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CONTENTS

Effect of amlodipine on blood and aortic tissue concentration of endothelin in male rabbits receiving atherogenic diet

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ABSTRACT

Background: Different factors are involved in the induction and progress of atherosclerosis. One of these factors is endothelin-1. Since, in atherosclerotic vessels, there are certain obvious changes, with abnormality in the transfer of calcium ions, some researchers have suggested that calcium channel blockers can slow down the process of atherosclerosis. In this study, we evaluated the effects of amlodipine and/or a high cholesterol diet on the blood and aortic concentration of endothelin in rabbits.

Materials and Methods: Thirty-six male New Zealand white rabbits were divided into four groups: the normal control group, normal diet plus amlodipine group, high-cholesterol diet group, and high-cholesterol diet plus amlodipine group. After 8 weeks all animals were anesthetized and blood or tissue samples were collected.

Results and Conclusions: Eight weeks of amlodipine treatment significantly reduced total cholesterol, low density lipoproteins (LDL), and triglycerides (TG) in the hypercholesterolemic diet group. Although amlodipine treatment tended to enhance HDL/LDL and HDL/cholesterol ratios in the mentioned group, these effects were not statistically significant. The observed significant increase in plasma high density lipoprotein cholesterol (HDL-C) and decrease in TG is considered to be the main effect of amlodipine treatment on the serum lipid profile in the control group. The plasma level of endothelin-1 in the atherosclerotic model group was significantly increased as compared to the control group ($P < 0.01$). After treatment with amlodipine, the ET-1 level reduced significantly in the control and high-cholesterol diet rabbits ($P < 0.01$). A high-cholesterol diet induced atherosclerotic lesions and thickening of the intima in the thoracic aorta. Amlodipine consumption reduced atherotic injuries in high-cholesterol diet rabbits. There were no lesions in the normal diet groups or the normal diet with amlodipine group. High cholesterol causes increase in plasma and tissue endothelin. Amlodipine treatment reduced the levels of total cholesterol, LDL, and TG and, in a high lipid intake situation reduced endothelin levels in plasma and aortic tissue. Our data shows that amlodipine treatment may be considered as one of the important interventions for prevention and regression of atherosclerosis.

KEY WORDS: Amlodipine, atherosclerosis, endothelin-1

Atherosclerosis is a leading cause of mortality and morbidity in the developed world and most of the developing countries.[1] Atherosclerosis is a complex process and is possibly caused by a high-fat diet and a sedentary lifestyle.[2] Hypercholesterolemia is one of the most important risk factors for atherosclerosis, which promotes functional and structural vascular injury.[3] Atherosclerosis is a progressive and systemic vascular disorder that initiates molecular and cellular events that are triggered by endothelial dysfunction, resulting in decreased nitric oxide production, increased ET-1 production and cyclooxgenase activity, and inflammation.[4,5]

The 21-amino acid peptide endothelin-1 (ET-1) is produced by vascular endothelial cells from the 38-amino acid precursor peptide, big ET-1, by the action of endothelin converting enzyme (ECE).[6] ET-1 may contribute to the progression of several cardiovascular disorders such as congestive heart failure, hypertension, and ischemic heart disease.[6] It has also been speculated that ET-1 is important in atherosclerosis.[7] Besides its vasoconstrictor effects ET-1 also contributes to cell proliferation, thereby promoting vascular growth and atherogenesis.[6] The expression of ET-1 is enhanced in smooth muscle cellular macrophages of human atherosclerotic plaques.[8] Many components of human atherosclerosis lesions, such as endothelial cells, macrophages, and smooth muscle

cells, express ET-1. These findings indicate that ET-1 may be involved in the pathophysiology of atherosclerosis.[9]

Calcium channel blockers (CCBs) have been suggested as a deterrent for cardiovascular diseases and atherosclerosis, and their antiatherogenic effects have been described in patients with coronary artery disease.[10] A variety of studies, performed in humans and animals, have indicated that CCBs can influence the natural progression of atherosclerosis.[11-13]

Amlodipine is a dihydropyridine, which contains a charged amino group and a lipid partition coefficient of about 1200, reflecting its marked ability to partition to the cell membrane; it can inhibit calcium permeability in vascular smooth muscle cells (SMC) and reduce atherosclerotoic lesions.[14] However, this effect could not be confirmed by others[15] and remains subject to controversy. In some animal studies the effect was indifferent[16] and the antiatherosclerotic potential of CCBs is under debate.

Amlodipine can also positively influence risk factors that are associated with atherosclerosis, the mechanisms of which are not known. To the best of our knowledge, evaluation of the effects of amlodipine and/or a high cholesterol diet on blood and tissue concentration of endothelin has not been done in a rabbit model before. Therefore, the present study was carried out to evaluate amlodipine as an antiatherosclerotic via its effect on ET-1 in hypercholesterolemic New Zealand rabbits.

Materials and Methods

Animals and diet

Forty male New Zealand white rabbits (1.4 kg at the start of the study) were divided into four groups: normal control group (NC), normal group receiving amlodipine (NA), high-cholesterol diet group (HC), and high-cholesterol diet with amlodipine group (HA). The control group was fed normal rabbit chow, whereas the high cholesterol diet groups were fed a 2% high-cholesterol diet made by adding cholesterol powder (Merck Company) to normal food. The NA and HA groups received amlodipine powder (Arya Company, Iran) 5 mg/kg/day. All animals were housed in an environmentally controlled room

The rabbits were anesthetized at the end of the experiments by injecting ketamine (25 mg/kg, i.v.) and sodium pentobarbital (20 mg/kg, i.v.) via the marginal ear vein. Blood samples were drawn from the inferior vena cava and were stored in tubes for estimation of the serum lipid profile. They were also stored in tubes containing EDTA (10 mmol/1 final concentration) on ice for estimation of plasma endothelin. After centrifugation (15 min, 4°C), plasma (1 ml) was stored at –80°C for analyses. The plasma ET-1 was measured with a special kit (Titer Zyme® EIA kit, No: 030806265).

Tissue samples

The thoracic aorta was immediately isolated and homogenized for measuring ET-1 (homogenize solution: 20 mol/ut Hcl + 1 mol/ut HCOOH). The homogenized solution was centrifuged (10 min, 3000 rpm, 6°C) and the light supernatant was taken and stored at –80°C. The tissue ET-1 was measured with a special kit (No: 030806265) after lyophilizing with a lyophilizator (Christ Aplphal4).

Histological studies of blood vessels

The thoracic aorta was immediately isolated and placed in 10% formalin. Briefly, after tissue processing, several serial sections of blood vessel segments (6 μm thick) were stained by standard hematoxylin-eosin and studied by light microscopy.

Serum lipid profile

Serum lipid profile, including total cholesterol and TG, were determined by enzymatic methods using an automatic analyzer (Abbott, Alcyon 300, USA).

Statistical analysis

The data is expressed as mean ± SEM; statistical computations are calculated using SPSS 10 for Windows (SPSS Inc., Chicago, IL, USA). The results among the four groups were analyzed by ANOVA. $P < 0.05$ was taken to indicate statistical significance.

Results

Serum lipid profile

Our results clearly demonstrate that eight weeks of the (2%) high-cholesterol diets significantly increased serum total cholesterol, LDL-C, HDL-C, and TG. These observations indicate that atherogenic diets induce hypercholesterolemia in the experimental New Zealand rabbit model. Although amlodipine treatment enhanced HDL/LDL and HDL/cholesterol ratios in this group, these effects were not statistically significant. The observed significant increase in plasma HDL-C and decrease in TG is considered to be the main effect of amlodipine treatment on the serum lipid profile in the control group [Table 1].

ET-1 level

The plasma level of ET-1 in the atherosclerotic model group was significantly increased as compared with the control

Table 1

Comparison of the serum lipid profile changes (mg/dl) among four groups of New Zealand rabbits administered amlodipine and/or high cholesterol diet

Variable	*NC*	*NA*	*HC*	*HA*
Total cholesterol	49.13 ± 0.6	40.3 ± 0.8	860.3 ± 0.6*$	524.5 ± 5.8*#$
LDL	7.23 ±1.39	13.13 ± 0.20	722 ± 0.86*$	451.43 ± 6.70*#$
HDL	14 ± 0.73	19.83 ± 0.54*	49 ± 0.63*$	48.33 ± 0.95*$
TG	95.50 ± 1.7	81 ± 0.50*	466.6 ± 2.5*$	138.6 ± 1.8*#$
HDL/LDL	2.47 ± 0.60	1.50 ± 0.05	0.07 ± 0.001*$	0.11 ± 0.002*$
HDL/CHOL	0.35 ± 0.02	0.4 ± 0.007*	0.06 ± 0.001*$	0.09 ± 0.001*$

Data are expressed as mean ± SEM (n = 9) for each group, Differences of $P < 0.05$ were considered significant, *NC *vs* NA, HC, and HA; #HC *vs* HA; $NA *vs* HC and HA, NC - Normal diet control; NA - Normal diet with amlodipine; HC - High cholesterol diet control; HA - High cholesterol diet with amlodipine

group ($P < 0.01$). After treatment with amlodipine for 8 weeks ET-1 level reduced significantly in the control ($P < 0.01$) and high-cholesterol diet rabbits ($P < 0.01$). High-cholesterol diet increased the tissue level of ET-1 as compared to the control group ($P < 0.01$). Amlodipine administration significantly reduced the tissue levels of endothelin in control and high-cholesterol diet rabbits ($P < 0.01$) [Table 2].

Histological findings

Eight weeks of a 2% high-cholesterol diet induced atherosclerotic lesions and thickening of the intima in the thoracic aorta of all the animals in the HC group. The internal layer was increased and the cells appeared yellowish-white due to the accumulation of lipids. Hypertrophy of endothelial cells and accumulation of lipids in the endothelial layers, with calcification in the media, indicates induction of atheroma. Amlodipine consumption reduced atherotic injuries in high-cholesterol diet rabbits. There were no lesions in the normal diet group or the normal diet with amlodipine group [Figures 1-3].

Figure 1: Example of standard H and E staining of aorta for evaluating atherosclerotic lesions in 2% high-cholesterol diet group. No lesions were observed in control groups. 2% high-cholesterol diet induced atherosclerotic lesions; the diameter of the internal layer was increased and the cells, due to accumulation of lipids, are seen as yellowish white. Hypertrophy of endothelial cells and accumulation of lipids in endothelial layers, with calcification in the media, indicates induction of atheroma. I = intima, M = media, A = adventitia, CA = calcification area, EC = endothelial cell

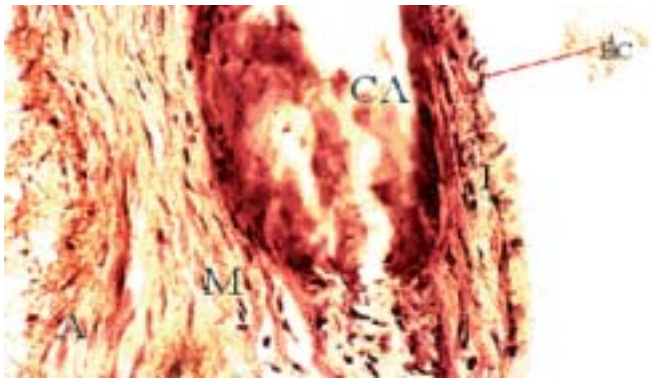


Table 2

Comparison of plasma and tissue endothelin changes among four groups of New Zealand rabbits administered amlodipine and/or high cholesterol diet

Group	*Plasma endothelin (pg/ml)*	*Aorta tissue endothelin (pg/100 mg tissue)*
Control	0.56 ± 0.01	0.02 ± 0.003
Amlodipine	0.39 ± 0.01*	0
Cholesterol diet	0.8 ± 0.04*	1.15 ± 0.02*$
Amlodipine and cholesterol diet	0.6 ± 0.01$#	0.95 ± 0.02*$#

Data are expressed as mean ± SEM (n = 9) for each group, Differences of $P < 0.05$ were considered significant, *NC *vs* NA, HC, and HA; #HC *vs* HA; $NA *vs* HC and HA, NC - Normal diet control; NA - Normal diet with amlodipine; HC - High cholesterol diet control; HA - High cholesterol diet with amlodipine

Discussion

Our results indicate that 8 weeks of a 2% high-cholesterol diet increased all lipid fractions and induced formation of atherosclerotic lesions, including thickening of the intima and/or macrophage foam cell formation, in the thoracic aorta. Because excessive cell calcium transport contributes to many cellular changes in atherogenesis, it has been proposed that the pharmacologic calcium blocker, amlodipine, may be effective in slowing the progression of atherosclerosis.[17] The key finding of this study was that the second generation dihydropyridine, amlodipine, is able to inhibit progression of preexisting atherosclerotic plaque; the formation of ET-1 was also significantly higher in atherosclerotic rabbits. These changes with amlodipine are similar to those reported in rabbits, swine, monkeys, and humans.[18] Based upon these studies, it appeared that CCBs would be most effective if administered concomitantly with the atherogenic stimuli (i.e., cholesterol). Since amlodipine is highly lipophilic, the drug

Figure 2: Example of standard H and E staining of aorta for evaluating atherosclerotic lesions in 2% high-cholesterol diet group. No lesions were observed in control groups. 2% high-cholesterol diet induced atherosclerotic lesions. (Calcification in lipid background with foam cells of medial area, calcification of inflammatory cells, and accumulation of cells in the lateral side indicates induction of atheroma). I = Intima, M = media, A = adventitia, CA = calcification area, FC = foam cell

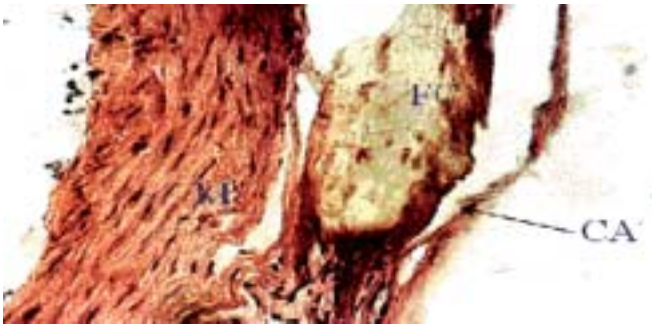
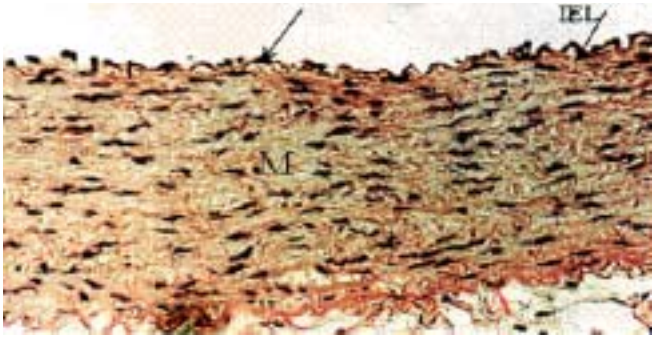


Figure 3: Example of standard H and E staining of aorta for evaluating atherosclerotic lesions in 2% high-cholesterol diet with amlodipine group. Treatment with amlodipine produced a decrease in atherotic injuries - seen as a reduction in the diameter of the endothelial layer and a marked reduction in the number of foam cells; accumulation of lipids was also reduced and inflammatory cells were absent. This indicates that amlodipine treatment was effective in decreasing the atherosclerotic process. I = intima, M = media, A = adventitia, CA = calcification area, EC = endothelial cell, IEL = intra elastic layer



can be rapidly absorbed in the atheroma of atherosclerotic lesions; it accumulates locally and acts more effectively in the atheromatous artery. If the lesions have already begun to form, CCBs usually showed little or no effect.[8] Because of marked increase in calcium permeability in SMC during the development of atherosclerotic lesions, a role for CCBs in the prevention of these lesions would seem reasonable. However, many reports failed to confirm this effect and the role of CCBs in atheroprotection was not established.[19]

The search for a CCB that might inhibit atherogenesis revealed a variety of interesting actions of the second generation dihydropyridine, amlodipine. Because excessive cell calcium transport contributes to many cellular changes in atherogenesis, it has been proposed that antagonists may be effective in slowing the progression of atherosclerosis and heart diseases.[17] Although how amlodipine improves atherosclerosis is still unclear, several possible mechanisms for the anti-atherogenesis (i.e., effects of amlodipine) have been proposed: recruitment of macrophages, lipid oxidation, and proliferation of SMC that are calcium dependent and may be influenced by amlodipine.[17]

ET-1 contributes to vasoconstriction and cell proliferation, thereby promoting vascular growth and atherogenesis.[20] ET-1 may be an early marker and mediator of endothelial dysfunction, leading to enhanced vasoconstrictor responses and contributing to the development of atherosclerotic lesions.[21] Several observations have linked hypercholesterolemia with the endothelin system and progression of atherosclerosis.[22] Increased ET-1 level due to a high-cholesterol diet may be attributed to high levels of lipids and some lipoproteins (LDL) produced by high-cholesterol diets. Recently, it has been reported that oxidized lipids can also induce endothelin converting enzyme-1 expression in human endothelial cells.[3] In our study, hypercholesterolemia produced by a high-cholesterol diet might have contributed to enhanced ET-1 formation via increase of lipids and LDL.

ET-1, via its chemoattractant properties, plays an important role in the recruitment of cells in the early stages of plaque development. ET-1 has mitogenic effects on smooth muscle cells and fibroblasts, thus contributing to the fibroproliferative stage of the process. Its effect on fibroblasts and connective tissue formation is also likely to play an important role in the stability of the atherosclerotic plaques. It was reported that local upregulation of ET-1 may play an important role in the pathogenesis of graft arteriosclerosis. The endothelin receptor antagonist, bosentan, could protect against this pathologic damage.[23] There is a close relationship between hypercholesterolemia and atherosclerosis and it has been suggested that atherosclerotic lesions might depend on increased lipid profiles. The high level of endothelin in hypercholesterolemic rabbits suggests that native circulating lipoproteins are important stimuli for both ECE-1 and ET synthesis. Studies have shown that prepro ET-1 and ET-1 release was stimulated by lipoprotein in endothelial cells.[24]

Thus, reduction of ET-1 by amlodipine has the potential to inhibit atherosclerotic plaques and can decrease atherosclerotic lesions. This study shows that amlodipine treatment reduces the inflammatory response and the size of macrophage foam cells. These findings, however, do not show concordance with human studies evaluating the effect of CCB treatment on atherosclerotic plaque progression.[25,26]

Our data show that amlodipine treatment may be considered as one of the important mechanisms for the prevention and regression of atherosclerosis. In conclusion, our findings suggest that blocking the ET system may provide a new and useful tool for antagonizing the proatherogenic effect on vascular function and indicate an antiatherosclerotic mechanism of action for amlodipine.

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