Lipoprotein-a Reduction by Ascorbate

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Abstract

High plasma levels of lipoprotein-a constitute a strong risk factor for cardiovascular disease. Lipoprotein-a is primarily found in the plasma of man and other species that are unable to synthesize ascorbate endoge-nously. Here it is shown that ascorbate, a strong physiological reducing agent, lowers elevated lipoprotein-a plasma levels in man.

Introduction

Lipoprotein-a [Lp-a] is associated with an increased risk of atherogenesis and thrombo-genesis. Recently it was proposed that Lp-a is a surrogate for ascorbate (1). This proposal suggested a role of ascorbate in the regulation of Lp-a synthesis: namely, that increased intake of ascorbate, a strong natural reducing agent, would lower Lp-a plasma levels. N-Acetylcysteine (NAC) was then also proposed to lower Lp-a plasma levels and was reported to do this to a variable degree (2,3). The effect of ascorbate in lowering Lp-a plasma levels was studied in a clinical pilot study with the results reported here.

Patients, Materials and Methods

Eleven outpatients with coronary heart disease and elevated Lp-a levels consented to participate in this study. The patients received 9 grams of ascorbic acid (Bronson Pharmaceuticals, La Canada, California) per day for a period of 14 weeks. Plasma Lp-a levels were determined at the beginning and at the end of the study. Lp-a plasma levels were determined by a sandwich ELISA method with monoclonal capture antibodies against apo-a and monoclonal peroxidase-labeled antibodies against the apoB-100 portion of the Lp-a molecule (4). The antibodies were a gift from Dr. J. C. Fruchart, Lille, France.

Results

In this study ascorbate was found to lower Lp-a plasma levels on average by 27% with a median value also of 27% (Table 1). Two of the 11 patients showed no decrease of Lp-a during this time period. Lp-a in the same plasma samples was also measured with immunological assays using monoclonal antibodies against the apo-a portion of the Lp-a molecule for both, capturing and revealing (radioimmunoassay [RIA], Pharmacia Diagnostics; anti-apo-a sandwich ELISA). Changes in Lp-a plasma concentrations were measured for RIA mean +2%, median -7.5 % and for ELISA mean -4%, median -12%. The mean values for vitamin C plasma levels were 48.6 uM at the beginning and 94.4 uM at the end of the study.

Table 1

Patient No.	Time (weeks)	Lp(a) Plasma Levels (mg/dl)	Change of Plasma Lp(a) %
1	0 14	87 43	-51
2	0 14	92 59	-36
3	0 14	104 76	-27
4	0 14	36 27	-25
5	0 14	93 40	-43 *
6	0 14	27 18	-33
7	0 14	29 27	-7
8	0 14	60 44	-26
9	0 14	69 48	-30
10	0 14	48 56	+16
11	0 14	110 126	+14
			-27%

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Discussion

Two factors may account for the differences between the assay including an antibody against apoB and the assays using exclusively anti-apo-a antibodies. One factor could be the variation in epitopes of the apo-a molecule as a result of the variation of the molecular size determined by the genetic isoforms. This factor was largely excluded in this study by determining the apo-a isoforms by means of SDS PAGE and subsequent immunoblotting with anti-apo-a antibodies.

The second possible factor accounting for these differences is the effect of reducing agents on the intramolecular disulfide bonds of the apo-a molecule. This factor is discussed here in more detail. Apo-a has been proposed to function as a proteinthiol (1) and the disulfide bonds of the repetitive plasminogen kringle IV structure are known to have different dissociation constants. Elevated plasma concentrations of reducing agents such as ascorbate or NAC could alter the epitope constellation of the apo-a molecule in vivo by reducing some of the many disulfide bonds to sulfhydryl groups. Under this condition, assays using only anti-apo-a antibodies could give falsely positive results, dependent on the specific epitopes they recognize in the repetitive kringle structures of the apo-a molecule.

In contrast, an assay measuring the apoB portion of the Lp-a molecule should provide more reliable results since apoB contains less disulfide bonds and in addition has a constant molecular size. This conclusion could also explain the fact that the only two studies reporting a lowering of Lp-a plasma levels with reducing agents included assays using anti-apoB antibodies for detection (2, and this paper). In contrast, assays exclusively based on antibodies against apo-a gave variable results in the presence of reducing agents (2,3).

From in vitro studies with NAC it was recently concluded that supraphysiological concentrations of reducing agents above 1 mM decrease the immunoreactivity for Lp-a (5). The extrapolation of these results to the in vivo situation must, of course, be handled with care. The highest molar concentration of ascorbate measured in the study reported here was 154 uM, a level that does not decrease the immunoreactivity of apo-a or Lp-a. The effect of physiological levels of ascorbate on the reduction of disulfide bonds of the apo-a molecule as well as the possible immunological implications need further investigation.

The results of the clinical study reported here, namely that dietary ascorbate supplementation reduces Lp-a plasma levels, was supported by in vitro studies in our laboratory with human liver cells (HepG2, data not shown). In metabolic studies using S35 methionine increasing concentrations of ascorbate in the cell culture medium decreased the amount of Lp-a secreted by these cells. Ascorbate concentrations up to 2.25 mM did not reveal any dissociation of apo-a from apoB. It is, therefore, concluded that the effect of ascorbate on Lp-a plasma levels is the result of a decreased rate of synthesis of Lp-a particles in the liver.

In conclusion, ascorbate is a physiological reducing agent involved in the metabolic regulation of Lp-a synthesis. Dietary supplementation of ascorbate, as an adjunct to conventional therapy, should contribute to reducing elevated Lp-a plasma levels and the risk of cardiovascular disease. Prolonged supplementation of ascorbate may be required to achieve these effects.

References

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