Research Article

Antiatherosclerotic Effect of L- thyroxine and Verapamil Combination on Thoracic Aorta of Hyperlipidemic Rabbits

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ABSTRACT

Atherosclerosis which results from gradual deposition of lipids in medium and large arteries is a leading cause of mortality worldwide. This study was done to determine the effect of L- thyroxine with verapamil combination on the development of atherosclerosis in thoracic aorta of hyperlipidemic induced rabbits. Twenty four healthy rabbits were randomly divided into three equal groups: first group was given standard diet, second group fed with high cholesterol diet (%2 cholesterol) and third group was given high cholesterol diet (2% cholesterol) and treated by L-thyroxine combined with verapamil for eight weeks. The lipid profile and heart rate were measured for all groups. At the end of study, Histopathological examination of thoracic aorta was done in all groups**.** The high cholesterol diet cause highly significant difference in total serum cholesterol (TC), LDL, VLDL, TG levels and body weight in addition to atherosclerotic changes of thoracic aorta. The using of L-thyroxine combined with Verapamil induced highly significant reduction in TC, LDL, VLDL, TG levels and body weight while histopathological examination showed decrease atherosclerotic changes in thoracic aorta. This indicates that combination of L-thyroxin with verapamil can effectively prevent the progress of atherosclerosis without the cardiac side effect of L-thyroxine (tackycardia). This is likely due to antihyperlipidemic, and antioxidant effects of this combination.

Keywords: Atherosclerosis, L-thyroxine, verapamil, hyperlipidemia, lipid profile.

INTRODUCTION

Atherosclerosis is a chronic vascular disease and a leading cause of death in the western world. It is well established that hyperlipidemia and oxidative stress (OS) are major contributors to atherogenic development. [1] The retention of low-density lipoproteins (LDL) in the arterial wall [2] and their oxidation by reactive oxygen species (ROS) initiates a complex series of biochemical and inflammatory reactions.[3] Oxidized LDL (ox-LDL) is internalized by macrophages through the scavenger receptors, leading to foam cell formation.[4] Furthermore, oxidized cholesterol products present in blood and in arterial plaques increase cholesterol biosynthesis, affect plasma membrane structure, cell proliferation, and cell death, and promotes atherosclerosis development. [5]

Increased atherosclerosis risk in hyperlipidemic patients may be a result of the enhanced oxidizability of their plasma lipoproteins. [6]

Pathogenesis

Atherosclerosis is a chronic pathological condition, and can take decades to develop severe atheromatous lesions in humans. [7] Foam cells appear even in early stages of atherosclerosis, and the accumulation of large numbers of foam cells is often observed in advanced lesions. [8] Low density lipoprotein (LDL) oxidation is a key process in early atherogenesis [9] and thus, inhibition of LDL oxidation is considered to be antiatherogenic. Very low density lipoprotein (VLDL) and high density lipoprotein (HDL) oxidation also occurs during oxidative stress and may also contribute to atherogenesis.[10] Calcium and cholesterol deposition is the hallmark of atherosclerotic lesions in the arterial wall.[11] Earlier experiments have examined whether inorganic compounds known to interfere with Ca^{2+} fluxes could reduce the extent and gravity of experimentally induced atherosclerotic lesions. Studies on calcium-chelating agents and lanthanum, an inorganic calcium antagonist, showed that they effectively reduced the formation of atherosclerotic plaques.[12]

MATERIALS AND METHODS

Twenty four healthy domestic rabbits, weighing (800 – 1100 grams) were used in this study. They were supplied by the animal house of the college of medicine / Al-Nahrain University. They were kept at room temperature (27°C ±1°C) and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 4 weeks and were fed with a standard pellet diet and water *ad libitum*. The rabbits were randomly divided into three groups. Normal control group (Group 1) received standard chew diet. The other two groups (G2 and G3) received high cholesterol diet consisting of standard pellet diet (92%), cholesterol (2%), cholic acid (1%), and coconut oil (5%) mixed by ether, for 8 successive weeks.[13] At the end of this period, the weight of each of the rabbits was measured and daily consumptions were monitored. After induction of hyperlipidemia the third group was treated with L-thyroxine in a dose of (50 µg/day) given as single oral dose just before food, Combined with verapamil in single

dose of (16 mg/kg/day) orally for 21 days. Lipid profile, body weight and histopathological examination of thoracic aorta were done for all groups.

RESULTS

Comparison between parameters of the normal control group (G1) and those after induction of hyperlipidemia (G2)

When comparison was done between normal control group (G1) and those which undergone induction of hyperlipidemia (by using high cholesterol diet) (G2), the calculated results showed highly significant difference ($p<0.001$) in parameters (Total serum cholesterol, LDL, VLDL, TG, and body weight), significant difference ($p<0.05$) in HDL, as shown in table (1).

Comparison between parameters of hyperlipidemic group (G2) and hyperlipidemic group treated with combination of L-thyroxine and Verapamil (G3)

Total serum cholesterol, LDL, HDL, VLDL, TG, and body weight were measured for hyperlipidemic group (G2) and hyperlipidemic group treated with combination of L-thyroxine and Verapamil (G3). As shown in table below (table- 2) highly significant ($p<0.001$) difference in total serum cholesterol, LDL, VLDL, TG, and body weight was noticed, while non significant ($p<0.05$) difference was noticed in HDL.

Table 1: Comparison of parameters between normal control group (G1) and hyperlipidemic group (G2) (Results presented in mean ± SEM)

Parameters	(G1) Control Group	(G2) Induction Group
Total cholesterol (mg/dl)	43.82 ± 2.29	360.8 ± 11.93**
LDL (mg/dl)	22.4 ± 2.29	307.39 ± 17.84**
HDL (mg/dl)	23.3 ± 1.07	14.85 ± 1.22*
VLDL (mg/dl)	16.78 ± 1.04	62.83 ± 4.16**
TG (mg/dl)	83.91 ± 5.22	314.17 ± 20.82**
Wt (gm)	978.75±22.86	1537.5±39.8**

*= Significant ($p<0.05$) difference; **= Highly significant ($p<0.001$) difference

Table 2: Comparison between parameters of induction group (G2) and hyperlipidemic group treated with combination of L-thyroxine and Verapamil (G3). (Results presented in mean±SEM)

Parameters	(G2) Induction Group	(G8) L-thyroxine + verapamil Group
Total cholesterol (mg/dl)	360.8±11.93	116.56±3.95**
LDL (mg/dl)	307.39±17.84	92.66±3.15**
HDL (mg/dl)	14.85±1.22	17.97±0.87
VLDL (mg/dl)	62.83±4.16	20.24±2.7**
TG (mg/dl)	314.17±20.82	101.23±13.62**
Wt (gm)	1537.5±39.8	1162.5±33.74**

**= Highly significant ($p<0.001$) difference

Histopathological examination:

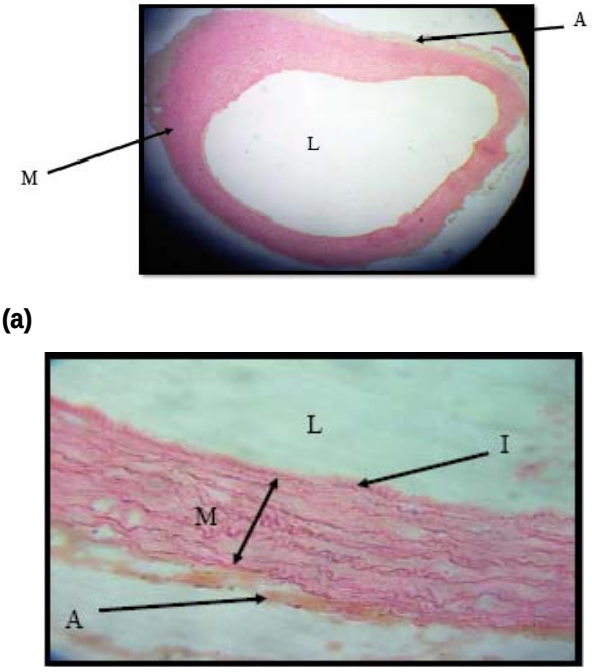

Figure 1: a & b- Transverse section of the thoracic aorta of control normal group (G1) showing normal histological features of the tunica intima (I), tunica media (M) & tunica adventitia (A). (L) Represent vascular lumen, aX10, bX40, stained with H&E.

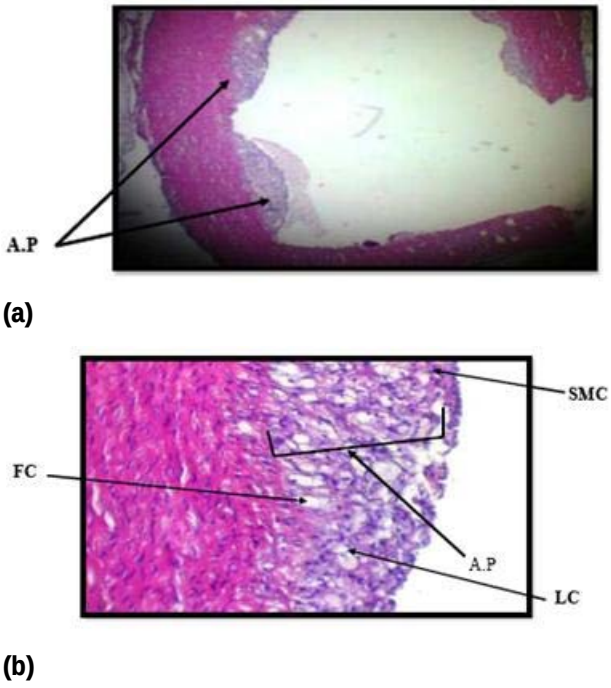

Figure 2: a & b- Transverse section of the thoracic aorta of induction group (G2) show an atherosclerotic plaque (A.P) at the thoracic aorta and narrowing of the leumen. The plaque which is an intimal lesion composed of a fibroblast, smooth muscle cells (SMC), lymphocyte (LC), foam cells (FC), Distribution of macrophages in the plaque. (a)X10 (b)X40.(H&E).

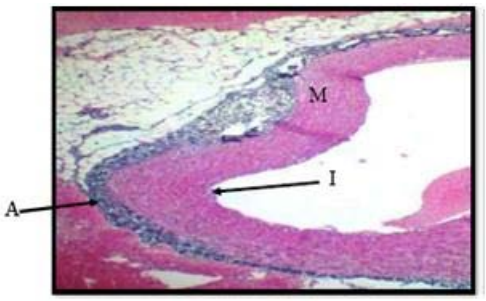

Figure 3: Transverse section of the thoracic aorta of hyperlipidemic group treated with L- thyroxine and Verapamil showing improvement of atherosclerotic changes, no plaque, normal tunica media (M), tunica adventatia (A) and mild disruption of tunica intima (I). X10,(H&E).

Methods of statistical analysis

Statistical analysis was performed by using SPSS (Statistical Package for social Science; Version 14), and Microsoft Excel Worksheet 2007. Crude data was analyzed to obtain mean and standard error of mean (SEM). Student paired t- test was used. P- Value was dependent < 0.05 as level of significance.

DISCUSSION

The hyperlipidemia is a worldwide health problem of major concern due to its accompanying rise in other metabolic disorders. [14] Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is identified as dyslipidemia, to describe the manifestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, dyslipidemia can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). [15] Hyperlipidemia plays a major role in atherogenesis and it is an important risk factor for atherosclerosis.[16] Atherosclerosis is the leading cause of mortality in developed countries. This complex disease can be described as an excessive inflammatory, fibro fatty, proliferative response that leads to damage of the arterial wall. Many believe that it can be induced from simple dysfunction of endothelial lining as occurs with hyperlipidemia. [17]

Successful induction of atherosclerosis in experimental animals was achieved by using different modified diets and chemicals to provide typical feature of atherosclerosis. Rabbits have become the most largely used experimental model to evaluate the development of atherosclerosis because they are very sensitive to cholesterol rich diet and accumulate large amount of cholesterol in their plasma. Their use as experimental models is highly relevant and brings information on factors that contribute to the progression and regression of this condition that can be applied to humans.[18]

In the present study, feeding of rabbits with high fat diet (2% cholesterol) in group 2, 3, for 8 weeks resulted in marked hyperlipidemia with a highly significant increase in serum concentration of total cholesterol (TC), triglyceride (TG), LDL, and VLDL, while HDL serum concentration was significantly decreased compared with normal control group (G1). These results are in agreement with those reported by Vidal, *et al.*, 2007. [19]

The dramatic increase in lipid profile parameters is attributed to the way in which rabbits respond to a high cholesterol diet. As cholesterol intake increase, bile acids reabsorption increase too which leads to increase its uptake by the liver. The resultant elevation in liver cholesterol content leads to an increase in VLDL production, a decrease in lipoprotein receptor activity, and an accumulation of cholesteryl ester rich VLDL and LDL in the plasma. These changes in plasma lipoproteins lead to the development of advanced atherosclerotic lesions. [20] These changes noticed in hyperlipidemic group may be due to demodulating lipid metabolism, especially by decreasing β-oxidation and increasing cholesterol synthesis and oxidative stress by decreasing free radical scavenger enzyme gene expression. [21] Also, Rui-Li *et al.*, 2006 [22] reported that high fat diet (HFD) induced abnormal increases in lipid peroxidation, serum concentrations of total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol, and a decrease in high-density lipoprotein cholesterol concentration in addition to decreased lipoprotein lipase activity, accompanied by a depressed antioxidant defense system.[23]

The weight gain in HFD group was highly significant than in normal control group, reflecting the influence of HFD at end of induction period (8 weeks).These results was in accordance with those of previous studies which showed that diets rich in fat not only induce obesity in humans but also make animals obese. [24] The factors that may contribute to obesity induced by a diet rich in fat include failure to adjust oxidation of fat to the extra fat in the diet, [25] increase in adipose tissue lipoprotein lipase activity, [26] increased meal size and decreased meal frequency,[27] as well as overconsumption of energy attributed to high energy density of the diet, [28] orosensory characteristics of fats and poorly satiating properties of the high-fat diets. [29]

Reviews of dietary obesity describe potential mechanisms of body weight and food intake regulation involving the central nervous system– mainly the hypothalamus – neuropeptides such as ghrelin and neuropeptide Y, and hormones such as insulin and leptin. [30] Adipose tissue per se is considered to be an endocrine organ that secretes cytokines such as IL-6 and TNFa; thus obesity could possibly be regarded as a chronic inflammatory disease. [31] Also in other studies, it is known that rabbits fed a cholesterol-rich diet accumulate cholesterol in nearly all organs, and that the content of cholesterol in the heart doubles within 2 to 3 months.[32] The cholesterol appears as intracellular lipid droplets[33] and is also incorporated into cell membranes, resulting in an alteration in

membrane structure and function.[34] The weight of the heart of the rabbits fed the high cholesterol diet increased by 10%.[35]

The highly significant decrease in total serum cholesterol, LDL, VLDL, and TG is partly due to the lipid lowering effect of L- thyroxine in addition to mild antioxidant effect of verapamil.[36] This combination was used to augment the up regulation of LDL receptor in liver and extrahepatic tissues to decrease the serum cholesterol because both of the drugs (L-thyroxine and verapamil) produce this effect but in deferent mechanism as mentioned by previous studies.

Generally, hypothyroidism is associated with increased levels of serum triglycerides, cholesterol and LDL cholesterol and *vice versa* hyperthyroidism is associated with their decreased levels.[37]

Thyroid hormones (such as 3, 3_, 5-triiodo-L-thyronine; T3) are important regulators of lipid metabolism and metabolic rate. They exert their physiological effects by binding to specific nuclear receptors, the thyroid hormone receptors (TR) α and β, which are widely distributed throughout the body. β isoform is the major TR expressed in liver, whereas α isoform is the major TR expressed in the heart. Beneficial effects of TR activation include lowering of low-density lipoprotein cholesterol and a reduction in whole body adiposity and weight.[38] Thyroid hormone stimulated increases in metabolic rate in liver could potentially lead to reduced liver lipid content. However, this beneficial effect could be counteracted by increased lipogenesis in liver[39] or lipolysis in adipocytes either of which could lead to deposition of lipids in the liver.[40]

The beneficial decrease in cholesterol after TR activation is driven solely by TR activation in hepatocytes, the only cell in the body capable of cholesterol disposal.[41] T3 reduces liver triglycerides and raise acyl-carnitines in plasma. T3 treatment resulted in an increase in liver mitochondrial respiration and changes in hepatic gene expression.[42] It increase catecholamine-induced lipolysis rates in adipocytes and increased plasma free fatty acid levels *in vivo*. This lipolysis in adipocytes may have fully or partially counteracted the beneficial hepatic activities. T3 treatment induce changes in gene expression in liver that lead to increased mitochondrial β-oxidation, but T3 treatment appears to over whelm the hepatic catabolism of triglycerides by mobilizing free fatty acid or triglycerides from the periphery.[43]

Ca^{+2} channel blockers, in addition to their well characterized inhibitory effect on voltage-dependent L-type Ca^{2+} channels,[44] have been tentatively used in the prevention of ischemic myocardial injury and atherosclerosis.[45] Moreover, antioxidant effects of dihydropyridine Ca2+ channel blockers have recently been described.[36]

Effect of atherogenic diet on histopathological findings

In the present study, histopathological examination of aorta of normal control animals did not reveal any sign of abnormality with normal intact intima, media and adventia. In contrast, histopathological examination of aorta of high cholesterol diet fed animals showed an atherosclerotic plaque at the thoracic aorta and narrowing of the leumen. The plaque which is an intimal lesion composed of a fibroblast, smooth muscle cells, lymphocyte, foam cells, and distribution of macrophages in the plaque (figure 2).

These findings are in agreement with previous studies of Peng, *et al.*[46] and Zhang, *et al*[47] who found that rabbits fed atherogenic diet for 8 weeks reveal an 84% increments in their aortic plaque size. Atherosclerosis is a chronic pathological condition, and can take decades to develop severe atheromatous lesions in humans.[7] Foam cells appear even in early stages of atherosclerosis, and the accumulation of large numbers of foam cells is often observed in advanced lesions. Because macrophages are a differentiated cell type derived from monocytes, foam cells should therefore have a defined lifetime and they could be replaced by other foam cells during the development of the atherosclerotic lesion. It is, however, poorly understood what happens in macrophages after they change into foam cells. Lipid droplets in foam cells regress when cholesterol acceptors such as apolipoprotein A-I or HDL are present in sufficient quantities, thus suggesting that lipid droplets are not stable stores of excess amount of lipids but, rather, are metabolically active.[48]

Intracellular accumulation of lipid droplets is a remarkable feature of foam cells in atherosclerosis.[49] The initial steps of foam cell formation have been extensively studied.[47] Scavenger receptors expressed by macrophages bind and take up modified LDL but not native LDL. Modified lipoproteins are taken up extensively by macrophages, because the recycling systems of scavenger receptors are not down regulated. Components in modified lipoproteins including cholesteryl ester (CE) are hydrolyzed in lysosomes. The resulting free cholesterol is transferred to the endoplasmic reticulum and then re-esterified to cholesterol ester, which accumulates in the cytosol to form intracellular lipid droplets.[50]

Effect of combination of L- thyroxine and verapamil on histopathological findings

Histopathlogical results indicated that combination of L-thyroxine and verapamil significantly reduced atherosclerotic lesions of thoracic aorta, when compared to the high cholesterol diet group because both of these drugs have antiatherosclerotic effect by different mechanisms.

The formation of atherosclerotic plaques is believed to be multifactorial. However, cholesterol deposition, cellular proliferation and migration, increased cellular matrix,

calcium overload and platelet aggregation are among the most common findings. All these processes are affected by calcium antagonists.[51] Among the other risk factors, as identified by classical epidemiology, are dyslipidemia, vasoconstrictor hormones incriminated in hypertension, oxidative stress and pro-inflammatory cytokines.[52]

L-thyroxine can decrease or remove these factors except inflammation because it had antihyperlipidemic and antioxidant activity[43] but had no anti-inflammatory effect.[53] Many previous studies discussed the anti atherosclerotic effect of L-thyroxine. Thyroid hormone has direct anti-atherosclerotic effects such as blood vessel dilatation, production of vasodilatory molecules, and inhibition of angiotensin II receptor expression and its signal transduction, these data suggest that thyroid hormone inhibits atherogenesis through direct effects on the vasculature as well as modifying risk factors for atherosclerosis.[54]

Verapamil may have reduced atherosclerosis in rabbits by affecting calcium-dependent cellular processes, decreasing the shear stress on the arterial endothelium, inhibiting platelet function or by decreasing calcium ion concentration in smooth muscle cells. Verapamil may have suppressed a number of cellular functions that are calcium-dependent and known to be important in the genesis of atherosclerosis.[55]

The mechanism by which calcium antagonists exert their anti-atherosclerotic activity is not clear. One of the early stages in the pathogenesis of the atherosclerotic lesion is the accumulation of cholesterol in the arterial wall. Calcium antagonists reduce aortic cholesterol, but this effect is not mediated by a reduction in plasma lipid concentrations, suggesting that calcium antagonists do not have a major impact on overall lipoprotein catabolism. However, several studies using cultured cells indicate that calcium antagonists modify cellular lipid metabolism in cells of the arterial wall.[56] This effect apparently involves both cholesterol delivery to the cells, by affecting the receptor-mediated catabolism of lipoproteins, and the intracellular cycle of cholesterol. Previous studies demonstrated that verapamil up regulates the expression of low-density lipoprotein (LDL) receptors, thus increasing binding and internalizing of LDL in cultured fibroblasts, arterial smooth muscle cells and endothelial cells.[57]

CONCLUSION

The using of L-thyroxine and Verapamil can produce antiatherosclerotic effect on thoracic aorta of hyperlipidemic rabbits without the cardiac side effect of L-thyroxine (tackycardia).

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