



Ascorbic acid supplementation does not lower plasma lipoprotein(a) concentrations

Jennifer L. Jenner *, Paul F. Jacques, Leo J. Seman, Ernst J. Schaefer

Lipid Metabolism Laboratory (JLJ, LJS, EJS) and the Epidemiology Program (PFJ),

Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA

Received 19 April 1999; received in revised form 6 September 1999; accepted 29 September 1999

Abstract

Elevated plasma concentrations of lipoprotein(a) (Lp[a]) are associated with premature coronary heart disease (CHD). Lp(a) is a lipoprotein particle consisting of low-density lipoprotein (LDL) with apolipoprotein (apo) (a) attached to the apo B-100 component of LDL. It has been hypothesized that ascorbic acid supplementation may reduce plasma levels of Lp(a). The purpose of this study was to determine whether ascorbic acid supplementation at a dose of 1 g/day would lower plasma concentrations of Lp(a) when studied in a randomized, placebo-controlled, blinded fashion. One hundred and one healthy men and women ranging in age from 20 to 69 years were studied for 8 months. Lp(a) values at baseline for the placebo group ($n = 52$) and the ascorbic acid supplemented group ($n = 49$) were 0.026 and 0.033 g/l, respectively. The 8-month concentrations were 0.027 g/l (placebo) and 0.038 g/l (supplemented group). None of these values were significantly different from each other. In addition, no difference in plasma Lp(a) concentration was seen between the placebo and supplemented groups when only subjects with an initial Lp(a) value of ≥ 0.050 g/l were analyzed. Our data indicate that plasma Lp(a) concentrations are not significantly affected by ascorbic acid supplementation in healthy human subjects. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Vitamin C; Lipoproteins; Lp(a)

1. Introduction

Lipoprotein(a) (Lp[a]) is a plasma particle that was first identified by Berg in 1963 [1]. Lp(a) is composed of a low density lipoprotein (LDL) particle and a highly glycosylated apolipoprotein (apo), apo(a), disulfide-linked to the apo B-100 of the LDL. Most prospective studies identify Lp(a) as a risk factor for coronary heart disease (CHD) [2–4].

Rath and Pauling linked Lp(a) concentrations with ascorbic acid concentrations by hypothesizing that the former may be a surrogate for the latter [5]. Subsequently, these investigators showed that administration of ascorbic acid to guinea pigs deficient in ascorbic acid resulted in a decrease of apo(a) accumulation in the arterial walls of the animals [6]. Although these findings have not been reproduced by other investigators, an interest in the possibility of using ascorbic acid supple-

mentation to lower plasma Lp(a) concentrations in humans was generated.

To assess whether ascorbic acid would reduce Lp(a) concentrations in plasma, we examined the effect of ascorbic acid supplementation in 101 healthy subjects over an 8-month period in a randomized, blinded, placebo-controlled fashion.

2. Methods

Subjects were part of a larger trial designed to examine the effects of ascorbic acid supplementation on plasma cholesterol concentrations [7]. The study was approved by the Human Investigation Review Committee of New England Medical Center and Tufts University. Exclusion criteria for the study included an elevated plasma ascorbic acid (PAA) concentration (> 80 fmol for men and > 90 fmol for women), a body mass index (BMI) > 31 kg/m² for men and > 33 kg/m² for women, a high density lipoprotein (HDL) choles-

* Corresponding author. Tel.: +1-617-5563228; fax: +1-617-5563103.

terol level > 1.4 mmol/l for men or > 1.7 mmol/l for women, and a plasma total cholesterol level > 6.7 mmol/l. Individuals who were current smokers, had a history of diabetes, heart disease, or liver disease, or used lipid-lowering medications or ascorbic acid supplements were also excluded. Of the 138 subjects recruited for the original study there was sufficient plasma from 101 subjects for measurement of Lp(a).

Subjects were randomized to receive either two 500 mg ascorbic acid tablets (Hoffman–LaRoche) or two matching placebo tablets per day. Subjects were instructed to take one tablet in the morning and one tablet in the evening for a period of 8 months. Fasting blood samples were obtained at baseline and at 8 months. PAA concentrations and returned pills were used to determine compliance. Plasma for ascorbic acid analyses was stabilized immediately after sample collection and then frozen at -70°C until the subject had finished the trial. Ascorbic acid concentrations were determined using a procedure adapted from the method described by Roe and Keuther [8]. Plasma Lp(a) was measured by an enzyme-linked immunosorbent assay (ELISA) (Macra Lp(a), Terumo Corp.,

Elkton, MD, now being marketed by Wampole Laboratories, Cranberry, NJ). Measurements of total cholesterol, HDL cholesterol, and triglyceride in plasma were carried out using enzymatic methods as previously described [7].

Student *t*-tests were performed to detect any differences between the placebo and supplemented groups at baseline and 8-month values. Analysis of covariance (ANCOVA) was used to adjust the Lp(a) concentrations at 8 months for differences in baseline Lp(a) concentrations between the ascorbic acid group and the placebo group and to test for differences between the groups at 8 months. Because triglyceride and Lp(a) distributions were skewed, these data were log-transformed prior to analysis. Analyses were performed with SPSS statistical software [9]. Geometric means and associated confidence intervals (CI) are presented as the anti-logarithm of the transformed means and CI.

2.1. Results

The baseline characteristics of the placebo ($n = 52$) and the supplemented ($n = 49$) groups are described in Table 1. Females comprised approximately 30% of both groups; they were combined with the males in all statistical analyses because there were no gender differences with respect to Lp(a) concentrations or the relationship between ascorbic acid and Lp(a). There were no differences between the groups in any of the baseline characteristics.

Presented in Table 2 are the geometric mean Lp(a) concentrations of the placebo and ascorbic acid groups at baseline and at 8 months. There were no statistically significant differences between the supplemented or placebo groups at either time-point with regard to Lp(a) concentrations.

We also tested whether 35 subjects that had baseline plasma Lp(a) concentrations ≥ 0.050 g/l were more responsive to ascorbic acid supplementation. Seventeen of these subjects were taking placebo and 18 were taking the supplement. The geometric mean baseline Lp(a) concentrations were 0.095 and 0.099 g/l ($P = 0.84$) for subjects taking the placebo and ascorbic acid supplement, respectively. In both groups, the Lp(a) concentration at 8 months was 0.110 g/l ($P = 0.71$).

To examine whether baseline PAA concentration had an effect on the response of Lp(a) to ascorbic acid supplementation, the subjects were dichotomized into two groups: those with PAA < 55 $\mu\text{mol/l}$ ($n = 61$) and those with \geq PAA 55 $\mu\text{mol/l}$ ($n = 40$). The baseline and month 8 Lp(a) concentrations were not different between these groups when analyzed according to their supplementation status (data not shown).

Table 1

Baseline characteristics of the placebo and ascorbic acid supplemented groups

	Placebo ($n = 52$)	Ascorbic acid ($n = 49$)
Age ^a	41 \pm 11	42 \pm 10
BMI (kg/m ²) ^a	25.5 \pm 3.0	25.9 \pm 2.4
Lipoprotein(a) (g/l) ^b	0.027 [0.003, 0.211]	0.029 [0.008, 0.193]
Total cholesterol (mmol/l) ^b	5.21 [3.36, 6.44]	4.94 [3.70, 6.54]
LDL cholesterol (mmol/l) ^b	3.30 [1.89, 4.42]	3.01 [2.04, 4.42]
HDL cholesterol (mmol/l) ^b	1.19 [0.75, 1.47]	1.11 [0.85, 1.55]
Triglycerides (mmol/l) ^b	1.21 [0.58, 3.39]	1.06 [0.65, 2.38]

^a Mean \pm SD.

^b Median [5th, 95th percentiles].

Table 2

Geometric mean plasma Lp(a) concentrations (g/l) of the placebo and ascorbic acid groups a; baseline and at 8 months

	Placebo ($n = 52$)	Ascorbic acid ($n = 49$)	<i>P</i> -value
Baseline ^a	0.026 (0.019–0.036)	0.033 (0.025–0.044)	0.25
Month 8 ^b	0.027 (0.019–0.038)	0.038 (0.029–0.050)	0.15
Month 8 ^c	0.031	0.034	0.31

^a Geometric mean (CI).

^b Unadjusted geometric mean (CI).

^c Geometric mean adjusted for baseline Lp(a).

3. Discussion

The purpose of the present study was to determine whether ascorbic acid supplementation at the dose of 1 g/day for 8 months would lower Lp(a) concentrations in a normal population in a randomized, placebo-controlled fashion. Ascorbic acid, as a relatively nontoxic and inexpensive antioxidant, would be an ideal candidate for lowering Lp(a) without the adverse side effects of other therapies. Unfortunately, our data indicate that ascorbic acid supplementation at the dose studied does not significantly lower Lp(a) concentrations. We concede that the concentrations of Lp(a) in this healthy population are relatively low. The normal distribution in Caucasians is highly skewed with approximately 60% of individuals having Lp(a) < 10 mg/dl [10]. However, we do feel that our conclusion is correct. Mensink et al. reported significant diet-related decreases in Lp(a) concentrations that initially were comparable to those in the present study indicating that even at relatively low levels, Lp(a) is susceptible to change [11].

Our results are in agreement with those of two other studies investigating the potential Lp(a)-lowering effect of ascorbate supplementation [12,13]. The first study involved patients with premature CHD [12]. Forty-three subjects were given either 4.5 g ascorbate or placebo daily for 12 weeks. There was no change in Lp(a) concentrations at the end of the treatment period despite a 120% increase in plasma ascorbate concentrations. While important, this study is limited to people with existing premature CHD. Lp(a) has been reported to be an acute-phase reactant [14] and interleukin 6-responsive elements have been identified in the apo(a) gene promoter [15]; therefore, the metabolism of Lp(a) in subjects with chronic disease may be altered due to various factors of the immune response.

The second study used 124 healthy individuals and the dose of ascorbic acid was either 1 or 2 g daily for a 1-month period followed by a 1-month 'washout' period [13]. There was no reduction in plasma Lp(a) concentrations after treatment or after the washout period. However, the study was not blinded or placebo-controlled, the supplementation period was short (1 month), and compliance was not determined.

Although ascorbic acid appears to have no effect on plasma concentrations of Lp(a), it may still indirectly play an important role in the metabolism of this lipoprotein particle. The reduced apo(a) accumulation in the arterial wall matrix of the guinea pig after ascorbate supplementation observed by Rath and Pauling may not necessarily have been due to reduced circulating Lp(a) levels. The presence of ascorbic acid could have led to a more rapid and complete repair of artery walls after injury, making them less susceptible to de-

position of Lp(a) and other lipoproteins. It is thought that oxidized lipoproteins in the arterial wall lead to the formation of foam cells [16]. These cells in turn, lead to the formation of fatty streaks, the earliest lesion associated with atherogenesis. The antioxidant properties of ascorbic acid may help to prevent lipoprotein oxidation and thus, foam cell formation. In this manner ascorbic acid may contribute to the protection against CHD that human populations with high intakes of ascorbic acid via fruits and vegetables appear to experience [17]. Further studies are needed to determine the mechanism(s) for this protection. However, the findings of this study indicate that ascorbic acid cannot be recommended as an Lp(a)-lowering agent.

Acknowledgements

This work was supported by Hoffmann-LaRoche and by the US Department of Agriculture, Agricultural Research Service under contract number 53-SK06-01. Jennifer Jenner received support from NIH Training Grant # T32AG00209.

References

- [1] Berg K. A new serum type system in man — The Lp system. *Acta Pathol Microbiol Scand* 1963;59:369–82.
- [2] Schaefer EJ, Lamon-Fava S, Jenner JL, et al. Lipoprotein(a) levels and risk of coronary heart disease in men: The Lipid Research Clinics Coronary Primary Prevention Trial. *J Am Med Assoc* 1994;271:999–1003.
- [3] Bostom AG, Gagnon DR, Cupples LA, et al. A prospective investigation of elevated lipoprotein(a) detected by electrophoresis and cardiovascular disease in women: The Framingham Heart Study. *Circulation* 1994;90:1688–95.
- [4] Bostom AG, Cupples LA, Jenner JL, et al. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. *J Am Med Assoc* 1996;276:544–8.
- [5] Rath M, Pauling L. Hypothesis: lipoprotein(a) is a surrogate for ascorbate. *Proc Natl Acad Sci USA* 1990;87:6204–7.
- [6] Rath M, Pauling L. Immunological evidence for the accumulation of lipoprotein(a) in the atherosclerotic lesion of the hypoascorbemic guinea pig. *Proc Natl Acad Sci USA* 1990;87:9388–90.
- [7] Jacques PF, Sulsky SI, Perrone GE, Jenner J, Schaefer EJ. Effect of vitamin C supplementation on lipoprotein cholesterol, apolipoprotein, and triglyceride concentrations. *Ann Epidemiol* 1995;5:52–9.
- [8] Roe JH, Keuther CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative de-hydro-ascorbic acid. *J Biol Chem* 1943;147:399–403.
- [9] Anonymous. *SPSS Reference Guide*. Chicago, IL: SPSS Inc. 1990.
- [10] Jenner JL, Ordovas JM, Lamon-Fava S, et al. Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels: The Framingham Offspring Study. *Circulation* 1993;87:1135–41.

- [11] Mensink RP, Zock PL, Katan MB, Hornstra G. Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein(a) levels in humans. *J Lipid Res* 1992;33:1493–501.
- [12] Bostom AG, Hume AL, Eaton CB, et al. The effect of high-dose ascorbate supplementation on plasma lipoprotein(a) levels in patients with premature coronary heart disease. *Pharmacotherapy* 1995;15:458–64.
- [13] Munoz JA, Garcia C, Quilez JL, Andugar MA. Effect of vitamin C on lipoproteins in healthy adults. *Ann Med Interne* 1994;145:13–9.
- [14] Maeda S, Abe A, Seishima M, Makino K, Noma A, Kawade M. Transient changes of serum lipoprotein(a) as an acute phase protein. *Atherosclerosis* 1989;78:145–50.
- [15] Wade DP, Clarke JG, Lindahl GE, et al. 5' control regions of the apolipoprotein(a) gene and members of the related plasminogen gene family. *Proc Natl Acad Sci USA* 1993;90:1369–73.
- [16] Steinberg D. A critical look at the evidence for the oxidation of LDL in atherogenesis. *Atherosclerosis* 1997;131:S5–7.
- [17] Bendich A, Langseth L. The health effects of vitamin C supplementation: a review. *J Am Coll Nutr* 1995;14:124–36.