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Original Contribution

Dietary coenzyme Q10 does not protect against cigarette smoke-augmented atherosclerosis in apoE-deficient mice

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ABSTRACT

Dietary coenzyme Q10 reduces spontaneous atherosclerosis in the apoE-deficient mouse model of experimental atherosclerosis. We have shown previously that exposure to sidestream cigarette smoke (SSCS) enhances atherosclerotic lesion formation in apoE-deficient mice. The aim of the present study was to determine if CoQ10 protected against SSCS-mediated atherosclerosis. Female apoE-deficient mice were fed a saturated fat-enriched diet (SFD) alone, or supplemented with 1% wt/wt coenzyme Q10 (SFD-Q10). Mice in each diet group were exposed to SSCS for 4 hrs/day, 5 days/week in a whole-body exposure chamber maintained at 35 ± 4 mg smoke particulates/m^3. Mice kept in filtered ambient air served as controls. Mice were euthanized after either 6 or 15 weeks of SSCS exposure and following measurements were performed: i) lung 7-ethoxyresorufin-O-deethylase (EROD) activity; ii) plasma cholesterol and CoQ10 concentrations; iii) aortic intimal area covered by atherosclerotic lesions; and, iv) pathological characterization of lesions. Lung EROD activity increased in SSCS mice of both diet groups, confirming SSCS exposure. Plasma concentrations of CoQ10 in SFD-Q10-fed mice were increased markedly in comparison to SFD-fed mice. Plasma cholesterol concentrations and distributions of cholesterol in lipoprotein fractions were unaffected by SSCS exposure. Dietary supplementation with CoQ10 significantly reduced atherosclerotic lesions in control mice. As reported previously, exposure to SSCS increased the size of lesions in apoE-/- mice at both time points. However, dietary supplementation with CoQ10 had no effect on atherosclerotic lesions augmented by SSCS exposure. The results suggest a role of oxidative processes in smoke-augmented atherosclerosis that are different than those mitigated by CoQ10.

Introduction

Increased oxidative stress has been implicated in the development of vascular disease and its functional importance in the evolution of atherosclerosis has been long recognized [1–4]. Exposure to cigarette smoke is a major risk factor for atherosclerotic vascular diseases. While the mechanisms through which tobacco smoke contributes to development and progression of atherosclerotic disease are poorly understood, it is generally believed that a high oxidative stress associated with tobacco smoke exposure plays an important role in promoting disease [5–7].

Efforts to protect against oxidative stress and cardiovascular disease by antioxidant intervention, particularly alpha tocopherol, have yielded mixed results [4,8–10]. Various studies have indicated an important role of mitochondria in generating endogenous oxidative stress which in turn may influence development and progression of atherosclerosis [11–13]. Natural ubiquinone or Coenzyme Q10 (CoQ10) is a lipid-soluble endogenous antioxidant in mitochondria that is composed of a quinoid moeity with isoprenoid side chain [14]. It is an essential component of the mitochondrial electron transport chain [15] and acts as a lipid antioxidant either directly in its reduced form, ubiquinol, or in recycling of radical forms of vitamin E [16]. Dietary CoQ10 is readily reduced to ubiquinol in the body which is the predominant form present in the plasma [17]. It possesses strong inhibitor activity against lipid peroxidation in tissues and membranes and is more efficient than vitamin E in protecting LDL oxidation [18]. Beneficial effects of dietary CoQ10 in cardiovascular disease have been reviewed [17,19].

In rodents, the main CoQ is CoQ9 which is a shorter chain homologue of CoQ10. Feeding CoQ10 to animals is known to increase the levels of both CoQ9 and CoQ10 in the plasma, tissues and mitochondria [20,21]. Studies have also shown that dietary supplementation with CoQ10 reduces the development of atherosclerotic lesion formation in atherosclerosis-prone apoE-deficient mice [22,23]. We have previously shown that exposure to sidestream cigarette smoke (SSCS) accelerates the development of atherosclerotic plaque formation in apo-E deficient mice [24]. The present study was performed to determine if dietary Co-Q10 supplementation will protect against SSCS-induced acceleration of atherosclerotic lesion formation in this model.

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Materials and methods

Animals

Female apoE-deficient mice (8-9 wks old) were purchased from the Jackson Laboratory (Bar Harbor, ME). Food and water were available to the mice *ad libitum*. Weekly body weights were maintained to assess any differences in growth rates. All procedures performed in this study had the prior approval of the University of Kentucky Institutional Animal Care and Use Committee.

Diets

Purified Teklad fat enriched diet (milk fat 21% wt/wt, cholesterol 0.15% wt/wt; TD 88137; SFD), and the same diet supplemented with 1% wt/wt coenzyme Q10 (CoQ10-SFD) were prepared by Harlan-Teklad, WI. Pure CoQ10 (natural ubiquinone, trans configuration) was supplied by Tishcon Corp (NY). Diets were obtained in batches prepared at 6 week intervals which were stored at 4 °C. Animals were divided into two diet groups of 30 each: one maintained on SFD and the other on CoQ10-SFD. Fresh diets were provided on alternate days.

Smoke exposures

Each diet group was divided into two equal subgroups: control and SSCS. SSCS groups were exposed for a total of 4 hrs/day, 5 days a week, for up to 15 weeks, as described previously [24,25]. Briefly, the inhalation exposures were carried out in a whole-body exposure chamber. Sidestream cigarette smoke was generated from the University of Kentucky 2R4F reference cigarettes. The total suspended particulate level in the chamber averaged 35 ± 4 mg/cubic meter. Sham control groups were exposed to filtered ambient air.

Exposure markers

Inhalation of SSCS by mice was monitored by urinary cotinine measurement by an ELISA and the measurement of 7-ethoxyresorufin-O-deethylase (EROD) activity in lung microsomes at the termination of the studies. Exposure to cigarette smoke induces this CYP1A1-linked enzyme which we have used to ascertain exposure of animals to cigarette smoke particulates [24].

Plasma measurement

Blood was collected from mice under light anesthesia and serum was obtained. Concentrations of total cholesterol by enzymatic assays were performed with commercially available kits (Wako Chemical Co). Lipoprotein cholesterol distributions among lipoproteins were determined by FPLC size-exclusion chromatography on 50 µl of serum from individual mice as described previously [26]. Serum concentrations of total CoQ10 were quantified by HPLC [27].

Quantification of atherosclerosis

The entire aorta from arch to iliac bifurcation was carefully removed for plaque area measurement. Atherosclerosis was quantified by intimal area measurements of the atherosclerotic lesions by *en face* method, as described previously [28,29]. After removing the extraneous fat, atherosclerotic lesions on the intimal aortic surface of apoE -/- mice were measured under a dissecting microscope, equipped with a Nikon digital camera and quantified with Image Pro (Media Cybernetics Inc.) software.

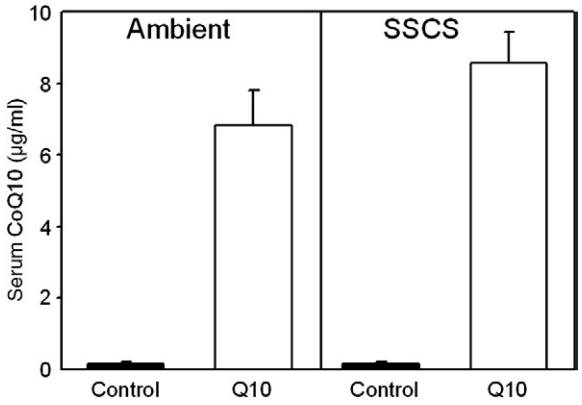

Fig. 1. Serum concentrations of CoQ10 in apoE -/- mice exposed to ambient air and SSCS for 15 weeks. Mice were fed a saturated fat-enriched diet (closed bars) or one supplemented with CoQ10 (open bars). Histobars are means of n = 10 measurements and bars are SEMs.

Characterization of atherosclerotic lesions

Lesions were sectioned in the aortic sinus as described previously [28] and stained by histological and immunochemical techniques, as described previously [26,30].

Statistics

Mean and standard error of mean (SEM) were calculated for each parameter. Data was analyzed using Sigma Stat using parametric or nonparametric analysis as appropriate. $P<0.05$ was considered statistically significant.

Results

Mice maintained on either SFD or CoQ10–SFD gained body weights at a similar rate between control and smoke-exposed animals in both diet groups (data not shown). Total serum CoQ10 concentrations were markedly increased by the dietary supplementation in groups exposed to both ambient air and SSCS (Fig. 1). These data demonstrate that there was efficient absorption of CoQ10 in mice and that SSCS exposure had no effect on the serum concentrations.

Dietary CoQ10 did not significantly influence the excretion of cotinine in urine in SSCS exposed mice (concentrations ranged from 1.6 to 3 µg/mg creatinine). A ~5 fold increase in pulmonary EROD activity occurred in SSCS exposed mice on both diets, indicating effective inhalation of smoke particulates (Fig. 2).

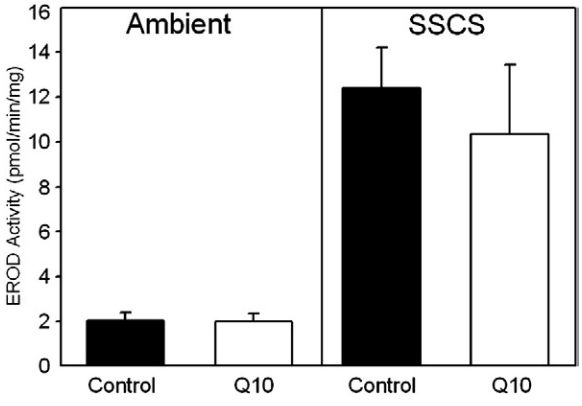

Fig. 2. Pulmonary EROD activity of apoE -/- mice exposed to ambient air and SSCS for 15 weeks. Mice were fed a saturated fat enriched diet (closed bars) or one supplemented with CoQ10 (open bars). Histobars are means of n = 10 measurements and bars are SEMs.