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Vitamin E therapy results in a reduction in HDL function in individuals with diabetes and the haptoglobin 2-1 genotype

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ABSTRACT

Objective: Vitamin E provides cardiovascular protection to individuals with diabetes and the haptoglobin 2-2 genotype but appears to increase cardiovascular risk in individuals with diabetes and the haptoglobin 2-1 genotype. We have previously demonstrated that the haptoglobin protein is associated with HDL and that HDL function and its oxidative modification are haptoglobin genotype dependent. We set out to test the hypothesis that the pharmacogenetic interaction between the haptoglobin genotype on cardiovascular risk might be secondary to a parallel interaction between the haptoglobin genotype and vitamin E on HDL function.

Research design and methods: Fifty-nine individuals with diabetes and the haptoglobin 2-1 or 2-2 genotypes were studied in a double-blind placebo controlled crossover design. Participants were treated with either vitamin E (400 IU) or placebo for 3 months and crossed over for an equivalent duration. Serum was collected at baseline and after the completion of each treatment. HDL functionality as well as HDL associated markers of oxidation and inflammation were measured after each interval in HDL purified from the cohort.

Results: Compared to placebo, vitamin E significantly increased HDL function in haptoglobin 2-2 but significantly decreased HDL function in haptoglobin 2-1. This pharmacogenetic interaction was paralleled by similar non-significant trends in HDL associated lipid peroxides, glutathione peroxidase, and inflammatory cargo.

Conclusion: There exists a pharmacogenetic interaction between the haptoglobin genotype and vitamin E on HDL function (clinicaltrials.gov NCT01113671).

The oxidative hypothesis of atherosclerosis suggests that oxidative modification of lipoproteins may be responsible for the development of atherosclerotic cardiovascular disease (CVD) [1]. This hypothesis is supported by data showing that oxidized lipoproteins can promote foam cell formation and macrophage activation [1], that antioxidants can prevent atherosclerosis in animals [1] and that endogenous levels of antioxidant enzymes are lower in individuals with atherosclerosis [2]. However, attempts to test this hypothesis in placebo controlled clinical trials have shown that vitamin E provides no cardiovascular benefit [3,4]. One reason why these studies may have failed was the lack of patient selection for antioxidant therapy. One population, which might benefit from antioxidant therapy is that defined by a polymorphism in an antioxidant gene which confers inferior antioxidant protection.

The haptoglobin (Hp) protein is an antioxidant due to its ability to neutralize the oxidative activity of hemoglobin (Hb) [5]. In man there exists two classes of alleles at the Hp locus denoted 1 and 2. The protein product of the Hp 2 allele is an inferior antioxidant compared to the Hp 1 allele product [6]. The Hp 2-2 genotype has been associated with a 2- to 5-fold increased risk of incident CVD in individuals with diabetes (DM) in seven independent studies [7–13].

There appears to exist a pharmacogenetic interaction between the Hp genotype and vitamin E on the development of CVD in indi-

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viduals with DM [13]. Not only does vitamin E appear to provide substantial cardiovascular benefit to Hp 2-2 DM [9,13] individuals but it also appears to promote CVD in Hp 2-1 DM individuals [12].

We have recently demonstrated that the Hp protein is associated with HDL. Additionally, we have shown that HDL function and the oxidative modification of HDL are Hp genotype dependent [14,15]. We hypothesized that the pharmacogenetic interaction of the Hp genotype and vitamin E on CVD might be the result of a parallel interaction of the Hp genotype and vitamin E on HDL function and structure. In order to test this hypothesis we characterized the structure and function of HDL from individuals with DM and the Hp 2-1 or Hp 2-2 genotypes in a double-blind randomized placebo controlled cross-over study.

1. Materials and methods

1.1. Crossover study design

The study was registered as clinical trial NCT01113671 and approved by the institutional ethics committee of the Rambam Medical Center, Haifa, Israel. All participants provided informed consent. The primary aim of the study was to determine if there existed a pharmacogenetic interaction between the Hp genotype and vitamin E on HDL function. Subjects were recruited from the ICARE cohort. We initially hoped to achieve a study size of 30 subjects of each Hp type (Hp 1-1, 2-1 and 2-2) in order to provide 80% power to observe a statistically significant interaction between the genotypes assuming that there would be a 20% improvement in HDL function in Hp 2-2 and no effect in the other Hp types. Targeted enrollment was achieved only for Hp 2-1 and Hp 2-2 (42% and 49% of population) but not for Hp 1-1 individuals (9% of population) and therefore analyses are reported only for Hp 2-1 and Hp 2-2. 36 Hp 2-1 and 32 Hp 2-2 DM individuals were consented and enrolled in the study. Participants were randomized to receive either placebo or vitamin E (400 IU natural source d-alpha-tocopheryl acetate/day) for 3 months followed by crossover. Serum was collected at baseline and after each treatment and stored at −80 °C. Analyses are reported on 31 Hp 2-1 and 28 Hp 2-2 participants who completed the protocol.

1.2. Isolation of HDL by affinity chromatography

Serum was diluted in an equal volume of 0.5 M NaCl in PBS and loaded onto a polyclonal anti-$ApoA_1$ sepharose column. After washing with PBS the HDL was eluted with tris-glycine (0.1 M, pH 2.5) followed immediately by neutralization with 1 M tris, pH 9.0.

1.3. Cholesterol efflux

Serum was assessed for its ability to promote the efflux of ^{3}H-cholesterol from J774 A.1 macrophages as previously described [14]. Results presented are normalized to HDL levels and are expressed as the percentage of cholesterol efflux.

1.4. Structural analysis of HDL

In this study the structural analysis of HDL consisted of the assessment of several components of the HDL particle: ApoA1, lecithin-cholesterol acyltransferase (LCAT), complement component 3 (C3), lipid peroxides, ApoE and glutathione peroxidase (GPx).

1.5. Assessment of HDL associated proteins

C3 [16], LCAT [16] and ApoE [16] were assessed in affinity-purified HDL by western blot. GPx-3 [17] was assessed in affinity-purified HDL by ELISA.

1.6. HDL-associated lipid peroxides and redox active iron

Total lipid peroxides associated with HDL and redox active iron were measured as previously described [14].

1.7. Serum CRP and adiponectin

Serum high sensitivity C-reactive protein (hs-CRP) and adiponectin levels were assessed by ELISA.

1.8. CD163 expression

Expression of CD163 by peripheral blood monocytes (PBMs) was examined by flow cytometry. Cells were treated with APC conjugated α-CD14 and PE conjugated α-CD163 antibodies and analysis was performed on a CyAN ADP analyzer.

1.9. Serum lipid profile and $ApoA_1$

HDL, total cholesterol and $ApoA_1$ were measured as previously described [18]. Nitration of ApoA1 was determined by western blot with results normalized to ApoA1.

1.10. Serum paraoxonase 1 (PON1) activity

Catalytic activities of PON1 was determined by using 5-(thiobutyl)-butyrolactone as substrate [19].

1.11. Statistical analysis

Results are reported as mean ± SE. Comparison of parametric values between groups was performed using Student's *t*-test or paired *t*-test as appropriate, with a p of ≤ 0.05 considered significant. Non-parametric values were compared with χ^2-test. Interaction testing was performed as described in an online Appendix. Briefly, we computed the change in efflux and relative change in efflux (change divided by baseline). Modeling was done with two observations for each patient (placebo and vitamin E) We computed the least square means with 95% confidence intervals with the PROC MIXED procedure in SAS 9.2.

2. Results

2.1. Baseline characteristics of Hp 2-1 and Hp 2-2 participants

Study participants with Hp 2-1 and Hp 2-2 genotypes did not differ in their baseline demographics and in the characteristics or management of their DM (Table 1 online supplement). At baseline, both $ApoA_1$ and serum stimulated cholesterol efflux (RCT) were significantly increased in Hp 2-1 as compared to Hp 2-2 study participants ($ApoA_1$: Hp 2-1, 156.8 ± 3.4 vs. Hp 2-2, 146.7 ± 3.7 $p = 0.05$; RCT: Hp 2-1, 15.4 ± 1.1% vs. Hp 2-2, 11.8 ± 0.8%, $p = 0.01$) (Table 2 online supplement).

2.2. Vitamin E improves RCT in Hp 2-2 but inhibits RCT in Hp 2-1

While in the Hp 2-2 cohort vitamin E was associated with a significantly higher RCT as compared to placebo (12.1 ± 0.81% vitamin E vs. 11.1 ± 0.64% placebo, $n = 28$; $p = 0.05$); in the Hp 2-1 cohort vitamin E was associated with a significantly lower RCT as compared to placebo (13.9 ± 1.1% vitamin E vs. 15.4 ± 1.0% placebo, $n = 31$; $p = 0.04$). There was a statistically significant interaction between the Hp genotype (Hp 2-1 vs. Hp 2-2) and vitamin E on RCT ($p = 0.006$). There was no significant effect of vitamin E on LCAT, $ApoA_1$ or HDL in Hp 2-1 or Hp 2-2 participants (Tables 1 and 2).