death with doses 20 mg/Kg/day or higher. There was not mortality among animals that received the same statin doses without a cholesterol-rich diet.

Table 1
Mice survival after high doses of rosuvastatin plus a cholesterol-rich diet

Ro mg/day	0	4	5	Day 6	7	17	18	30
0	100	100	100	100	100	100	100	100
20	100	100	100	100	83	83	66.4	50
50	100	100	50	16.6	16.6	16.6	16.6	16.6
100	100	100	66.4	16.6	16.6	16.6	16.6	16.6
200	100	100	0	0	0	0	0	0
400	100	100	16.6	0	0	0	0	0

Ro rosuvastatin, mg/Kg/day.

Effect of medium doses of rosuvastatin on mice with a cholesterol- rich diet

It was tested a 20 mg/Kg/day dose of Ro in mice with CD or HD. The animals were sacrificed at different periods of time. A daily analysis was performed in order to observe any morphologic change in hepatocytes and the respiratory function of their mitochondria. The body weigh was registered and also the liver weigh in relation to body weight.

Table 2
Liver weight of mice treated with hypercholesterolemic diet plus rosuvastatin 20 mg/Kg/day

Group	Liver weight (g)
CD	1.71 ± 0.26
HD	1.85 ± 0.21
CD + Ro 1 day	1.86 ± 0.15
HD + Ro 1 day	1.65 ± 0.16
HD + Ro 3 days	2.59 ± 0.79[a]
HD + Ro 5 days	3.30 ± 0.62[a]

CD control diet; HD hypercholesterolemic diet; Ro rosuvastatin 20 mg/Kg/day; [a]-Key of significance compared with HD ($p < 0.05$).

The experiment included six animals per group that were sacrificed on days 1, 3 and 5 of treatment. Macroscopically, the livers of the statin-treated mice were friable, with appearance of steatosis. Table 2 shows that the liver weight of animals treated with HD + Ro 20 mg/Kg/day increased significantly after 3 and 5 days of treatment.

Biochemical parameters

Serum triacylglycerols decreased after 3 to 5 five days of treatment with HD + Ro 20 mg/Kg/day, in the days 3 (47 ± 2.82 mg/dL) and 5 (53 ± 0 mg/dL) versus HD (97 ± 2.82 mg/dL). On the contrary, serum cholesterol levels increased significantly on days 3 and 5 of HD + Ro 20 mg/Kg/day (761.5 ± 0.7 mg/dL and 813 ± 1.06 mg/dL, respectively), versus HD values (202 ± 5.65 mg/dL). On the other hand, HDL-C diminished on days 3 and 5 after HD + Ro 20 mg/Kg/day (16 ± 1.06 mg/dL and 14 ± 0.14 mg/dL, respectively) compared with HD group values (59.2 ± 0.63 mg/dL).

Table 3
Respiratory control in hepatocyte mitochondria from mice treated with hypercholesterolemic diet plus rosuvastatin 20 mg/Kg/day.

Oxygen	**consumption**	**nanoatom/min/mg**	**protein**
Group	**State 4**	**State 3**	**Respiratory Control**
CD	0.15 ± 0.02	0.53 ± 0.08	3.45 ± 0.07
HD	0.23 ± 0.02	0.72 ± 0.10	3.05 ± 0.07
CD + Ro	0.19 ± 0.00	0.62 ± 0.07	3.10 ± 0.28
HD + Ro/1 day	0.25 ± 0.03	0.71 ± 0.01	2–80 ± 0.28
HD + Ro/3 days	0.10 ± 0.01	0.30 ± 0.10	2–90 ± 0.56
HD + Ro/5 days	0.09 ± 0.13	0.09 ± 0.13[a]	0.95 ± 0.63[a]

CD control diet; HD hypercholesterolemic diet; Ro rosuvastatin 20 mg/Kg/day; Key of significance: [a]compared with HD ($p < 0.05$).

An unexpected result was a traumatic decrement in ALT and AST serum activity that were almost undetected on days 3 and 5 after treatment with HD + Ro 20 mg/Kg/day vs HD values (108 ± 5.02 U/L and 274 ± 10.61 U/L; respectively). The respiratory function was decreased after 5 days of treatment HD + Ro 20 mg/Kg/day (0.09 ± 0.13 and 0.95 ± 0.63) with respect group HD (0.72 ± 0.10 and 3.05 ± 0.07) (Table 3).

Another part of the experiment included the evaluation in terms of absorbance of the 3-hydroxy-methylglutaryl-CoA/mevalonate ratio in the liver homogenate [22]. This ratio was high after 3 days of HD + Ro 20 mg/Kg/day treatment and even higher after 5 days of treatment (3.93 ± 1.30), versus the HD mice group (2.53 ± 0.31) ($p < 0.05$) (Fig. 1).

Microscopy observations

The study was performed in hematoxylin/eosin-dyed liver tissue after 1, 3 and 5 days of HD + Ro 20 mg/Kg/day treatment. The liver of CD + Ro 20 mg/Kg/day (Fig. 2b) or HD (Fig. 2c) showed no alterations. On the contrary, the livers of HD + Ro 20 mg/Kg/day obtained after 1 to 5 days of treatment showed an early steatosis with abundant lipid drops. There was not necrosis (Figs. 2d, 2e, 2f).

Effect of moderate doses of rosuvastatin given to male mice along with a cholesterol-rich diet.

The Ro doses utilized were 0, 1, 2.5 and 5 mg/Kg/day during different periods of time. Mice were sacrificed every day and the morphological and physiological studies were performed as explained in the previous section. The animals that received HD + Ro 5 mg/Kg/day did not show important differences in their body weight in comparison with other groups, however their liver weight increased significantly from

day 5 to day 11, a fact that was apparent when we compared the percentage of liver weight relative to body weight. A similar result was obtained when it was used a dose of 2.5 mg/Kg/day, for 15 days (Table 4), but not when Ro 1.0 mg/Kg/day was administered for 59 days.

Table 4
Liver weight and its relation to body weight in hypercholesterolemic mice treated with 2.5 mg/Kg/day of rosuvastatin for 15 days

Length of treatment (day)	Liver weight (g; m ± SD)	% of liver weight relative to body weight
1	1.20 ± 0.27	5.0
3	1.41 ± 0.39	6.52
5	1.50 ± 0.31	7 – 06
7	2.04 ± 0.38	9.06
9	2.73 ± 0.71	10.95
11	2.63 ± 0.79	11.25
13	2.48 ± 0.25	10.59
15	2.40 ± 0.35	11.23
M, media and SD standard deviation		

The treatment with HD + Ro 5 mg/Kg/day produced an impaired respiratory function. The HD + Ro 2.5 mg/Kg/day also produced a lower respiratory control and higher oxygen consumption. On the other hand, when the animals received a HD + Ro 1.0 mg/Kg/day treatment, even for two months, they did not show alteration of the mitochondrial function (Table 5).

Table 5
Oxygen consumption by the liver mitochondria from hypercholesterolemic mice treated with rosuvastatin 1.0, 2.5 or 5 mg/Kg/day

Rosuvastatin treatment mg/Kg/day	Length of treatment (day)	Respiratory Control
CD	0	3.44
HD + Ro 5	3	2.20
HD + Ro 5	5	1.79
HD + Ro 2.5	5	1.80
HD + Ro 2.5	9	2.20
HD + Ro 2.5	15	2.40
HD + Ro 1	59	3.56
HD	59	3.22
CD + Ro 1	59	2.96
CD control diet, HD hypercholesterolemic diet, Ro rosuvastatin		

The microscopic observations of hepatocyte mitochondria showed that the HD + Ro 5 mg/Kg/day was harmful. After 11 days of treatment it was observed a loss of the mitochondrial architecture pattern, the matrix with empty spaces, some mitochondria with donut form (Fig. 3a), and a generalized damage scarcely compatible with life. After 5 days of treatment with HD + Ro 2.5 mg/Kg/day the liver mitochondria (Fig. 3b) show an important structural damage and a copious inter mitochondrial material, but scarce lipid drops.

When the HD + Ro 1 mg/Kg/day treatment was administered for 59 days, the liver mitochondria (Fig. 3c) showed in general a normal morphology with a few mitochondria exhibiting a horseshoe form; the mitochondrial architecture was comparable to that of CD mice (Fig. 3d). The treatment with either CD + Ro 1 mg/Kg/day (Fig. 3c) or HD alone (Fig. 3f) did not produce adverse morphological changes during the study term. HD + Ro 5 mg/Kg/day treatment (Fig. 3a) was harmful for mitochondria which showed loss of their architecture pattern, matrix with empty spaces and some organelles with donut-like form. The HD + Ro 2.5 mg/Kg/day treatment (Fig. 3b) displayed copious mitochondrial damage and disorganized organelle structure. Mitochondria obtained after 59 days of HD + Ro 1 mg/Kg/day (Fig. 3c) showed a general conserved structure with few organelles having a horseshoe aspect. Mitochondria Fig. 3d, 3e and 3f are the corresponding controls.

Discussion

The aim of this study was to make an analysis of the undesirable effects of moderate, median and high doses of rosuvastatin in CD-1 male mice that received a cholesterol-rich diet, focusing in the

morphological and functional changes on hepatocyte mitochondria.

Rosuvastatin is a powerful synthetic statin that is not exempted of undesirable effects in doses used in human being. If a human with 70 Kg body weight employs a rosuvastatin dose of 40 mg/day, he or she is using a 0.57 mg/Kg/day dose. We administered different doses the smallest one was equivalent to 1 mg/Kg/day. Doses higher than this produced several undesirable effects in our mice model, especially in the liver, the main site of statin accumulation. Undesirable side effects have also been reported in the skeletal muscle of humans where myositis and even rhabdomyolysis can be produced. The studies of statins toxicity in the skeletal muscle have been greatly privileged [23]. Although it has been reported that the use of statins at low doses helps to recover the histological architecture of the liver in patients with non-alcoholic fatty liver[24], it has also been observed that in patients with medium doses and complicated with sepsis, rosuvastatin seems to accelerate liver damage and it has already been reported that in mice with dystrophin protein deficiency, which received doses of 10 mg / kg of rosuvastatin, the areas of myonecrosis and inflammation increased significantly, while in mice without alteration in dystrophin and subjected to the same doses, the level of NF-Kβ, TNF-α and creatine kinase activity increased significantly [25] a reason to think that high doses rosuvastatin could potentiate the expression of some genes that encode proteins of inflammation pathways such as those already mentioned. It has also been reported that at medium doses rosuvastatin modulates the expression of some molecules such as NF-Kβ [26]. Even at these same doses rosuvastatin seems to inhibit the overexpression of this protein by previous inflammatory processes in the hippocampus [27]. Its effects at high doses on these proteins are not well known. On the other hand, beside the cholesterol-lowering effect of statins, it has been suggested that they can be used as an associated therapy for COVID-19 due to their anti-inflammatory action that might reduce the risk of cardiovascular complications caused by SARS-CoV-2 virus [28, 29]. Rosuvastatin and other statins are under a close examination.

In this study, it was observed that high doses of rosuvastatin affected liver mitochondria architecture and mitochondrial respiration. It was compared the oxygen consumption in states 3 and 4 of respiration. The phosphorylation in state 3 was stimulated by the addition of 200 mM ADP to the incubation medium. The state 3/state 4 ratio diminished when mice received rosuvastatin doses 2.5 mg/Kg/day or higher. Studies carried out with simvastatin in rats and humans with high doses reported a decrease in the functioning of complex I of the respiratory chain [30] that considerably decreases the transport of electrons captured by NADH towards complex III of the respiratory chain, therefore the mitochondrial energy load would depend mostly of the electrons captured by FADH and entering complex II, which would result in a lower production of ATP by ATP synthase under these conditions. Additionally, the inhibition of the enzyme 3-hydroxy-3 methyl glutaryl CoA reductase, decreases the formation of coenzyme Q [31], which is essential for the transport of electrons to complex III, that would affect the Q cycle in this complex, which is important for the regeneration of coenzyme Q. All these factors together contribute to a decrease in ATP synthesis.

In animal models it has been observed diseases that affect mitochondrial function, that also alter the transport of metabolites to the mitochondria (especially ADP) [32] so it would be important to evaluate

whether rosuvastatin in high doses has a possible effect on the expression or the operation of ANT 1 and 2 conveyors; though we did not explore the intimal mechanism of the respiratory chain alteration. However, there is information regarding the changes that other statins induce in muscle mitochondria function [33, 34, 35]. The 3-hydroxy-methylglutaryl-CoA / mevalonate ratio in the liver homogenate was high from the third day and very high after the 5th day, this result reflects the decrease in the activity of the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase

The role of some molecules in inflammatory processes at the mitochondrial level is already known, NF-Kβ plays a very important role in many of the functions of the mitochondria, and it has been observed that its over-activation is related to inflammatory processes and cancer [36]. Furthermore, NF-Kβ is closely related to the functioning of antioxidant proteins expressed in mitochondria such as superoxide dismutase, catalase and glutathione peroxidase, which are very important enzymes for the regulation of free radical genesis and proper functioning of mitochondrial energy metabolism [37]. Furthermore, rosuvastatin has been reported to have beneficial cardioprotective effects at low doses in mice, and at high doses this effect is lost, and even harmful effects are observed [38].

Rosuvastatin is minimally metabolized through CYP 450 enzymes [11]. The abnormal drug metabolism produces a large number of free radicals and lipid peroxidation resulting in oxidative stress and cells damage. Rosuvastatin reduces serum lipids and cholesterol in the body which results in CoQ_{10} deficiency; CoQ_{10} is the antioxidant that can protect biological membranes from free radicals and lipid peroxidation. CoQ_{10} deficiency might be traumatic after high statin doses. In our study, a decrease in HDL-cholesterol was observed from the third day of treatment with doses of 20 mg / kg / day; The dose-dependent effect of statins on the reduction of LDL cholesterol has previously been reported [39], however for HDL-C, this relationship seems to have no relevance [40], although it was reported a low increase in HDL-C after treatment with atorvastatin, in the same study a decrease in the capacity for cholesterol efflux by the ABCA1 protein was observed [41], which is important in the genesis of HDL lipoproteins. Therefore, rosuvastatin in high doses could significantly affect the efflux of cholesterol by this protein and thus reduce the amount of HDL-C.

In electron microscopy, a loss of the normal structure of the mitochondria (rosette-shaped mitochondria) and alterations in the continuity of the inner mitochondrial membrane and matrix with spaces is observed. In light microscopy, hepatocytes with ballooning degeneration and cells with Mallory-Denk bodies were observed; from the third day of treatment with rosuvastatin at a dose of 20 mg / kg / day, these changes occur in response to a severe depletion of ATP [42], and they are the macroscopic consequences that high doses of rosuvastatin have on mitochondrial metabolism.

Conclusion

Our study contributes to define the morphological and functional alterations of mice hepatocyte mitochondria after moderate or high doses of rosuvastatin. These alterations are not necessarily

translated to human being but can be considered by clinicians and their patients when they employ this kind of drugs. Further studies are necessary to translate these findings to humans.

Abbreviations

Rosuvastatin (Ro), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), EGTA Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid), ADP (adenosine diphosphate), HDL-C (high density lipoprotein cholesterol),, AST (aspartate aminotransferase), ALT (alanine aminotransferase), nuclear factor κB (NF-κB), Coronavirus Disease 2019 (COVID-19), Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2), Nicotinamide Adenine Dinucleotide (NADH), Flavin Adenine Dinucleotide FADH, Adenosine triphosphate (ATP), ATP-binding cassette transporter A1(ABCA1).

Declarations

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Authors' contributions

Juan Díaz, conceptualization, methodology, fund acquisition, original draft preparation; Isela Juárez, conceptualization, supervision, fund acquisition, writing-reviewing; Alejandro Marín, data curation, writing reviewing; Alma Zetina, investigation; Andrés Castell, investigation, supervision; Jorge Blé, writing-reviewing; Rodrigo Miranda, investigation.

Conflict of interest

The authors declare no conflict of interest

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Figures

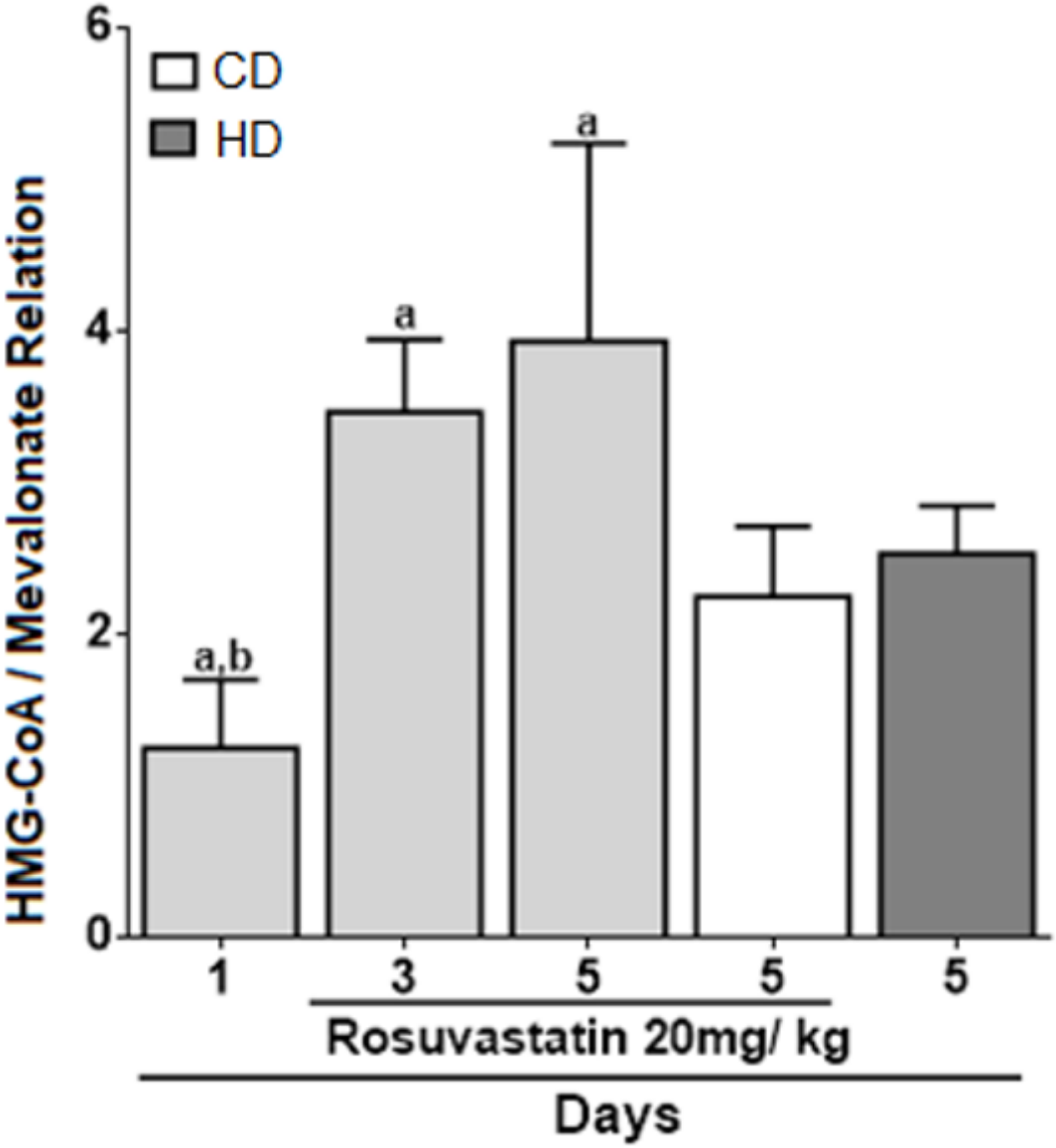

Figure 1

Absorbance of 3-hydroxymethylglutaryl-CoA/mevalonate ratio in the liver homogenate. CD control diet, HD hypercholesterolemic diet. Key of significance: acompared with CD and b with HD ($p<0.05$).

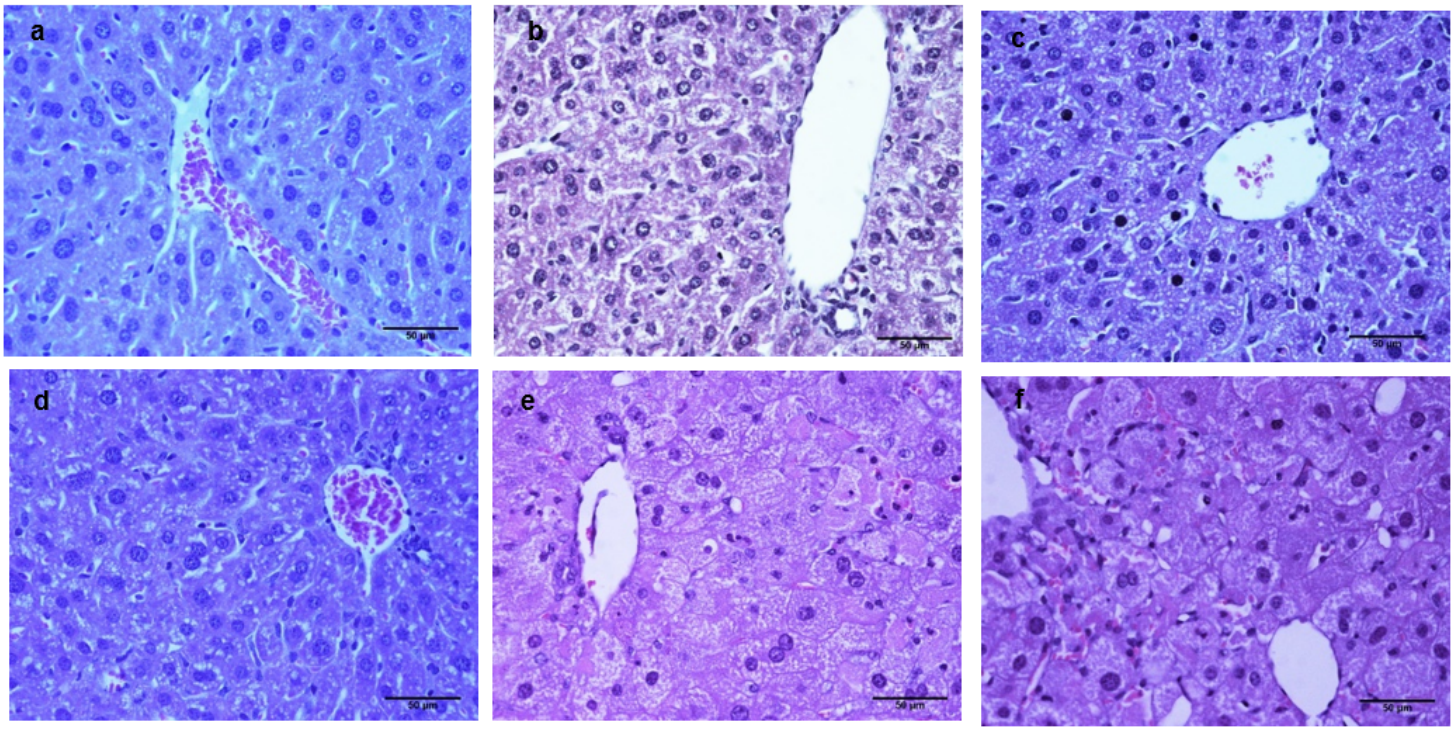

Figure 2

Microscopy images of liver tissue from mice after cholesterol-rich diet and rosuvastatin. Mice liver slices were stained with hematoxylin-eosin. 1) CD; 2) CD + Ro; 3) HD; 4) HD + Ro one day; 5) HD + Ro 3 days; 6) HD + Ro 5 days. CD, control diet; HD, cholesterol-rich diet; Ro, rosuvastatin 20 mg/Kg/day. The liver of CD + Ro 20 mg/Kg/day obtained after 1, 3 and 5 days of treatment manifest early steatosis and significant changes in the cellular structure. Sinusoidal dilatation (4, 5, 6), some cells with ballooning degeneration, pyknotic nuclei (5, 6), Mallory Denk bodies (5) and hepatocytes with loss of their nuclei (5, 6) are observed

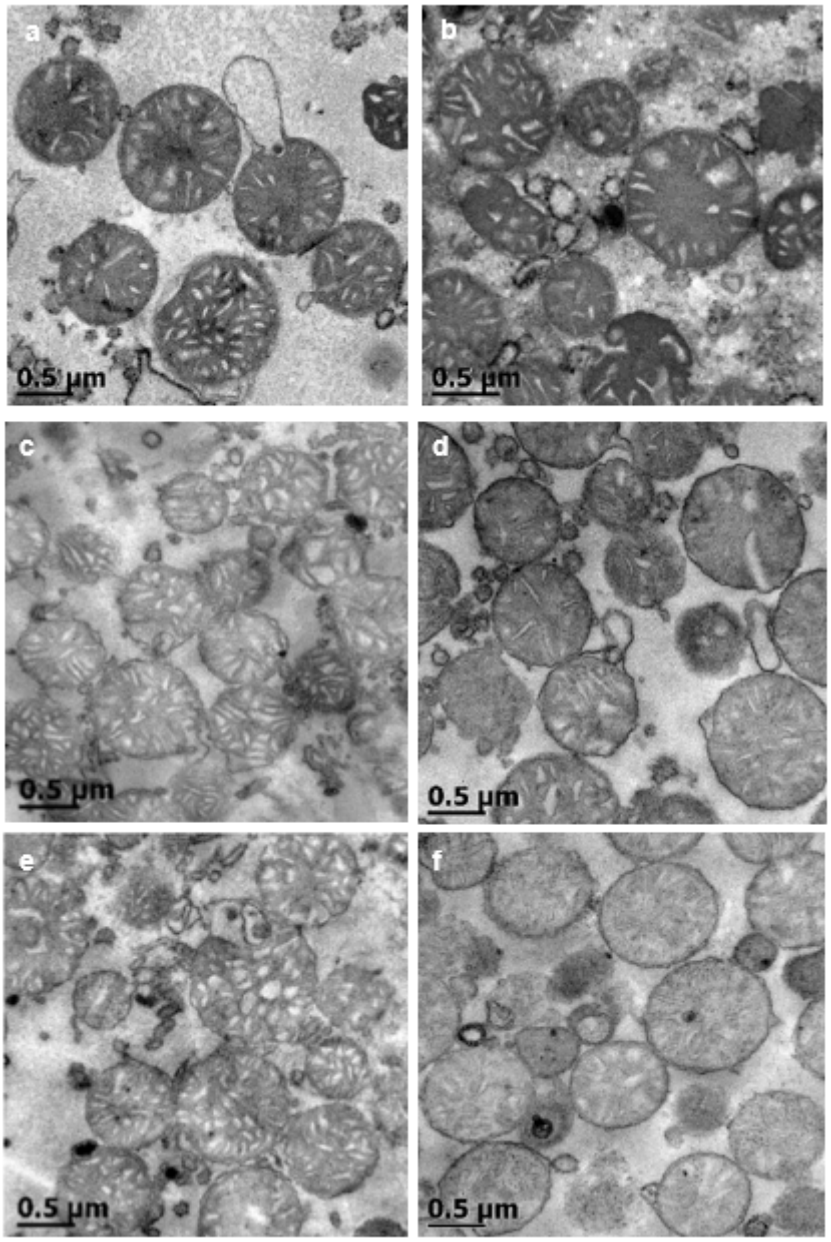

Figure 3

Electron microscopy of hepatocytes mitochondria from mice after cholesterol-rich diet and rosuvastatin. Liver mitochondria from male mice that were treated with A) HD + Ro 5 mg/Kg/day 11 days; B) HD + Ro 2.5 mg/Kg/day 5 days; C) HD + Ro 1.0 mg/Kg/day 59 days; D) CD 59 days; E) CD + Ro 1 mg/Kg/day 59 days; F) HD 59 days. CD, control diet; HD, cholesterol-rich diet; Ro, rosuvastatin.