Evaluation the Effects of Amlodipine on Atherosclerosis Progression in High Cholesterol-Fed Male Rabbits.

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ABSTRACT
The objective of present study was to assess the effect of amlodipine on atherosclerosis via interfering with inflammatory and oxidative pathways. Twenty four local domestic male rabbits were involved in this study. The animals were randomly divided into three groups which include: Group I rabbits fed normal chow (oxoid) diet for 12 weeks. Group II rabbits fed with 1% cholesterol enriched diet. Group III rabbits fed with 1% cholesterol enriched diet together with amlodipine (5 mg/kg once daily before morning feed). Blood samples were collected before zero time and every four weeks of experimental diets for measurement of serum triglycerides, total cholesterol, high density lipoprotein cholesterol, high sensitive C-Reactive Protein, endothelin-1 and intracellular adhesion molecule-1 level. At the end of 12 weeks the aorta was removed for measurement of aortic Malondialdehyde, reduced glutathione and aortic intimal thickness. Amlodipine treatment did show an insignificant effect on lipid parameters compared with induced untreated group (P > 0.05). While it was significantly reduced the elevation in endothelin-1, high sensitive C-Reactive Protein, intracellular adhesion molecule-1 level, aortic MDA and aortic intimal thickness compared with induced untreated group (P < 0.05), and they restore aortic reduced glutathione level (P < 0.05). Amlodipine may decrease atherosclerosis progression in hypercholesterolemic rabbit via interfering with oxidative and inflammatory pathways without affecting lipid parameters.

Keywords: amlodipine, inflammatory markers, oxidative stress, atherosclerosis.

INTRODUCTION
Atherosclerosis is the main cause of mortality and morbidity in the developed world and most of developing countries1. Atherosclerosis is a difficult process, and it is possibly caused by high-fat diet and sedentary lifestyle2. Hypercholesterolemia is one of the most essential risk factors for atherosclerosis and cardiovascular disease. Atherosclerosis is a progressive structural and functional vascular disorder that initiates molecular and cellular events activated by endothelial dysfunction, resulting in reduced nitric oxide production, increased endothelin-1 [ET-1] synthesis and cyclooxygenase activity and inflammation3. The incidence of atherosclerosis is 3-4 times greater in diabetics than non-diabetics patient at comparable plasma cholesterol concentrations4. It has been extensively demonstrated that inflammation plays an essential role in atherogenesis and mediating all stages of this disease from initiation through progression and, finally, the thrombotic complications of atherosclerosis5. Inflammation contributes to the development and progression of atherosclerosis and the therapeutic potential of some anti-inflammatory agents have been evaluated for possible anti atherosclerotic activity. Recent researches suggested that some drugs with anti-inflammatory properties appear to have a helpful effect on atherosclerosis or subsequent risk for cardiovascular events5. Calcium channel antagonists have been suggested as a deterrent of cardiovascular disease and atherosclerosis, and their anti-atherogenic action have been described in patients with coronary artery disease7. A variety of studies done in humans and animals have indicated that calcium channel blockers can affect the natural progression of atherosclerosis8. Amlodipine, a third generation calcium channel-blocking agent is a long-acting dihydropyridine calcium antagonist that is lipophilic and have a charged amino group and a lipid partition coefficient of about 1200. This reflects its ability to penetrate to the cell membrane, and it can prevent calcium permeability in vascular smooth muscle cells (SMC) and decrease the atherosclerotic lesions9. In some animal studies, the effect has been indifferent and the anti-atherosclerotic effect of CCBs and underlying mechanisms are under question10. Amlodipine can also positively effect risk factors that are associated with atherosclerosis, but all the mechanisms by which it might protect are not known. Previously, we have found that high-cholesterol regimen lead to a series of histopathological changes in the aortic artery such as atheroma formation, rupture of intima and calcification of media in some loci of aortic tissue and amlodipine treatment improved all alterations11. Therefore, the goal of this study was to view of amlodipine as an anti-atherosclerotic effect.

MATERIALS AND METHODS
Twenty four local domestic male rabbits were involved in this study. The animals were randomly divided into three
groups include. Group I rabbits fed normal chow (oxid) diet for 12 weeks. Group II rabbits fed with 1% cholesterol enriched diet for 12 weeks. Group III rabbits fed with 1% cholesterol enriched diet together with amloidipine (5 mg/kg once daily before morning feed for 12 weeks).

The blood samples were collected before (0 time) and every four weeks on experimental diets for measurement of serum triglycerides (TG), total cholesterol (TC), HDL-C, endothenin-1 (ED-1), high sensitive C-Reative Protein (hsCRP) and intracellular adhesion molecule-1 (ICAM-1) level.

At the end of 12 weeks the aorta was removed for measurement of aortic Malondialdehyde (MDA), reduced glutathione (GSH) and aortic intimal thickness.

**RESULTS**

There was a slight insignificant increase in body weight of amloidipine receiving group suggesting that food consumption probably was similar in all the groups and cholesterol or amloidipine had no effect on body weight. Compared with the control, levels of TC, TG, HDL-C, LDL-C, atherogenic index, hsCRP, ED-1, ICAM-1, aortic MDA and aortic intimal thickness were increased and aortic GSH were decreased in the animals with atherogenic diet ($P < 0.05$). Amloidipine treatment don’t show significant effect on lipid parameters compared with induced untreated group ($P > 0.05$). Amloidipine reduced the elevation in hsCRP, ED-1, ICAM-1, aortic MDA and aortic intimal thickness compared with induced untreated group ($P < 0.05$). Also they restore aortic GSH level ($P < 0.05$) as shown in Table (1), Table (2) and Table (3).

![Table 1: Changes of rabbit's serum lipid profile (TC, TG and HDL) of the three experimental groups. The data was expressed as Mean ± SEM (N=8 in each group) Using paired T-test.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>TC(mg/dl)</th>
<th>TG(mg/dl)</th>
<th>HDL(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Zero time</td>
<td>80 ± 1.92</td>
<td>36.3 ± 4.56</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>85 ± 4.26</td>
<td>35.6 ± 4.2</td>
</tr>
<tr>
<td>Induced untreated</td>
<td>Zero time</td>
<td>95 ± 1.75</td>
<td>35 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>995 ± 31*</td>
<td>348 ± 16.3*</td>
</tr>
<tr>
<td>Amlodipine 5mg/kg</td>
<td>Zero time</td>
<td>86 ± 2.36</td>
<td>37.2 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>989 ± 11.9*</td>
<td>335 ± 10.2*</td>
</tr>
</tbody>
</table>

*p<0.05

![Table 2: Changes of rabbit’s serum Inflammatory markers (hs-CRP, ED-1 and ICAM-1) of the three experimental groups. The data was expressed as Mean ± SEM (N=8 in each group) Using paired T-test.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Hs-CRP(mg/l)</th>
<th>ED-1(pg/ml)</th>
<th>ICAM-1(pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Zero time</td>
<td>2.8 ± 0.7</td>
<td>0.41 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>2.9 ± 0.9</td>
<td>0.45 ± 0.2</td>
</tr>
<tr>
<td>Induced untreated</td>
<td>Zero time</td>
<td>3.1 ± 0.5</td>
<td>0.45 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>22.6 ± 2.4*</td>
<td>1.6 ± 0.5*</td>
</tr>
<tr>
<td>Amlodipine 5mg/kg</td>
<td>Zero time</td>
<td>3.2 ± 0.2</td>
<td>0.42 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>12 weeks</td>
<td>11.4 ± 1.7*</td>
<td>0.84 ± 0.2*</td>
</tr>
</tbody>
</table>

*p<0.05

![Table 3: The means of rabbit’s aortic Oxidative stress parameters (MDA and GTH) and aortic intimal thickness of the three experimental groups at the end of experiment. The data was expressed as Mean ± SEM (N=8 in each group) Using paired T-test.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Aortic MDA µmole/gm aorta</th>
<th>Aortic GTH nmole/mg</th>
<th>aorta Aortic intima thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.1 ± 0.24</td>
<td>37.3 ± 2.6</td>
<td>29.4 ± 3.7</td>
</tr>
<tr>
<td>Induced untreated</td>
<td>10.2 ± 0.51*</td>
<td>19.8 ± 1.5*</td>
<td>356.2 ± 32.5*</td>
</tr>
<tr>
<td>Amlodipine 5mg/kg</td>
<td>4.7 ± 0.64**</td>
<td>31.6 ± 2.7**</td>
<td>211.3 ± 22.5**</td>
</tr>
</tbody>
</table>

*p<0.05
** p<0.05 as compare to induced untreated
Figure 1: Effect of amlodipine 5 mg/kg/day treatment on serum lipid profile (TC, TG and HDL) levels (mg/dl) during the experimental treatment.

Figure 2: Effect of amlodipine 5mg/kg/day treatment on serum hs-CRP (mg/l), serum ED-1 (pg/ml) and serum ICAM-1 (pg/ml) in comparisons to the two control group (normal and induced untreated).

Figure 3: Effect of amlodipine 5mg/kg/day and treatment on aortic MDA µmole/gm aorta, aortic GTH nmole/mg aorta and aortic intimal thickness level (µm) in comparisons to the two control group (normal and induced untreated).
Our results indicated that 12 weeks consumption of 1% high-cholesterol diet increased all serum cholesterol profile fractions and induced development of atherosclerotic lesions including the thickening of the intima and/or accumulation of lipid droplets under endothelial cells in carotid artery. Moreover, the results of the current study shown that the level of ET-1 and other inflammatory markers were significantly higher in the atherosclerotic rabbits and amlodipine treatment could significantly reduce it. Thus, our results support the hypothesis that amlodipine treatment changed the development of carotid artery lesion.

The key finding of this research was that, amlodipine is able to suppress the progression of atherosclerotic plaque in our rabbits. These changes are similar to those found in rabbits, swine, monkeys and humans by using amlodipine.

Since amlodipine is highly lipophilic, it can be rapidly absorbed in the atheroma of atherosclerotic lesions, accumulating locally, and it acts more efficiently in atheromatous artery. Because of marked increase in calcium permeability in smooth muscle cells during the expansion of atherosclerotic lesions, a role for CCBs in the avoidance of lesions would seem reasonable. But many reports failed to confirm this action and the atheroprotection role of CCBs was not established.

Amlodipine improves atherosclerosis is still unclear, several probable mechanisms of the anti-atherogen action of amlodipine have been proposed: lipid oxidation, recruitment of macrophages and proliferation of smooth muscle cells that are calcium dependent and may be affected by amlodipine.

Effects of amlodipine on study parameters:

In this study we demonstrated that treatment with amlodipine appeared to has no significant change on lipid parameters in comparison with the induced untreated group and this may be due to that the change in lipid parameter affected by high fat diet overrides any changes expected from amlodipine.

Moreover in the current study treatment with amlodipine was significantly reduced the elevation of inflammatory markers (hs-CRP, ED-1 & ICAM-1) in atherosclerosis model of hypercholesterolemic rabbit suggesting that amlodipine reduce vascular inflammation induced by high cholesterol diet.

Our study based on fact that inflammation has an essential role in atherogenesis which would make it a promising target for future therapy. Our finding is in agreement with who had found that there were significant decrease in ED-1 level in cholesterol-fed rabbits treated with amlodipine.

Also this is in agreement with who had found that there was a significant decrease in TNF-α level in Zucker Metabolic Syndrome Rats treated with amlodipine.

In addition to that in the current study amlodipine had significantly reduced aortic MDA level suggesting decrease in reactive oxygen species and subsequent lipid peroxidation. Also amlodipine had significant effect on aortic GSH levels where prevents GSH reduction in hypercholesterolemic rabbit, and thus, maintain antioxidant balance which is essential for vascular protection against lipid peroxide. Thus the potent anti-inflammatory action of amlodipine might contribute to its anti-oxidative effect through reduction of proinflammatory acute phase protein (hsCRP).

It has also been found that amlodipine decrease oxidative stress by restoring copper/zinc-containing SOD activity in the heart in hypertensive rats. Moreover, amlodipine has been shown to enhance nitric oxide (NO) production via stimulation of the formation of endothelial nitric oxide synthase (NOS).

In the present study there was a significant reduction of aortic intima thickness in amlodipine treated rabbits as compared with that in the induced untreated group. This
is in agreement with who demonstrated that amlodipine treatment by reducing the ET-1 may contribute to reducing the progression of atherosclerotic disease\textsuperscript{14,15} and who suggested that amlodipine not only inhibits atherosclerotic lesion formation, but also regresses atherosclerosis, and that these effects are at least partly due to inhibition of oxidative stress and inflammatory response\textsuperscript{20,21}.

Atherosclerosis is now widely established as a chronic inflammatory process. Low-grade inflammation, enhanced oxidant stress and lipid peroxidation. The athero-protective effect of amlodipine is may be due to interfering with inflammatory and oxidative pathways in hypercholesterolemic rabbit.

The mechanism by which amlodipine regresses atherosclerotic lesions is not yet clear. In the current study, administration of amlodipine was decreased the expression of ICAM-1 and ED-1, suggesting that amlodipine inhibited the infiltration of monocytes/macrophages into the atherosclerotic lesions. It has been reported that amlodipine decreased the DNA synthesis enhanced by basic fibroblast growth factor through suppression of extracellular signal regulated kinase activity in human vascular smooth muscle cells\textsuperscript{22}.

It has recently been reported that macrophage apoptosis is associated with decreased lesion progression, and that amlodipine repressed aortic wall hypertrophy in spontaneous hypertensive rats through improved apoptosis suggesting that apoptosis is involved in the regulation of atherosclerosis\textsuperscript{23}. These results suggest that both the suppression of VSMC proliferation and the enhancement of apoptosis might be involved in the regression of atherosclerotic lesions by amlodipine\textsuperscript{24}. Amlodipine is highly lipophilic, it can be rapidly absorbed in the atheroma of atherosclerotic lesions, accumulating locally, and it acts more effectively in atheromatous artery\textsuperscript{14}.

Because of marked increase in calcium permeability in smooth muscle cells during the formation of atherosclerotic lesions, a role for CCBs in the avoidance of lesions would seem reasonable. But many reports failed to confirm this effect and the atheroprotection role of CCBs was not established\textsuperscript{8}.

Because excessive cell calcium transport contributes to many cellular changes in atherosogenesis, it has been proposed that calcium channel blockers may be effective in slowing the progression of atherosclerosis and heart diseases\textsuperscript{15}.

Although how exactly amlodipine improves atherosclerosis is still unclear, several possible mechanisms of the anti-atherogen effects of amlodipine have been proposed: lipid oxidation, recruitment of macrophages and proliferation of smooth muscle cells that are calcium dependent and may be affected by amlodipine. Furthermore, we demonstrated that amlodipine prevent lipid peroxidation as shown by lowering serum MDA level which may be further contribute to its protective effect against hypercholesterolemic atherosclerosis.

REFERENCES

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