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ANTI-ATHEROSCLEROTIC EFFECTS OF ATORVASTATIN: A RANDOMISED CONTROL TRIAL

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ABSTRACT

This study was undertaken to investigate the Antiatherosclerotic effects of atorvastatin in treating cases of atherosclerosis associated with hyperlipidemia. Forty local domestic rabbits were assigned to five groups (eight rabbits in each group): Group I is the control; group II, rabbit fed 1% cholesterol-diet (induced untreated group) and group III, 1% cholesterol-diet + zofenopril (0.5 mg/kg daily orally). The remaining 16 atherogenic rabbits were randomly allocated into two groups, eight animals each; first group received atherogenic diet only for the next four weeks and served as an atherogenic control (Group IV). In addition to atherogenic diet, the other group received atorvastatin for the next four weeks (Group V). Blood samples were collected for serum lipids. Histopathological and histomorphometrical examination of the aorta was done at the end of 12 weeks to assess the atherosclerotic changes and aortic intima-media thickness respectively. Results showed a significant improvement in the levels of lipid parameters and the aortic intimal thickness in the atorvastatin treated group compared to the atherogenic control group ($P < 0.001$). This study illustrated the beneficial anti-atherogenic effects of atorvastatin in treating atherosclerosis associated with hyperlipidemia.

Keywords: Atorvastatin, Hyperlipidemia, Atherosclerosis.

INTRODUCTION

Atherosclerotic vascular disease, particularly coronary artery disease (CAD), cerebrovascular disease represents the single largest cause of death of men and women in the world. Overall, atherosclerosis and its complications are responsible for about 40% of deaths in the developed world and for about 30% of fatalities in developing nations [1-3]. Atherosclerosis is characterized by a complex of multifactorial pathogenesis resulting in strong inter-individual variations in disease progression and response to treatment [4]**.** Atherosclerosis involves several highly interrelated processes, including lipid disturbances, platelet activation, thrombosis, endothelial dysfunction, inflammation, oxidative stress, vascular smooth cell activation, altered matrix metabolism, remodelling, and genetic factors [5, 6].

The earliest recognizable evidence of atherosclerosis is the development of fatty streaks which over time evolve into atheroma. Fatty streak, consist of a sub-endothelial collection of foam cells, which are cholesterol-laden macrophages or smooth muscle cells [7]. The cholesterol accumulating in foam cells is known to be ultimately derived from circulating plasma lipoproteins. Low-density lipoprotein (LDL) must be oxidized before it can induce foam-cell formation. The rate of production of oxidized low-density lipoprotein (Ox-LDL) in the arterial intima is a function of the concentration of native LDL present. That concentration is proportional to the plasma LDL concentration [8].

Atorvastatin belong to the statin group which lower LDL-C by inhibiting the conversion of hydroxy methyl glutamyl CoA (HMG COA) to mevalonate, the rate limiting step in cholesterol biosynthesis. Competitive inhibition of HMG COA reductase is not only interferes with cholesterol biosynthesis but also reduces the production of intermediate metabolites, isoprenoids. Thus, the decreased isoprenylation, caused by statins, accounts for their pleiotropic , cholesterol-independent, effects. Atorvastatin is administered as an active compound (acid form). It is relatively lipophylic and has a high affinity for the active site of the enzyme [9-11].

MATERIALS AND METHODS

Preparation of animals

The experimental procedures and animal uses related to this study were approved by the Scientific and Ethical Committee of the College of Medicine –Kufa University, according to the guidelines for the care and use of laboratory animals in scientific research. A total of 40 New Zealand White Male Rabbits (*Oryctolagus cuniculus*), aged 3–4 months and weighing

2.0 to 2.4 kg, were used in this study. The animals were placed in Kufa Medical College's animal house. The rabbits were housed in an isolated room, in a group caging system, at controlled temperature (25±2 ◦C) and ambient humidity. Lights were maintained on a 12-h light/dark cycle. The animals were given free access to water and libitum.

Design of the study

After two weeks acclimatization period, a group of 8 rabbits **(Group I)** were randomly selected and sacrificed at the start of the experiment where baseline values of the study parameters were measured. Another 8 rabbits were selected and maintained on standard chow diet (4% fat, 18% protein, 60% carbohydrate, and 4% fibers) throughout the experiment (12 weeks) and served as a normal diet control **(Group II)**. The rest 24 rabbits were fed on an atherogenic diet for 8 weeks to induce atherogensis. At the end of 8^{th} week, a group of 8 atherogenic rabbits **(Group III)** were separated and sacrificed and served as an atherogenic-baseline group. Then the remaining 16 atherogenic rabbits were randomly allocated into two groups, eight animals each; first group received atherogenic diet only for the next four weeks and served as an atherogenic control **(Group IV)**. In addition to atherogenic diet, the other group received atorvastatin for the next four weeks **(Group V)**.

Animal model of atherosclerosis

Induction of hyperlipidemia and subsequent development of atherosclerosis were carried out by feeding the rabbits an atherogenic diet (4% cholesterol-enriched diet made by addition of cholesterol powder to chow pellets) for 8 weeks [12]**.**

Preparation of atorvastatin

Atorvastatin was used in a dose of 5mg/kg/day orally [1 3] . A 20 mg tablet (Avas-20, Micro Labs, Batch No. AVFH0035, India) was suspended in carboxymethylcellulose to produce 5% solution and doses were prepared from this stock solution and given to the rabbits according to body weight once daily through stomach tube.

Preparation and collection of the samples

At the end of the experiments, food was withheld for 16-18 hr and all animals were sacrificed. The chest was opened by thoracotomy. Blood was withdrawn and aorta was separated before following investigations were performed:

A) Lipid profile including total serum cholesterol (TC), triglycerides (TG), HDL- &

LDL-cholesterol (HDL-C & LDL-C). Serum lipid profiles were determined by enzymatic methods using an automatic analyzer (Abbott, Alcyon 300, USA) [14, 15].

B) Histopathological examination of the aorta for assessment of atherosclerosis. The aortic arch was exteriorized, cleaned of adherent fat and connective tissue excised. All specimens were immediately fixed in 10% formaldehyde solution. After fixation they were processed in usual manner, and embedded in paraffin for subsequent histomorphometeric examination for atherosclerosis [16].

Statistical analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 13.0 and Microsoft Excel (Office2007, Microsoft). Data were expressed as mean ± SEM. Post-hoc test was used to compare the mean values within each group at different time using LSD technique. Analysis of Variance (ANOVA) was used for the multiple comparison among all groups. The statistical significance, direction and strength of linear correlation between 2 quantitative variables were measured by Pearson's linear correlation coefficient. A significance differences were set at p value equal or less than the 0.05.

RESULTS

1. Effect of atherogenic diet on serum lipid profile and atherogenic index

Feeding an atherogenic diet to rabbits for eight weeks resulted in significant changes in serum lipid profile, as compared to baseline group. TC, TG, VLDL-C and LDL-C were significantly increased ($p< 0.001$) while HDL-C was significantly decreased ($p< 0.001$). Table (1)

Table (1): Changes in serum lipid profile of rabbits fed on atherogenic diet for 8 weeks, (N=8). The data expressed as means ± SEM

Parameters	Baseline values	After 8 weeks of atherogenic diet	P-Value
TC (mg/dl)	86.5 ± 3.354	546.875 ± 9.33	p< 0.001
TG (mg/dl)	94.125 ± 4.231	149.5 ± 2.405	p< 0.001
HDL-C (mg/dl)	38.375 ± 2.938	19.375 ± 0.532	p< 0.001
VLDL-C(mg/dl)	18.825 ± 0.846	29.9 ± 0.481	p< 0.001
LDL-C (mg/dl)	29.3 ± 3.226	496.35 ± 9.818	p< 0.001

Similarly, eight weeks of atherogenic diet produced significant ($p< 0.001$) rise in atherogenic index (27.301 ± 0.902 vs. 1.324± 0.156). Figure (1)

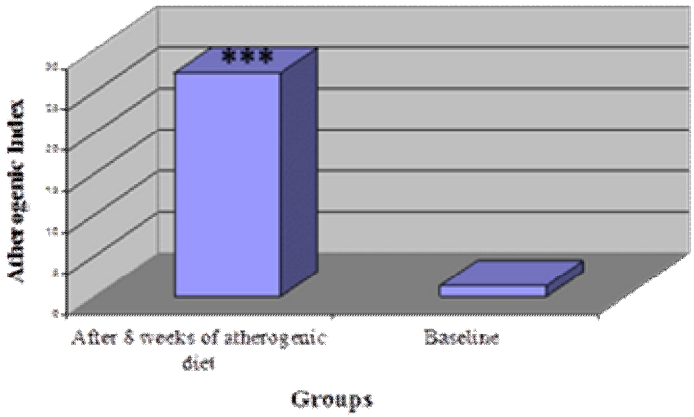

Figure (1): Mean changes of atherogenic index of rabbits fed on atherogenic diet for 8 weeks, (N=8 in each group), p<0.001.

2. Correlation between atherogenic index and aortic intimal thickness

Atherogenic index was found to have a statistically significant positive correlation with aortic intimal thickness ($r = 0.972$, $p < 0.01$).

3. Effect of 4 weeks drug treatment on serum lipid profile

As compared to atherogenic baseline group, the atorvastatin-treated group showed a significant decrease in total serum cholesterol, TG, VLDL-C and LDL-C ($p< 0.01$), while HDL-C was significantly increased ($p< 0.01$). Table (2)

Table (2): Effects of 4 weeks atorvastatin (5 mg/kg) treatment on serum lipid profile, (N= 8); The data expressed as means ± SEM.

Parameters	Before treatment	After treatment	P-value
TC (mg/dl)	546.875 ± 9.33	289.125 ± 8.463	p< 0.01
TG (mg/dl)	149.5 ± 2.405	129.5 ± 3.774	p< 0.01
HDL-C (mg/dl)	19.375 ± 0.532	25.875 ± 0.789	p< 0.01
VLDL-C (mg/dl)	29.9 ± 0.481	25.9 ± 0.754	p< 0.01
LDL-C (mg/dl)	496.35 ± 9.818	237.6 ± 8.825	p< 0.01

Atherogenic control group showed significant increases in total serum cholesterol, TG, VLDL-C and LDL-C ($p< 0.05$). No statistically significant changes were observed in the serum levels of HDL-C ($p>0.05$).Table (3)

Table (3): Changes in serum lipid profile of atherogenic control group, (N= 8); the data expressed as means ± SEM.

Parameters	Before treatment	After treatment	P-value
TC (mg/dl)	546.875 ± 9.33	606.25 ± 29.675	p< 0.05
TG (mg/dl)	149.5 ± 2.405	159.125 ± 3.6	p< 0.05
HDL-C (mg/dl)	19.375 ± 0.532	18.25 ± 0.59	p>0.05
VLDL-C (mg/dl)	29.9 ± 0.481	31.825 ± 0.72	p< 0.05
LDL-C (mg/dl)	496.35 ± 9.818	575.825 ± 29.976	p< 0.05

As compared to baseline group, almost no significant changes (p< 0.05) in lipid profile namely total serum cholesterol, TG, HDL-C, VLDL-C and LDL-C were seen in normal diet control group. Table (4).

Table (4): Changes in serum lipid profile of rabbits fed on normal diet for 12 weeks (normal diet control group), (N= 8); the data expressed as means ± SEM.

Parameters	Baseline values	After 12 weeks of normal diet	P-value
TC (mg/dl)	86.5 ± 3.354	98 ± 3.968	p< 0.05
TG (mg/dl)	94.125 ± 4.231	109.875 ± 5.422	p< 0.05
HDL-C (mg/dl)	38.375 ± 2.938	40.125 ± 3.522	p< 0.05
VLDL-C (mg/dl)	18.825 ± 0.846	22.716 ± 0.919	p< 0.05
LDL-C (mg/dl)	29.3 ± 3.226	35.158 ± 4.378	p< 0.05

4. Effect of 4 weeks atorvastatin treatment on atherogenic index

Atherogenic index was significantly decreased in hyperlipidemic groups treated with atorvastatin (10.241±0.433) as compared to atherogenic baseline group (27.301±0.902) at (p< 0.001).

5. Effect of 4 weeks drug treatment on aortic intimal thickness

In comparison to atherogenic baseline group, hyperlipidemic rabbits showed statistically significant decreases in aortic intimal thickness after 4 weeks treatment with atorvastatin (27.328±0.461) compared to the atherogenic baseline group (60.913±1.11) at (p< 0.001). The atherogenic control group showed a significant increase in aortic intimal thickness (figure 2) compared to the normal diet control group (figure 3); (p< 0.001).

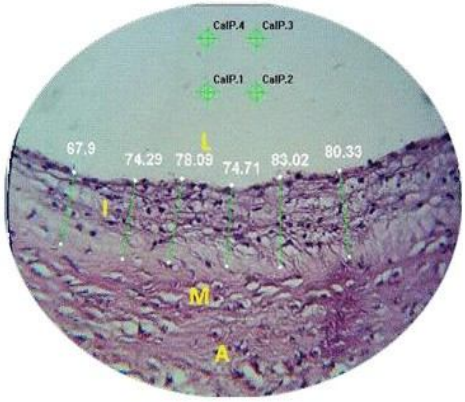

Figure (2): Photomicrograph of histomorphometeric section in aortic arch of rabbits fed on atherogenic diet for 12 weeks (atherogenic control) shows diffuse intimal thickening with lipid and collagen collection within the intima, atherosclerotic changes. (I) intima, (M) media, and (L) lumen. Stained with haematoxylin and Eosin (x100).

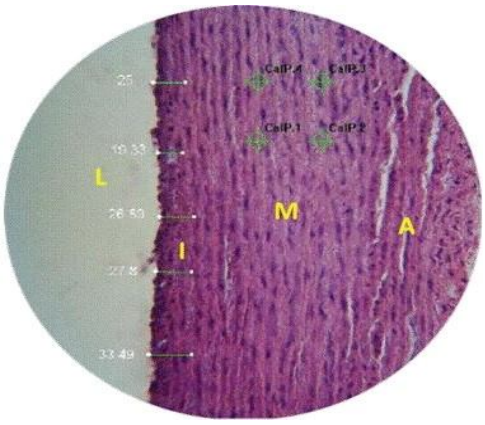

Figure (3): Photomicrograph of histomorphometeric section in aortic arch of rabbits fed on normal diet for 12 weeks (normal diet control) shows the normal intimal thickness and intact continuous endothelium. (I) intima, (M) media, (A) adventitia and (L) lumen. Stained with haematoxylin and Eosin (x 100).

Atorvastatin treated group revealed a significant differences ($p<0.001$) in aortic intimal thickness compared to the normal diet control group (figure4).

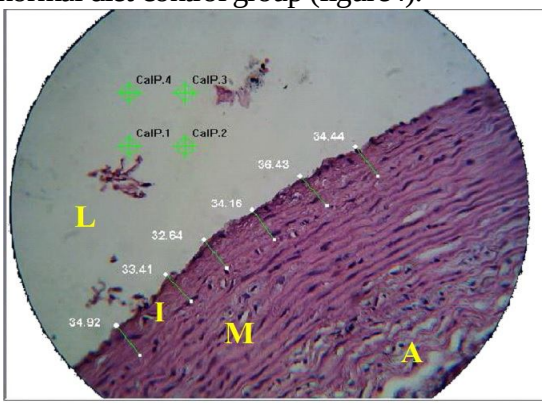

Figure (4): Photomicrograph of histomorphometeric section in aortic arch of atorvastatin treated hyperlipidemic rabbits. (I) intima, (M) media, (A) adventitia and (L) lumen. Stained with haematoxylin and Eosin (x100).

DISCUSSION

In this study, it was shown that keeping the rabbits on a cholesterol- enriched diet for 8 weeks significantly increased total cholesterol, LDL- C, TG and VLDL-C and significantly decreased the HDL-C levels. Atherogenic index was significantly increased in cholesterol fed rabbits as compared to normal diet rabbits. These findings are in agreement with that reported by [17, 18]. The significant changes in serum lipids and atherogenic index are anticipated and might be attributed to the exogenous cholesterol (atherogenic diet).

In addition, the histomorphometeric examination of the normal and the hyperlipidemic rabbits' aorta revealed that in normal rabbits, aorta wall has a uniform thickness with no bulging in the lumen and the endothelial lining is intact without any interruption (figure 3). On the other hand, hyperlipidemia caused marked alterations in the aortic endothelium. The average intimal thickness of aorta was multiplied almost 3.5 times as compared to average normal aorta (figure 2). Atherogenic index was additionally found to have a strong positive correlation with aortic intimal thickness. Similar findings were demonstrated by Hakimoglu, et al. In 2007[19].

Atorvastatin was found to produce a significant decrease in total cholesterol, LDL-C, TG and VLDL-C and a significant increase in HDL-C levels (Table 2). Atherogenic index was further decreased by atorvastatin treatment. This favorable modifying effect of atorvastatin on serum lipids was seen due to atorvastatin's lipid lowering effect due to HMG-COA reductase inhibition [20-22].

Moreover, it was noticed that there was a significant inhibition of progression of intimal thickening and hyperlipidemia by atorvastatin treatment. Histomorphometeric examination revealed that atorvastatin significantly reduced the aortic intimal thickness of hyperlipidemia rabbits. Similar observations were obtained by [23-25].

CONCLUSION

This study illustrated the beneficial anti-atherogenic effects of atorvastatin in treating atherosclerosis associated with hyperlipidemia.

Conflict of interest: The authors declare that they have no conflict of interest

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