ORIGINAL ARTICLE

Effect of Tocotrienols on Aortic Adventitia of Cholesterol-Fed Rabbits

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ABSTRACT

Objective: To identify the effect of tcotrienols on adventitial changes induced by high cholesterol diet in descending thoracic aorta of rabbits.

Study Design: Laboratory based randomized control trial.

Place and Duration of Study: The Anatomy department of Army Medical College Rawalpindi in collaboration with National Institute of Health, Islamabad. The study commenced on 10th March 2009 and completed on 10th September 2009.

Materials and Methods: Thirty adult male New Zealand White rabbits were randomly divided into three equal groups (I, II & III). Group-I consumed standard NIH diet while group-II was fed 2% high cholesterol diet. Group-III animals were given the same diet as to group II, however, tocotrienols 6 mg/kg body wt/day were also added to the diet. Following six weeks of experiment, aorta of every animal was dissected. Cross sections were taken from descending thoracic aorta and processed for light microscopic examination. In H&E and verhoeff stained slides, adventitial histomorphological changes were compared among the three groups.

Results: In rabbits on standard diet, tunica adventitia was a thin layer composed of loose network of collagen and elastic fibers which lacked lamellar pattern as that of media. Adventitial cells were relatively scanty. In contrast, aortic adventitia in group-II was thickened with increased number of inflammatory cells characterized by central round nucleus and foamy cytoplasm. Above mentioned histological changes were present in group-III but were of lesser degree than group-II. Mean±SD thickness of adventitia and inflammatory cells score was significantly greater in group-II &III when either was compared with group-I. However, group-III showed 19% ($p<0.05$) reduction in adventitial thickening and & 36% ($p<0.05$) lesser inflammatory cells score versus group-II.

Conclusion: Tocotrienols decrease adventitial thickening and inflammation induced by high cholesterol diet in aorta of cholesterol-fed rabbits.

Key Words: *Adventitia, High Cholesterol Diet, Rabbit Aorta, Tocotrienols.*

Introduction

Atherosclerosis is considered to be the most common physical burden globally.[1] Despite the use of dietary modifications and newer pharmacological approaches, very few effective measures exist for the prevention and treatment of this disease. Anti-atherogenic effects of tocotrienols (members of vitamin E family) are also unclear as yet.

Traditionally, atherosclerosis is considered as disease of intima with involvement of media in later stages of disease progression. Accordingly, intimal and medial thickening was accepted as the most appropriate predictor of atherosclerosis.[2] With the advancement of new era, however, it was noted that features of atherosclerosis definitely exist in the tunica adventitia[3] and adventitial inflammation is an early event in the process of atherogenesis.[4] Majesky et al labeled adventitia as a dynamic interface harboring progenitor cells which respond to arterial injury and then migrate into tunica intima.[5] Gutterman proposed that future managements for prevention of atherosclerosis should focus on drug therapies targeting adventitia of vessels.[6]

Vitamin E includes eight chemically distinct substances: four tocopherols and four tocotrienols (alpha, beta, gamma and delta). Heath benefits of vitamin E in the prevention and treatment of atherosclerosis have been postulated with conflicting results but more than 95 per cent studies of vitamin E were directed towards tocopherols and tocotrienols remain poorly understood.[7] Moreover, available data regarding tocotrienols is mainly limited to their potent hypocholesterolemic, antioxidant and anti-inflammatory properties[8,9] and thus addresses a major void in evaluation of their effect on histomorphological aspects of the atherosclerosis.

Therefore, this experimental study was designed to investigate the effect of this lesser known form of

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vitamin E (tocotrienols) on aortic adventitia of rabbits fed high cholesterol diet.

Materials and Methods

The present study was a laboratory based randomized control trial conducted in the Anatomy department of Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. All experimental procedures were approved by the institutional animal ethical committee. The study commenced on 10th March 2009 and completed on 10th September 2009. Inclusion criteria were thirty adult male New Zealand White (NZW) rabbits, 10-18 months old and weighing 1.5 to 2.5 kg. Female rabbits and animals with any evident pathology were excluded from the study. Each rabbit was kept in a separate cage, at a standard temperature of 21 ± 20C with 12 hour light/dark cycle at NIH. Each animal was given 100g/day standard NIH diet. Water was available ad libitum. After one week of acclimatization to experimental conditions, thirty rabbits were divided into three equal study groups (I, II&III) by using non-probability convenient sampling technique. Group-I continued standard NIH diet (100g / head/day) while each animal in group-II was given 2% high cholesterol diet {2g cholesterol powder (Applichem, Germany) mixed with 100g standard NIH diet/ head/day.[10] Group-III animals were fed the same diet as to group II, however, tocotrienols {mixture of 90% delta & 10% gamma (American River Nutrition, Inc. Hadley, MA. USA)} 6 mg/kg body wt/day[11] were also added to the diet. Composition of diet per animal in each group is given in table-I. After six weeks of experiment, rabbits were euthanized. Cross sections were taken from descending thoracic aorta and placed in 10% formol calcium. After 48 hours, tissues were processed for light microscopic examination. H&E staining was done for histomorphological examination. For morphometry, verhoeff van-Geisson stain was used for the delineation of collagen and elastin. Under 40X objective, at three point of maximal luminal narrowing, adventitial thickness (AT) was measured from media-adventitia interface (external elastic lamina) to the adventitia-periadventitia interface (outer edge of collagen containing dense fibrous tissue).[12] Mean of the three values was calculated for each cross section. Inflammation in adventitia was semiquantitatively scored accordoing to the following criteria: 0 = No inflammatory cells, 1 = inflammatory cells present in $\geqslant$ 25 to < 50 per cent circumference, 2 = inflammatory cells present in $\geqslant$ 50 to < 75 per cent circumference, 3 = inflammatory cells present in $\geqslant$ 75 per cent circumference. Inflammation was defined as presence of $\geqslant$ 25 mononuclear round cells with foamy cytoplasm per field with 40X magnification objective.[13,14]

Parametric data was analyzed using SPSS (Statistical package for social sciences) windows version 20. Quantitative data was expressed as Mean ± S.D. For each variable, group differences were compared by one way analysis of variance (ANOVA) followed by post hoc tukey test for intergroup comparison of parameters. All the results were considered statistically significant at a p-value less than 0.05.

Results

In rabbits on standard NIH diet, tunica adventitia was a thin layer composed of loose network of collagen and elastic fibers which lack lamellar pattern as that of media. Adventitial cells were relatively scanty and mainly suggestive of fibroblasts (Figure 1-a). Vasa vasora and nervi vascularis were also scattered amongst the fibers. In contrast, aortic adventitia in group-II appeared to be thickened especially beneath the intimal lesions (Figure 1-b). This thickening was associated with increased number of mononuclear round cells with central nucleus and foamy cytoplasm. These cells were forming aggregates in adventitia and penetrating medioadventitial interface (Figure 1-d). Above mentioned changes were present in group-III (Figure 1-c) but were of lesser degree than group-II

Mean±SD thickness of adventitia and inflammatory cells score was significantly greater in group-II &III when either was compared with group-I. However, group-III showed 19 % reduction in adventitial thickening and & 36% lesser inflammatory cells score versus group-II (Table-II).

Table I: Showing composition of diet per animal per day in each group

Components of diet	Group-I	Group-II	Group-III
Chickpea powder	77 g	77 g	77 g
Wheat bran powder	23 g	23 g	23 g
Cholesterol powder	—	2 g	2 g
Tocotrienols	—	—	12.5 mg

Table II: Showing comparison of adventitial thickness and inflammatory cells score between rabbits fed standard NIH diet (Group-I), 2% high cholesterol diet (Group-II), 2% high cholesterol diet+ tocotrienols (Group-III) for 6 weeks

Parameters	Adventitial thickness (µm)			Inflammatory cells score		
Groups	I	II	III	I	II	III
Mean value	4.63	9.30	7.53	0.00	2.20	1.40
Std. Deviation	0.777	1.451	0.737	0.00	0.918	0.516
SEM	0.245	0.459	0.233	0.00	0.290	0.163
p-value	Group-I versus Group-II=0.000 Group-II versus Group-III=0.002 Group-I versus Group-III=0.000			Group-I versus Group-II=0.000 Group-II versus Group-III=0.018 Group-I versus Group-III=0.000		

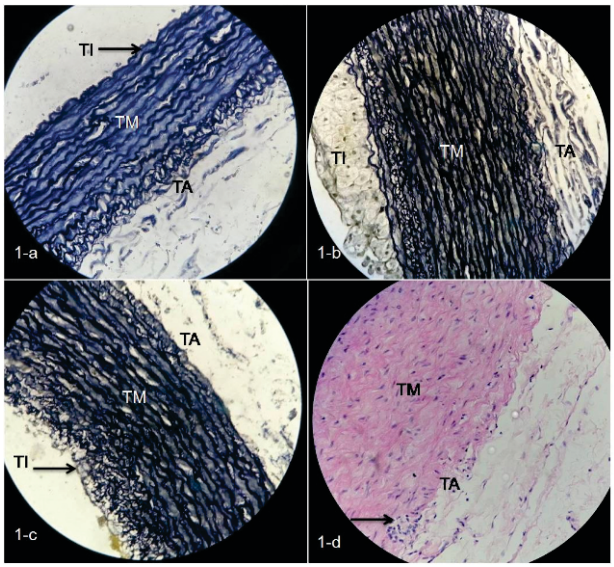

Fig 1: Aortic cross sections of rabbit fed standard NIH diet (1-a), 2% high cholesterol diet (1-b, 1-d,) & 2% high cholesterol diet+ tocotrienols (1-c) for 6 weeks (1-a, 1-b, 1-c: Magnification X400, Verhoeff Van Geisson stain; 1-d: arrow indicates inflammatory cells, H&E stain).
TI: Tunica intima, TM: Tunica media. TA: Tunica adventitia

Discussion

The present experimental study evaluated the effect of tcotrienols (mixture of 90% delta & 10% gamma) on adventitial atherosclerotic changes induced by high cholesterol diet in aorta of rabbits. Our results suggested that tocotrienols supplementation significantly decrease adventitial thickening and inflammation. Adventitial thickness displays a strong correlation with atherosclerosis risk factors especially dyslipidemias.[12,15] Atherogenic stimuli excites adventitial cells particularly fibroblasts, which in turn produce increase amount of extracellular matrix. These fibrobasts also generate an inflammatory response by releasing cytokines and chemokines.[16,17] Growth of vasa vasora further modulates arterial wall structure by acting as conduit for inflammatory and progenitor cells.[16] Adventitial thickening and inflammation, in response to high cholesterol diet, as observed in the current experiment, supports the findings of Gradus-Pizlo et al[17] who found significantly greater thickness of adventitia in patients with coronary atherosclerosis than those with normal arteries. This adventitial thickening was associated with enhanced number of mononuclear vacuolated cells especially in the outer half of vessel. According to dushkin,[18] these cells are considered as an attribute to generate inflammation. Maillaro & Taylor [19] reported that population of these inflammatory cells include lymphocytes, monocytes, macrophages and fibroblasts which work in concert to elicit an inflammatory response that progresses towards tunica intima. In reviewing the immune and inflammatory mechanisms regarding atherosclerosis, Galkina and Ley[20] stated that these cells are found in normal adventitia but their number expands in atherosclerotic lesions. Contrary to our findings, Deopujari and Dixit[21] reported that basic pathological changes occur in tunica intima and media and tunica adventitia is not affected in coronary artery disease. Tocotrienols mediated substantial reduction in adventitial thickening is quite close to the findings of Qureshi et al[22] whose striking results highlighted the atheroprotective properties of novel tocotrienols of rice bran by substantial reduction of 57 per cent, 33 per cent and 47 per cent in growth of atheromatous plaque in three genotypes of mice. Comparable results were seen in a recent local study which showed that tocotrienols significantly decrease atheromatous changes in diet induced diabetic BALB/c mice.[23] However, our findings are not consistent with Ismail et al[24] who found no beneficial effect on atherosclerosis development in six rabbits given palm tocotrienols plus 2 per cent cholesterol for 10 weeks compared with the six rabbits given cholesterol alone and six rabbits on regular diet. The difference can be accredited to relatively small sample size in their study and more severe nature of the disease.

As inflammation is the hallmark of atherosclerotic lesions.[25] Reduction in adventitial inflammatory cells, in our study, is in supportive context with Wu et al[26] who concluded that tocotrienols possess potent anti-inflammatory activity by suppressing the expression of inflammatory mediators in human monocytic cells. Many researchers have compared anti-inflammatory properties of various tocotrienols and tocopherols and found delta tocotrienol as the most potent isoform.[27,28] In a study on hypercholesterolemic subjects, delta tocotrienol was given in doses of 125, 250, 500, 750 mg/day for 4 weeks. In each concentration, delta tocotrienol was therapeutically effective in reducing biomarkers of oxidative stress and inflammation including serum nitric oxide, C-reactive protein, malondialdehyde, and δ-glutamyl-transferase.[9]

In conclusion, our experimental data suggests that tocotrienols exhibit significant potential in lowering adventitial atherosclerotic changes induced by high cholesterol diet in aorta of rabbits. The study had been carried out in an experimental model of atherosclerosis that differs from chronic lesions observed in human cases. Therefore, human trials should be considered necessary for final elucidation of tocotrienols as atherosuppressive agents. Moreover, mechanisms involved in suppression of atherosclerotic changes warrants further investigations.

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