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Chemistry

Hypothesis: Lipoprotein(a) is a surrogate for ascorbate

(vitamin C/atherosclerosis/coronary heart disease/cancer)

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ABSTRACT **The concept that lipoprotein(a) [Lp(a)] is a surrogate for ascorbate is suggested by the fact that this lipoprotein is found generally in the blood of primates and the guinea pig, which have lost the ability to synthesize ascorbate, but only rarely in the blood of other animals. Properties of Lp(a) that are shared with ascorbate, in accordance with this hypothesis, are the acceleration of wound healing and other cell-repair mechanisms, the strengthening of the extracellular matrix (e.g., in blood vessels), and the prevention of lipid peroxidation. High plasma Lp(a) is associated with coronary heart disease and other forms of atherosclerosis in humans, and the incidence of cardiovascular disease is decreased by elevated ascorbate. Similar observations have been made in cancer and diabetes. We have formulated the hypothesis that Lp(a) is a surrogate for ascorbate in humans and other species and have marshaled the evidence bearing on this hypothesis.**

Lipoproteins consist of particles, each of which is a globule of lipid molecules surrounded by an apoprotein shell. Lipoprotein(a) [Lp(a)] was discovered by Blumberg *et al.* (1) and by Berg (2). It shares with low density lipoprotein (LDL) its lipid and apoprotein composition—mainly apoprotein B-100 (apo B), consisting of a polypeptide chain of 4536 amino acid residues. The unique feature of Lp(a) is an additional glycoprotein, designated apoprotein(a) [apo(a)], which is linked to apo B by disulfide groups. The cDNA sequence of apo(a) (3) shows a striking homology to plasminogen, with multiple repeats of kringle 4, one kringle 5, and a protease domain. The isoforms of apo(a) vary in the range of 300 to 800 kDa and differ mainly in their genetically determined number of kringle 4 structures (3); apo(a) has no plasmin-like protease activity (4), but a serine protease activity has been demonstrated recently (5). Like plasminogen, Lp(a) has been shown to bind to lysine-Sepharose, immobilized fibrin and fibrinogen (6), and the plasminogen receptor on endothelial cells (7–9). This binding is inhibited by ε-aminocaproic acid, certain other amines, and plasminogen.

Lp(a) and Ascorbate in Different Species

Lp(a) has been detected in the plasma of humans, other primates (10), and the European hedgehog (*Erinaceus europeus*) (11). The presence of apo(a) in some other species cannot be excluded, since no comprehensive immunological or genetic screening has been reported yet. Most mammals synthesize ascorbate, usually in the range of 30–300 mg per day per kg of body weight. A few species, including humans, other primates, the guinea pig, and the Indian fruit-eating bat, have lost the ability to synthesize ascorbate. We observed that Lp(a) is found primarily in the plasma of those species that are unable to synthesize ascorbate. Vice versa, most mammals having an endogenous ascorbate supply lack detectable Lp(a) in their plasma.

It was the recognition of the correlation in mammal species of the two events, the loss of the ability to synthesize ascorbate and the detection of apo(a) and Lp(a) in the plasma of these species, that caused us to formulate the hypothesis that apo(a) serves as a surrogate for ascorbate. We have not found any earlier description of this hypothesis in the scientific literature.

The loss of the ability to synthesize ascorbate is the result of a mutation of the gene encoding for L-gulono-γ-lactone oxidase (GLO), which catalyzes the conversion of gulonolactone to ascorbate (12). In the case of the primates this mutation happened about 40 million years ago (13). Since ascorbate has numerous important metabolic functions and since the dietary ascorbate uptake was on average less than 10% of the amount synthesized by comparable animal species, this loss placed a great stress on the primates. This deficiency may have led to evolutionarily effective mutations to reduce this stress and in particular to acquire the ability to synthesize apo(a). Such a mutation may have occurred, in part, through the modification of another kringle-containing protein, such as plasminogen.

There is, however, another possibility. Other animals might be found in the future with functional genes for both apo(a) and GLO. In this case, it would be more likely that plasma ascorbate levels play a regulatory role in apo(a) synthesis.

Our hypothesis led to the prediction that the guinea pig, unable to synthesize ascorbate, would be found to produce detectable amounts of Lp(a). In fact, we were able to demonstrate apo(a) immunoreactivity in the blood of guinea pigs by SDS/PAGE and subsequent immunoblotting (unpublished observation). In another experiment the European hedgehog, known to have Lp(a) in its blood, was studied for its ability to synthesize ascorbate. One of our colleagues, C. Tsao, has shown that the hedgehog liver has not lost its ability to synthesize ascorbate (personal communication). This indicates that the genes for both apo(a) and GLO are present in the same animal. This observation supports the hypothesis of a regulatory role of ascorbate in the synthesis of apo(a).

Since the ability to synthesize apo(a) has survived millions of years in evolution, this protein must have one or more valuable functions. Some of these functions are discussed in the following sections.

Ascorbate and Lp(a) Strengthen the Extracellular Matrix and Promote the Healing of Wounds

Ascorbate is essential for the protection of the extracellular matrix system. This is in part due to the increased rate of synthesis of collagen when the ascorbate level is high. Ascorbate is required (one ascorbate molecule per hydroxyl

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Abbreviations: Lp(a), lipoprotein(a); LDL, low density lipoprotein; apo B, apoprotein B-100; apo(a), apolipoprotein(a); GLO, L-gulono-γ-lactone oxidase; CHD, coronary heart disease.

group) for many enzyme-catalyzed hydroxylation reactions, including the conversion of procollagen to collagen by the conversion of lysine and proline to hydroxylysine and hydroxyproline residues.

One of the manifestations of scurvy resulting from the extreme depletion of ascorbate is capillary fragility, followed by massive hemorrhages throughout the tissues (13). In such conditions inhibition of fibrinolysis would be advantageous. Because of its unique properties, Lp(a) is an ideal molecule to meet this requirement; apo(a), because of its homology to plasminogen, would target the Lp(a) particle to sites of increased vascular permeability. In this situation the ability of Lp(a) to competitively inhibit the binding of plasminogen to fibrin and the plasminogen receptor would be beneficial. In fact, Lp(a) has been shown to have antifibrinolytic properties (14).

It seems reasonable for us to propose that one way in which Lp(a) serves as a surrogate for ascorbate is the strengthening of the extracellular matrix in the blood vessels and other organs, particularly with low ascorbate concentrations. In fact, apo(a) has been detected in nonlesion areas of arterial wall from children (15). Lp(a) in the arterial wall would strengthen the arteries, but atherosclerosis would occur, as described later, if this function were to operate to too great an extent.

Another way in which Lp(a) functions as a surrogate for ascorbate is in accelerating the healing of wounds. Both high plasma ascorbate (references cited in pp. 136–140 of ref. 16) and high plasma Lp(a) (17) have been reported to accelerate the process of wound healing. A possible mechanism for Lp(a) is its binding to fibrin and other extracellular matrix components (5, 18), thereby compensating for a decreased rate in collagen formation, particularly when the ascorbate concentration is low.

Ascorbate, Uric Acid, and Lp(a) as Antioxidants

During evolution of primates a major factor in lengthening their life-span may have been improved protective mechanisms against damage by oxygen radicals. Free-radical-mediated lipid peroxidation seems to be critically involved in cardiovascular disease and in cancer, rheumatoid arthritis, and other pathological and degenerative processes, including aging. Ascorbate has been shown to completely protect plasma lipids against detectable peroxidative damage induced by aqueous peroxyl radicals, with other antioxidants (α-tocopherol, β-carotene, bilirubin, proteinthiols) being less effective (19). Other investigators have reported similar results for the guinea pig (20). The primates after having lost the ability to synthesize ascorbate may well have been under evolutionary pressure to develop other antioxidative mechanisms. Uric acid has been reported to be a moderately effective antioxidant, and the much increased level of urate in primates, in comparison with other animals, has been described as a response to the low level of ascorbate (21).

We now suggest that apo(a), with over 100 disulfide groups per molecule, is also effective as an antioxidant, acting also in this way as a surrogate for ascorbate. It would be especially effective in preventing peroxidation of lipids in the Lp(a) particle because of its presence in the shell surrounding the lipid sphere, where it could destroy the peroxyl radicals before they reach the lipids. Some of the internal disulfide groups in the kringles of apoprotein(a) might be reduced by ascorbate to thiol groups. Another possibility is the formation of disulfide radicals by adding or subtracting an electron, the latter giving a product analogous to the superoxide radical. Immunoblots of homogenized arterial wall taken at autopsy support this hypothesis (22). Twenty-four hours after death the apo(a) extracted was not degraded and had the same molecular size as the apo(a) in the premortem blood. In contrast, apo B is known to be partially degraded under these conditions.

Lp(a), Ascorbate, and Cardiovascular Disease

Ascorbic acid levels were found to be decreased in the plasma and leukocytes of coronary heart disease (CHD) patients (23). Furthermore, the concentrations of ascorbate in atherosclerotic lesions of human arterial wall are considerably lower than in the areas without lesions (24). In contrast, plasma Lp(a) was found to be elevated in CHD patients and patients with other forms of atherosclerosis (25, 26). There is a 1000-fold range of Lp(a) blood concentrations in human beings, determined largely by heredity (27, 28) and to some extent by environmental factors, especially nutrition (29). Lp(a) above 30 mg/dl doubles the risk of CHD, and if in addition LDL is elevated the risk is increased by a factor of 5 (30). There is no correlation between Lp(a) levels and cholesterol plasma levels, and in normolipemic CHD patients the only risk factor for CHD is found to be elevated Lp(a) (22). This observation indicates that Lp(a) can cause atherosclerosis without hyperlipidemia.

The importance of Lp(a) in human atherosclerosis has been revealed by a quantitative study of the amount of this lipoprotein in the wall of the ascending aorta of coronary bypass patients (22). Lp(a) deposition in the arterial wall was found to correlate with the extent of plaque development in both the human aorta and the coronary arteries (15). Furthermore, a selective accumulation of Lp(a) over LDL was established in both human arteries (22) and occluded coronary bypass vein grafts (31).

As discussed above, the development and retention of Lp(a) in evolution strongly support a beneficial role of Lp(a). The great range of concentrations of Lp(a) found in human plasma suggests that the control mechanisms for apo(a) synthesis at the optimum level have not yet been developed.

In addition, the atherogenicity of Lp(a) seems to be closely related to the ascorbate concentrations in plasma and tissue. We suggest that ascorbate deficiency increases plasma Lp(a). It is also known that ascorbate deficiency affects the integrity of the endothelial cell lining (32), thus promoting the infiltration of Lp(a) and other lipoproteins. On the basis of these considerations, we postulate that ascorbate can reduce or prevent the development of atherosclerosis by lowering plasma Lp(a), decreasing lipoprotein infiltration into the arterial wall, and preventing lipid peroxidation.

Ascorbate could prevent the atherogenicity of Lp(a) also in another way. Since the binding of Lp(a) to fibrin involves lysine groups, we suggest that, because of its involvement in hydroxylation reactions, ascorbate could convert these groups to hydroxylysine groups and thus contribute to preventing the attachment of Lp(a). The binding of Lp(a) might also be affected by chemical modification of the lysine-binding site of the Lp(a) particle itself and ascorbate could interfere with this modification.

The Guinea Pig as an Animal Model for Atherosclerosis

It is known that the formation of atherosclerotic plaques can be induced in the rabbit and other animals by feeding a high-cholesterol diet. This can also be done with the guinea pig. However, the guinea pig is in a remarkable way different. It has been reported that atherosclerotic deposits in the arteries of the guinea pig were formed on an ascorbate-deficient diet without additional cholesterol (33). We have verified that these deposits are not formed by guinea pigs given higher doses of ascorbate but are formed by the animals on an ascorbate-deficient diet without the administration of large amounts of cholesterol (unpublished experiments). Histological examinations showed that the atherosclerotic pro-

cess in the guinea pig resembles that in humans. Dissociation of the endothelial cells with parietal adhesion of coagulated lipemic plasma has been observed (34). Since we have been able to detect Lp(a) in the plasma of the guinea pig, we predict that Lp(a) will be found to be deposited in the arterial wall of hypoascorbemic guinea pigs and to contribute to plaque formation. Because of its similarity to man with respect to ascorbate and Lp(a) metabolism the guinea pig should be an ideal animal model for atherosclerosis research (ref. 16, p. 60).

Lp(a) and Ascorbate in Cancer and Diabetes Mellitus

Similar to the inverse correlation of Lp(a) and ascorbate in atherosclerosis, a high incidence of cancer is associated with low levels of ascorbate (35) and also high levels of Lp(a) (36). Similar observations were made in diabetes mellitus for ascorbate (37) and Lp(a) (38). Despite different causes, the progression of these diseases is dependent on the integrity and stability of the tissue, particularly the extracellular matrix (39). Ascorbate depletion in these pathological states will cause Lp(a) to increase and make up the deficiency at the sites of disease progression. We therefore predict that Lp(a) will be found in the vicinity of cancer processes.

Roles of Lp(a) and Ascorbate

A striking relationship of Lp(a) and ascorbate is that species that have lost the ability to synthesize ascorbate have detectable amounts of Lp(a) in their plasma. Plasma Lp(a) and ascorbate levels are inversely correlated in wound healing, atherosclerosis, cancer, diabetes, and other pathological conditions.

Additional evidence for the Lp(a)–ascorbate connection comes from another observation. In patients with trauma and after myocardial infarction, Lp(a) plasma levels were found to increase gradually, following acute phase proteins such as C-reactive protein, haptoglobin, and others, with a relatively late maximum at 2 weeks (17). Inversely, plasma ascorbate levels were found to decrease for approximately 2 weeks after myocardial infarction (23). The fall in ascorbate may be explained by mobilization of ascorbate at the site of the lesion, through migration of leukocytes.

Brown and Goldstein have suggested that Lp(a) might play a role in wound healing (40). We now suggest a broader role of Lp(a) in tissue maintenance and repair. In brief, we propose that Lp(a) is a late member in the chain of responses to cellular damage. Its role under physiological and pathophysiological conditions would be to reconstitute cellular and extracellular integrity. The fact that animals with plasma Lp(a) levels below the detection level do not suffer disadvantages strongly suggests that in its physiological role Lp(a) can be replaced. We therefore propose that not only is Lp(a) a surrogate for ascorbate, but also ascorbate is a surrogate for Lp(a).

Conclusion

We have marshaled the evidence that high levels of Lp(a) and low levels of ascorbate are associated with an increased incidence in mortality from cardiovascular disease, cancer, and other diseases. We suggest that Lp(a) levels may be decreased by ascorbate. There is epidemiological evidence (41) that dietary ascorbate supplementation is equally effective in reducing the mortality rate for heart disease, cancer, diabetes, and other diseases in the elderly. Moreover, preliminary studies have shown that the process of atherosclerosis in both guinea pigs (42) and humans (43) can be reversed by adequate amounts of ascorbate.

We have thus described a metabolic regulatory mechanism that may have significant implications for the most frequently occurring diseases in the industrialized countries. The application of this mechanism will significantly expand the scope of conventional therapy. It may lead the way to new approaches in the effective prevention and treatment of cardiovascular and other diseases.

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