EFFECT OF AMLODIPINE ON BLOOD AND RENAL TISSUE CONCENTRATIONS OF ENDOTHELIN IN MALE RABBITS RECEIVING AN ATHEROGENIC DIET

Mustafa Mohammadi[(1)], Fariba Mirzaei[(2)]

Abstract

INTRODUCTION: Recent studies indicate that endothelin-1 (ET-1) and abnormality in the transfer of calcium ions have a role in the atherosclerosis process. Amlodipine can influence the risk factors associated with atherosclerosis, but the possible protective mechanisms of ET-1 are not known. We evaluated the effects of amlodipine and/or high-cholesterol diet on blood and renal tissue concentration of endothelin, as well as the role of ET-1 in the pathophysiology of atherosclerosis in male New Zealand white rabbits.

METHODS: Thirty-six male New Zealand white rabbits were divided into four groups: The normal control group, normal group receiving amlodipine, high-cholesterol diet group and high-cholesterol diet plus amlodipine group. After 8 weeks, all animals were anesthetized and blood or tissues samples were colleted.

RESULTS: Amlodipine led to significant increase in plasma high-density lipoprotein cholesterol (HDL-C) and decrease in serum triglyceride (TG) in the control group. The plasma level of ET-1 in the atherosclerotic model group increased significantly compared with the control group (p<0.01). After 8 weeks of treatment with amlodipine, ET-1 levels decreased significantly in the control group (p<0.01) and high-cholesterol diet rabbits (p<0.01). Amlodipine administration significantly reduced the tissue levels of endothelin only in high-cholesterol diet rabbits (p<0.01). Eight weeks of high-cholesterol diet (2%) did not induce any atherosclerotic lesion in this artery, and amlodipine had no significant effect.

CONCLUSIONS: The increase of lipids and ET-1 in the renal artery and plasma with a high-cholesterol diet is not linked to the early stages of atherosclerotic plaque formation. Amlodipine can reduce levels of ET-1 and lipids, but the mechanisms remain to be determined.

Introduction

Atherosclerosis is the leading cause of mortality and morbidity in the developed world and most of the developing countries.[1] It is a complex process, and is possibly related to a high-fat diet and sedentary lifestyle.[2] Hypercholesterolemia is one of the most important atherosclerosis risk factors which promote functional and structural vascular injury.[3] Atherosclerosis is a progressive and systemic vascular disorder that initiates molecular and cellular events triggered by endothelial dysfunction, resulting in decreased nitric oxide production, increased ET-1 production and cyclooxygenase 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 endothelin converting enzyme (ECE).[6] ET-1, the most potent vasoconstrictive substance known today, exerts different biological effects in a large variety of cardiovascular diseases, including atherosclerosis.[7]. Besides its vasoconstrictor effects, ET-1 contributes to cell proliferation, thereby promoting vascular growth and atherogenesis.[6] Recent studies have demonstrated that ET-1 contributes to atheroma formation and has an important impact on the progression of atherosclerosis.[8] Furthermore, it has been shown that macrophages , endothelial cells, and smooth muscle cells produceET-1 locally in the atherosclerotic intima by,.[9,10] Taken together, in vitro observations suggest that ET-1, released in excess during atherosclerosis, might contribute to the development of atherosclerotic lesions.[8]

(1) Mustafa Mohammadi, Ph.D. Associate Professor, Department of Physiology, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Tel: +98 (411) 3364664, Email: m.mohammadin@yahoo.com
(2) Fariba Mirzaei, M.Sc., Department of Physiology, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Atherosclerosis is a progressive disorder and risk factor for atherosclerotic renovascular disease (ARVD). It has been shown that vascular obstruction increases linearly with atherosclerosis. Atherosclerosis, which usually involves the proximal third of the main renal artery accounts for 90% of cases of renal artery stenosis.[9] A growing body of evidence showed that renal ischemia secondary to atherosclerosis as a cause of renal failure in the elderly. Eligible patients can be randomized to a medical treatment with hypotensive drugs such as calcium channel blockers.[10].

Calcium channel blockers (CCBs) have been suggested as a deterrent of cardiovascular disease and atherosclerosis, and their antiatherogenic effects have been described in patients with coronary artery disease.[11]. A variety of studies performed in humans and animals have indicated that these drugs can influence the natural process of atherosclerosis.[12-14].

Amlodipine, a third generation calcium antagonist is a long-acting lipophilic dihydropyridine calcium channel-blocking agent which contains a charged amino group and has 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 atherosclerotic lesions.[15]. However, this effect could not be confirmed by others[16] and remains subject to controversy. In some animal studies, the effect was not significant[17] and the anti-atherosclerotic potential of CCBs is under debate. Amlodipine can also positively influence the risk factors associated with atherosclerosis, but the mechanisms by which it might exert a protective effect are not known.

We postulated that amlodipine alters the blood and tissue levels of ET-1 and the progression of carotid artery atherosclerosis; hence we evaluated the effect of amlodipine on the blood and tissue levels of ET-1 in hypercholesterolemic rabbits.

Materials and methods

A number of 36 male New Zealand white rabbits (1.4 kg of weight at the beginning) were divided into four groups of equal number, namely the normal control group (NC), normal group receiving amlodipine (NA), high-cholesterol diet group (HC), and high-cholesterol diet plus amlodipine group (HA). The control group was fed normal rabbit chow, whereas the high-cholesterol diet groups were fed with high-cholesterol diet (2%). Cholesterol powder (Merck Company, Germany) was added to normal food. NA and HE groups received amlodipine powder (Arya Company, Iran) 5 mg/kg/day. All animals were housed in an environmentally controlled animal room.

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

Proximal third of the main left renal artery was immediately isolated and then was homogenized to measure ET-1 (homogenized solution: 20 mmol/l hydrochloric acid +1 mol/l acetic acid). The homogenized solution was centrifuged (10 min, 3000, 6 °C) and the light supernatant was taken and stored at -80 °C until analyzes. Tissue ET-1 was measured using a special kit (No: 030806265) after lyophilizing with lyophilizator (Christ Aplphal 4).

Renal artery tissue was immediately isolated and placed in formalin 10%. Briefly after tissue processing, several serial blood vessel segments (6 μm in thickness) were stained by standard hematoxylin-eosin method and studied by light microscopy.

Serum lipid profile including TC, low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglyceride (TG) were determined by enzymatic methods using automatic analyzer (Abbott, Alcyon 300, USA).

Data were analyzed by SPSS, and are expressed as mean ± standard error of mean (SEM). Possible differences between groups were examined by one-way ANOVA and Tukey's post hoc test. P values of less than 0.05 were considered as statistically significant.

Results

Eight weeks consumption of 2% high-cholesterol diet significantly increased serum TC, LDL-C, HDL-C, and TG. These observations showed that an atherogenic diet led to hypercholesterolemia in our experimental New Zealand rabbit model. Although amlodipine treatment tended to enhance HDL-C/LDL-C and HDL-C/cholesterol ratios in the

mentioned group, these effects were not statistically significant. The significant increase observed in plasma HDL-C and the decrease in TG is considered to be the main effect of amlodipine treatment on serum lipid profile in the control group (Table 1).

The plasma level of ET-1 in the atherosclerotic model group increased significantly compared with the control group (p<0.01). ET-1 level decreased significantly in the control group (p<0.01) and in high-cholesterol diet rabbits (p<0.01) following eight weeks of treatment with amlodipine. High-cholesterol diet increased the tissue level of ET-1 significantly compared to controls (p<0.01). Amlodipine administration led to a significant reduction of endothelin levels only in high-cholesterol diet rabbits (p<0.01) (Table 2).

The 2% high-cholesterol diet did not induce any atherosclerotic lesion. Amlodipine treatment had any significant effect. No lesion was seen either in the NC or in the NA group (Figures 1, 2).

Discussion

In this experimental study, eight weeks of 2% high-cholesterol diet increased all components of serum cholesterol profile but did not induce the formation of atherosclerotic lesions. ET-1 formation was significantly higher in atherosclerotic rabbits and was significantly reduced with amlodipine treatment. Our results contradict reports suggesting that a high-cholesterol diet can induce atherosclerosis and that amlodipine can alter the development and progression of arterial lesions.[11]

Our key finding was that 2% high-cholesterol diet did not induce atherosclerotic plaque formation; this is in contrast to results reported from studies on rabbits, swine, monkeys and humans.[18]

The search for a CCB that might inhibit atherogenesis revealed a variety of interesting actions of the third generation of dihydropyridine calcium antagonist, amlodipine.

TABLE 1. Comparison of serum lipid profile alterations in four groups of New Zealand rabbits administered amlodipine and/or high-cholesterol diet.

Variable	NC	NA	HC	HA
TC(mg/dl)	49.13 ± 0.6	40.3±0.8	860.3±0.6 * $	524.5±5.8*#$
LDL-C(mg/dl)	7.23±1.39	13.13±0.20	722±0.86*$	451.43 ±6.70*#$
HDL-C(mg/dl)	14±0.73	19.83±0.54*	49±0.63 *$	48.33±0.95*$
TG(mg/dl)	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/TC	0.35±0.02	0.4±0.007*	0.06±0.001*$	0.09±0.001*$

Data are expressed as mean ± SEM (n=6) for each group.
P<0.05 was considered significant.
*NC vs. NA, HC and HA; # HC vs. HA; $ NA vs. HC and HA.
Abbreviations: NC=normal-diet control; NA=normal diet with amlodipine; HC=high-cholesterol diet control; HA=high-cholesterol diet with amlodipine; TC=total cholesterol,TG: triglycerides

TABLE 2. Comparison of plasma and tissue endothelin changes in renal artery among four groups of experiment

Group	Plasma endothelin (pg/ml)	Kidney tissue endothelin (pg/100 mg tissue)
Control	0.56± 0.01	0.83 ±0.02
Amlodipine	0.39 ±0.01*	0.74 ±0.05
Cholesterol diet	0.8 ±0.04*	71.7± 1.94*$
Amlodipine and cholesterol diet.	0.6 ±0.01$#	39.16 ±1.72*$#

Data are expressed as Mean ± SEM (n=9) for each group.
P<0.05 was considered significant.
*NC vs. NA, HC and HA; # HC vs. HA; $ NA vs. HC and HA.
Abbreviations: NC=normal-diet control; NA=normal diet with amlodipine; HC=High-cholesterol diet control; HA=high-cholesterol diet with amlodipine
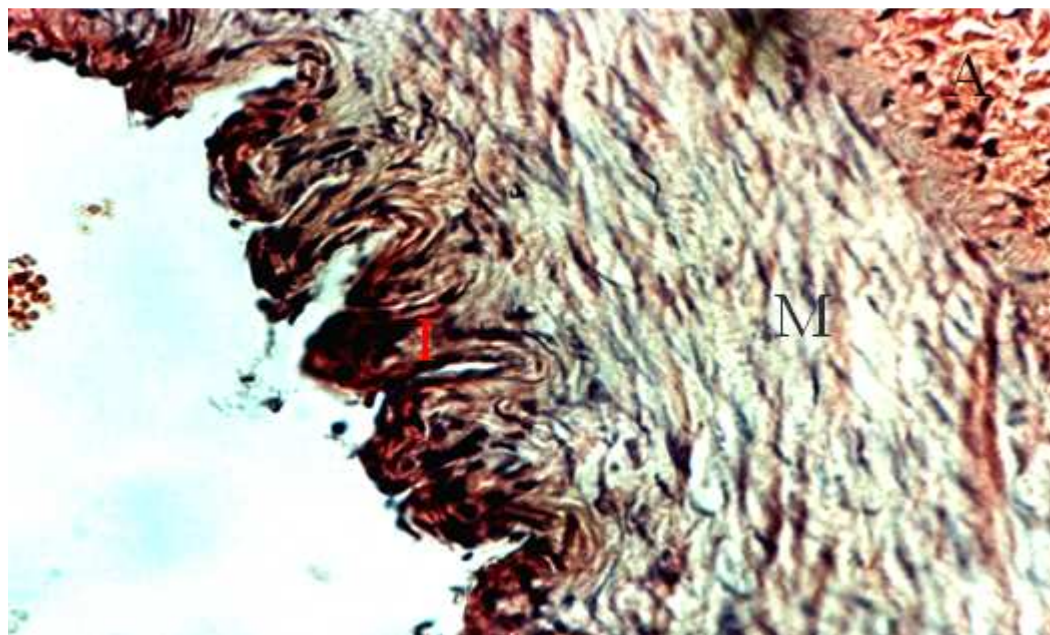
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FIGURE 1. Example of standard H & E staining of renal artery for evaluating atherosclerotic lesions in 2% high-cholesterol diet group. No lesion was observed in the control group. Consumption of 2% high-cholesterol diet did not induce any atherosclerotic lesions in this artery. All of these changes suggest that consumption of 2% high-cholesterol diet did not induce atherosclerosis lesions.
I=intima, M=media, A=adventitia, CA=calcification area, FC=foam cell; large scale 660
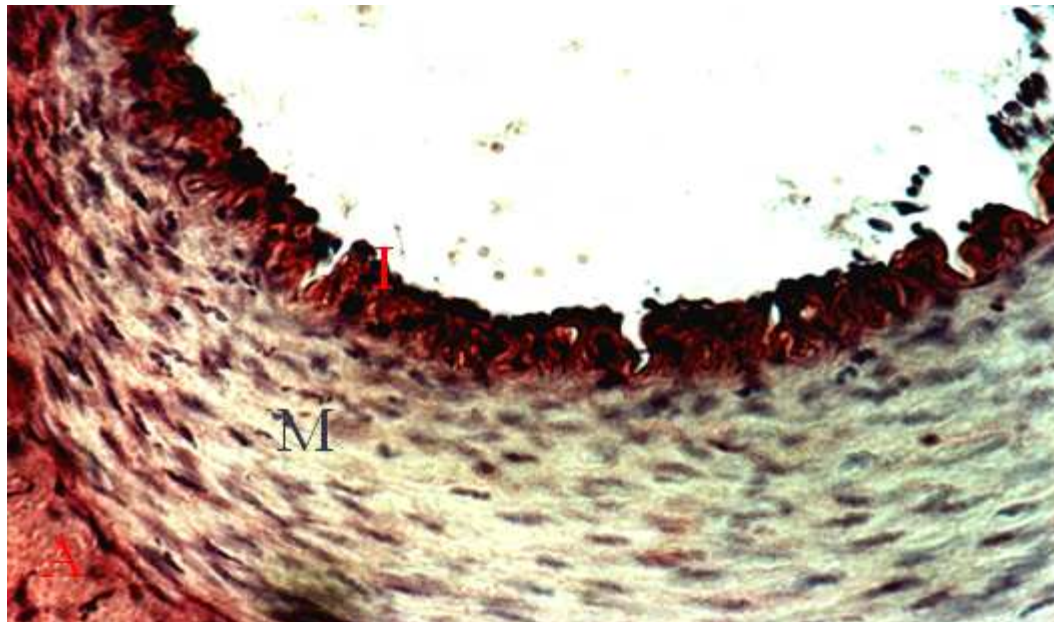

FIGURE 2. Example of standard H & E staining of carotid artery for evaluating atherosclerotic lesions in 2% high-cholesterol diet with the amlodipine group. No significant effects were observed following amlodipine treatment.
I=intima, M=media, A=adventitia, CA=calcification area, EC=endothelial cell, IEL=intra-elastic layer, large scale 660

Excessive cell calcium transport contributes to many cellular changes in atherogenesis, hence it has been proposed that antagonists may be effective in slowing the progression of atherosclerosis and heart disease.[19] We found no significant changes in the atherosclerotic process following the administration of 2% high-cholesterol diet or amlodipine.

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 following a high-cholesterol diet may be attributed to high levels of lipids and some lipoproteins (LDL). 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 through increasing lipids and LDL.

With its chemo-attractant properties, ET-1 plays an important role in the recruitment of cells in the early stages of plaque development. Its effect on fibroblasts and connective tissue formation is also likely to play an important role in the stability of the atherosclerotic plaques.[23] The high level of endothelin in hypercholesterolemic rabbits suggests that native circulating lipoproteins are important stimuli of ET-1 synthesis. ET-1 release was found to be stimulated by lipoprotein in endothelial cells.[24] In patients with symptomatic atherosclerotic vascular disease, plasma ET-1 concentrations are higher than in normal subjects, and a significant correlation is demonstrable between plasma ET-1 and the number of vascular sites exhibiting atherosclerosis.[25]

It has also been reported that local up-regulation of ET-1 may play an important role in the pathogenesis of graft arteriosclerosis and that a close relationship exists between hypercholesterolemia and atherosclerosis,[26] however, we suggest that formation of atherosclerotic lesions might not be completely dependent on increases in lipid profile components and/or ET-1.

The reduction of ET-1 by amlodipine has the potential to positively affect the atherosclerotic process. It cannot be confirmed that high levels of ET-1 and lipid profile are associated with atherosclerotic lesions. Further studies of the effect of a high-cholesterol diet and CCB treatment on atherosclerotic plaque progression in renal artery are warranted.[28,29]

In the current study, the increase of ET-1 in renal artery and plasma was not linked to early stages of atherosclerotic plaque formation. Thus, enhanced ET-1 production may not substantially contribute to cell growth and progression of atherosclerotic lesions. We suggest that amlodipine treatment can reduce levels of ET-1 as well as other lipid profile components; however, more studies are needed to understand whether amlodipine can contribute to prevention and/or regression of atherosclerosis or not.

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