Effects of the Calcium Channel Blocker Amlodipine on Serum and Aortic Cholesterol, Lipid Peroxidation, Antioxidant Status and Aortic Histology in Cholesterol-Fed Rabbits

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Abstract
Reactive oxygen metabolites and oxidized fatty acids are proinflammatory and are involved in the pathophysiology of atherosclerosis. Amlodipine, a unique third-generation dihydropyridine-type calcium channel blocker, seems to exert atheroprotective effects through its antioxidant properties related to its chemical structure and independent of its calcium channel-blocking effect. In this study, the interactions of amlodipine with major cellular antioxidants were investigated in order to elucidate the mechanisms underlying its atheroprotective effects. New Zealand white male rabbits were fed regular chow (group 1), chow with 1% cholesterol (group 2), regular chow plus 5 mg/kg/day amlodipine per os (group 3) and 1% cholesterol plus amlodipine (group 4) for 8 weeks. Total cholesterol, malondialdehyde (MDA) and vitamin E concentrations and catalase and superoxide dismutase (SOD) activities were determined in blood drawn before and after the experimental period. Aortic tissue was examined for atherosclerotic changes and aortic total cholesterol, MDA, catalase and SOD were determined. At the end of the 8-week treatment period, serum total cholesterol and plasma MDA were elevated in groups 2 and 4. In group 2, serum vitamin E and plasma SOD diminished (p < 0.05) and catalase increased (p < 0.05). In group 4, SOD activity increased at the end of treatment. MDA levels were lower and plasma SOD activities were higher in group 4 than in group 2. Aortic tissue investigations revealed higher total cholesterol and MDA concentrations and catalase activities in group 2 than in group 4, and the highest tissue SOD activity was recorded in group 4 (p < 0.05 for all comparisons). Morphological examination of aortic tissues exhibited endothelial disarrangement and lipid deposition in group 2. Histopathological alterations related to atherogenesis were less in group 4 than in group 2. Amlodipine seems to exert atheroprotective effects by reducing aortic cholesterol accumulation and blood and aortic lipid peroxidation, enhancing SOD activity both in blood and aortic tissue and suppressing the consumption of vitamin E. On the other hand, the suppression of catalase activity in blood and the aorta interferes with the drug's well-known antioxidant effects.
Introduction

Amlodipine is a charged dihydropyridine-type third-generation calcium channel blocker (CCB) which is well tolerated and widely used in the treatment of hypertension, myocardial ischemia and congestive heart failure [25]. There is growing evidence that this long-acting agent has atheroprotective activity in animal models and effectively inhibits lesion development in cholesterol-fed rabbits [2, 15, 23, 37], but has little effect on established lesions [34]. Novel actions of amlodipine, independent of its action on calcium channels, have been described which suggest that some of its atheroprotective effects may be due to its unique physical and pharmacokinetic properties. These include its highly lipophilic nature [lipid partition coefficient (K_p) of approximately 10^4] and strong partition into the cell membrane with a K_p of about 1,200, which enable this drug to restore cholesterol-induced membrane bilayer abnormality in the smooth muscle cell by restoring the membrane to its normal width in the atherosclerotic rabbit aorta [22, 35]. Other proposed mechanisms that may be responsible for the antiatherogenic effect of amlodipine involve the suppression of smooth muscle cell proliferation and connective tissue migration in the vascular wall [4], inhibition of oxygen free radicals involved in lipid peroxidation [5, 27, 31, 38] and prevention of vascular endothelial damage [13, 40]. The oxidoperoxidative transformation of lipids and an imbalance in the pro-oxidant/antioxidant equilibrium in favor of oxidative stress is of pivotal importance in the pathogenesis of cholesterol-induced atherosclerosis [5, 16]. Several lines of evidence suggest that calcium antagonists, particularly those of the dihydropyridine type, such as amlodipine, are potential antioxidants and could have beneficial effects in the prevention of atherosclerosis [7]. Amlodipine belongs to the chain-breaking group of antioxidants; its dihydropyridine ring donates electrons to the propagating radicals and reduces them to a nonreactive form [10]. Considering that disruption of the delicate balance between pro-oxidants and antioxidants has been implicated in the pathophysiology of atherosclerosis, and on the basis of the fact that the effectiveness of the antioxidant defense system, in which vitamin E is the major chain-breaking lipophilic antioxidant and the enzymes superoxide dismutase (SOD) and catalase are among the major contributors, determines the degree of oxidative stress [16], we speculated whether the antioxidant, atheroprotective effects of amlodipine are mediated via its effects on the activities of these enzymes and vitamin E. To test this hypothesis, we investigated the changes in antioxidant status (vitamin E, SOD and catalase) and lipid peroxidation products (malondialdehyde; MDA) in blood and aortic tissue from cholesterol-fed rabbits and control animals following 8 weeks of treatment with the CCB amlodipine.

Materials and Methods

Animals and Diets

Forty New Zealand white male rabbits ranging in weight from 2,000 to 2,500 g were divided into four groups of 10 animals each. Group 1 consisted of the control animals, which were fed with pelleted regular chow (basal diet). Group 2 received the same basal diet plus 1% pure cholesterol, group 3 received the basal diet plus 5 mg/kg/day amlodipine per os and group 4 received the same 1% cholesterol diet plus the same dose of amlodipine. All rabbits were kept for a 2-week adaptation period before being fed with their respective diet protocols for 8 weeks. At the end of the 2 weeks in which all animals were fed the basal diet, all animals were fasted overnight and blood was drawn from the ear vein for the determination of total cholesterol, lipid peroxides, vitamin E, SOD and catalase. After 8 weeks on the respective diets, the experimental period was terminated and all animals were fasted overnight. They were anesthetized and blood was collected by heart puncture. Rabbits were euthanized by pentobarbital injection (100 mg/kg) and the aortas were removed immediately and washed with ice-cold saline. The intimal-medial layer of each aorta was separated from the medial-adventitial layer and used for total cholesterol, SOD and catalase assays and measurement of lipid peroxides (as thiobarbituric acid-reactive substances). Another portion was prepared for the histological examinations. All animal protocols were approved by the Ege University Medical School Research Ethics Committee.

Chemicals and Reagents

α-Tocopherol, tocopherol acetate, nitroblue tetrazolium, xanthine, xanthine oxidase (0.65 units/mg protein) and SOD (3,100 units/mg protein) were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Ammonium molybdate, ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane (TEP) methanol, ethanol, n-hexane and tetrahydrofuran were obtained from Merck KGaA, Darmstadt, Germany. All other chemicals and reagents were of analytical or high-performance liquid chromatography (HPLC) grade.

Total Cholesterol Analysis

Serum total cholesterol concentrations were assayed on a Hitachi 704 automatic analyzer using a kinetic enzymatic method (Biocon Diagnostics, Vöhl-Marienhagen, Germany).

For aortic total cholesterol analysis, aortic tissue made to 10 volumes (w/v) with ice-cold Tris buffer (50 mM Tris, 0.1 M EDTA, 0.25 M sucrose, pH 7.5) was homogenized for 2 min using an IKA Labortechnik T25B homogenizer at a speed setting of 1,500 rpm. The homogenate was centrifuged for 60 min at 30,000 g in a Jouan KR22i ultracentrifuge at 4°C. The supernatant fraction was decanted and used to determine aortic total cholesterol content as described elsewhere [12]. Protein concentrations in the supernatants were measured using bovine serum albumin as standard [14].