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Dietary phytosterols reduce probucol-induced atherogenesis in apo E-KO mice

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Abstract

We have previously shown strong pro-atherogenic effects of probucol in apolipoprotein E-knockout (apo E-KO) mice. The aims of the present study were to investigate whether (a) dietary phytosterols reduce probucol-induced atherogenesis and (b) beneficial interactions exist between these agents. Male apo E-KO mice fed with an atherogenic diet supplemented with phytosterols or probucol or their combination for 14 weeks. Single therapy with either phytosterols or probucol resulted in a 25% reduction in plasma total cholesterol (TC) concentrations as compared to the control group. The effects of the combination therapy were more profound (60% reduction). While phytosterols reduced atherogenesis by 60%, probucol caused an increase of 150% in atherogenesis. Addition of phytosterols to probucol substantially reduced pro-atherogenic effects of probucol. This was associated with improved high density lipoprotein (HDL) concentrations. The ratio of TC to HDL cholesterol was markedly reduced in the combination therapy group as compared to the probucol-treated group. A strong positive association between the ratio of TC to HDL cholesterol and the extent of atherosclerotic lesions was observed. The coronary arteries of the probucol-treated group showed various stages of atherogenesis from infiltration of monocytes into intima to complete occlusion of the vessel by atheromatous lesions. Such pathological findings were not observed in the combination therapy group. Approximately 40% of the mice in the probucol-treated group and 10% of the animals in the combination therapy group developed skin lesions. Further studies warrant the investigation of the underlying mechanisms of the observed beneficial interactions between dietary phytosterols and probucol.

Keywords: Probucol; Phytosterols; Atherosclerosis; Apo E-KO mice

1. Introduction

Coronary artery disease (CAD) still remains one of the major causes of morbidity and mortality in Western societies. Among several modifiable risk factors, increased levels of low density lipoprotein (LDL) cholesterol and its oxidized form are well-associated with progression of the disease. In this regard, several therapeutic and/or life style modification strategies have shown benefit in serum lipoprotein concentrations, however, their benefits in cardiovascular disease development still need further documentation. Many large-scale clinical trials have documented benefits of lowering LDL–cholesterol levels by pharmaceutical agents in patients with multiple risk factors [1–6]. Dietary approach is more suitable in patients with mild hypercholesterolemia with no other known risk factors [7–9]. We have previously shown that dietary phytosterols significantly reduce plasma cholesterol concentrations and the extent of atherosclerotic lesions in apolipoprotein E-knockout (apo E-KO) mice regardless of presence or absence of dietary cholesterol [10,11]. Similarly, our analysis of clinical data showed that dietary phytosterols reduce total cholesterol (TC) and LDL cholesterol on average by 10 and 13%, respectively, with no significant effects on plasma triglyceride levels or high density lipoprotein (HDL)–cholesterol concentrations [12].

Abbreviations: apo E-KO, apolipoprotein E-kockout; CAD, coronary artery disease; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol

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Among many anti-atherogeneic drugs, probucol is distinguished due to its strong cholesterol-lowering and antioxidant activities, making this agent attractive for prevention of CAD [13–16]. However, probucol also reduces HDL cholesterol which may not be beneficial in CAD patients. This may be one of the main reasons that probucol is not in the market in several countries. Despite its HDL-lowering effects, anti-atherogenic effects of probucol have been reported in both humans and experimental animals [17–19]. In contrary, pro-atherogenic effects of probucol were recently observed in genetically modified animal models of atherosclerosis. For example, we and others have shown that probucol paradoxically promotes atherogenesis in apo E-KO mice despite its strong LDL cholesterol-lowering and antioxidant activities [11,20]. The reasons for this paradoxical activity are not known, except for reductions in HDL cholesterol and increased plasma fibrinogen levels [11,20]. The probucol-induced atherosclerotic lesions in apo E-KO mice are quickly developed and advanced over a short period of time. These features of atherosclerosis development suggest that probucol-induced atherosclerosis in this animal model can be used as a fast experimental approach for investigating the pathogenesis of atherosclerosis as well as the effects of pharmaceuticals and/or nutraceuticals on its prevention and/or treatment relatively quickly.

Our previous studies showed strong anti-atherogenic effects of dietary phytosterols in apo E-KO mice [10,11]. These effects of plant sterols are believed to be mainly mediated through reductions in plasma LDL and very low density lipoprotein (VLDL) cholesterol concentrations. In addition, we have shown that dietary phytosterols slightly reduce and increase plasma fibrinogen and HDL–cholesterol levels, respectively, in apo E-KO mice [11]. The latter effects of phytosterols are opposite to those of probucol in this animal model [11]. Thus, if pro-atherogenic effects of probucol in apo E-KO mice are mediated through increases and decreases in plasma fibrinogen and HDL–cholesterol levels, respectively, thus dietary phytosterols should be able to reduce probucol-induced atherosclerosis. The aims of the present study were: (1) to investigate whether dietary phytosterols reduce probucol-induced atherosclerosis in apo E-KO mice and (2) to test potential synergistic or additive effects of these two agents against atherogenesis in this animal model.

2. Materials and methods

2.1. Animals and diets

Thirty male 4-week-old apo E-KO mice were purchased from Jackson Laboratory and assigned to control ($n = 7$); probucol-treated ($n = 7$); phytosterol-treated ($n = 8$) and phytosterols + probucol-treated ($n = 8$) groups matched with their mean body weight and plasma total cholesterol levels as previously published [10,11]. Pico Lab mouse diet was supplemented with 0.2% (w/w) cholesterol (base diet) for the control group; this "base diet" was further supplemented with 2% (w/w) soybean-derived phytosterol mixtures containing 58% β-sitosterol, 19% campesterol, 13% dihydrobrassicasterol and 10% stigmasterol for the phytosterol-treated group or with 1% (w/w) probucol or a combination of phytosterols + probucol. The experiments were carried out over 14 weeks. Body weights were recorded weekly and blood samples taken at baseline and 4-week intervals from the jugular vein of lightly anesthetized animals. At sacrifice, the hearts and aortas were removed and fixed in 10% buffered formalin for histological examination. The Animal Care Committee at the University of Manitoba, Winnipeg, Canada, approved the experiments.

2.2. Lipid analyses

Total cholesterol, triglycerides (TG) and HDL–cholesterol levels were measured at baseline, during and at the end of the study using standard enzymatic methods [10,11]. Non-HDL–cholesterol levels (in this animal model the non-HDL cholesterol comprises mainly of β-VLDL and to a lesser extent LDL) were calculated by subtraction of HDL–cholesterol levels from TC levels; standard precipitation method was used to prepare HDL fraction [21]. All of lipid measurements were performed in duplicates using internal standard solutions provided by the manufactures (Thermo DMA, Arlington, TX, USA) as quality control. Our lipid measurements in standard solutions always showed values within 94–98% of actual values reported by the manufacturer.

2.3. Histology and morphometry evaluations of atherosclerotic lesions

Sections at the aortic roots were cut and stained with hematoxylin and eosin (H&E) and oil red O (ORO) for histological and morphometrical examinations. ORO-stained sections were used to estimate atherosclerotic lesion size using Image Pro-Plus software [11,22].

2.4. Statistical analyses

One-way ANOVA analysis followed by the Tukey test was used to determine the significant differences among the groups. Data are presented as mean and standard deviation (S.D.).

3. Results

3.1. Body weight

Body weight gain was comparable among all of four experimental groups over the experimental course. Over the