ApoA-I mimetics: tomatoes to the rescue

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In this issue of the *Journal of Lipid Research*, Chattopadhyay and colleagues (1) describe a novel mode of delivery of a bioactive apoA-I mimic expressed as a transgene in tomato plants. This is one of the first, if not THE first, example of the oral delivery of an anti-inflammatory peptide and could be regarded as an encouraging prototype of a method for the delivery of a variety of peptide agents for pharmacologic benefit. These statements may seem reasonably straightforward, but reaching this potential beneficial goal required a great deal of earlier work.

This group and others (2, 3) have experimented intensively with the potential advantages of the apoA-I mimetics in a variety of disease models including atherosclerosis. These apoA-I mimetics are largely based upon the amphipathic helical repeating structure of apoA-I, the major protein of HDL. Although the apoA-I mimetic peptides do not directly reflect the specific amino acid sequence of the apoprotein, their physical properties resemble those of apoA-I and the family of exchangeable apolipoproteins. The initial canonical 18 amino acid peptide was developed in the laboratory of Jere Segrest (4). This peptide has now been designated 2F, because it has two phenylalanines on its hydrophobic face. A number of variants of this parent peptide have been synthesized, many of which retain biological activity such as ability to prevent the oxidation of LDL by artery wall cells and activate LCAT (5). These range from 3F to 7F based upon the number of phenylalanine residues substituting for nonpolar residues on the hydrophobic face of the peptide. To increase the helicity and stability of these peptides, the ends were blocked by N-terminal acylation and C-terminal amidation. At least in the case of the 4F peptide, end blocking was important for its biological activity also.

The 4F peptide with four phenylalanines (at positions 3, 6, 14, and 18) has received the most attention over the past decade. It has been synthesized from L amino acids (L4F) or D amino acids (D4F). This distinction is very important, as L4F proved to be unstable when orally delivered to animal models, whereas D4F is much more stable, presumably because D4F is more resistant to trypsin digestion in the gastrointestinal tract. Thus, D4F given orally or by injection was efficacious as an anti-inflammatory peptide that also reduced atherosclerosis in either the apoE-deficient or the LDL receptor-deficient (Ldlr<sup>−/−</sup>) mouse models. On the other hand, L4F was effective only when given by injection.

The 4F peptide has been shown to be effective not only for the treatment of murine atherosclerosis but also for the treatment of a variety of other chronic inflammatory conditions in animal models. Were they to be employed in the treatment of human atherosclerosis, repeated administration would be required as the half-life of the peptides in vivo is quite brief. In addition, the use of peptides fabricated from D amino acids and blocked at both ends would be prohibitively expensive.

Recent evidence has suggested that the intestine is the likely site of action of the peptides in the reduction of atherosclerosis [reviewed in (6)]. This is based upon the comparison of D4F administered either orally or subcutaneously. The efficacy by either route was equal at the same administered dose, although the liver and plasma levels were 100- to 300-fold greater after subcutaneous administration. Only in the intestine and the feces were the levels of peptide comparable for both routes of administration. In one sense, this outcome is “counterintuitive” as the intestine would be the first port of call for the oral peptide but surely not for subcutaneous administered peptide. Could there be an entity that binds the peptide that could account for these results?

These experimental observations and concerns about the production and administration of mimetic peptides to humans led the investigators to seek a peptide that could be delivered to the intestine in its “natural” state. Chattopadhyay et al. first sought to identify a peptide made up of L amino acids that did not require end blocking for its stability or biological efficacy. By empirical study, the L6F (phenylalanines at 6, 10, 11, 14, 17, and 18) met their needs. Synthetic unblocked L6F incorporated into the diet was an effective anti-inflammatory and anti-atherogenic agent in a murine model of hyperlipidemia and atherosclerosis. They then sought an efficient method for the large scale production of the 6F peptide in a form that

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could be readily administered orally in mammals. They turned to *Agrobacterium tumefaciens* bacteria-mediated transfection (7) to generate transgenic tomato plants expressing the 6F mimetic peptide along with a plant signal peptide. More than 20% of the transformed tomato plants were positive for the transgene. The choice of tomatoes allows for the consumption of the uncooked transgenic plant tissue, thus precluding heat-induced inactivation of the peptide. The investigators used *Ldlr*−/− mice consuming Western-type diet mixed with lyophilized tomato fruit tissue to demonstrate that the 6F peptide synthesized by the transgenic tomato plants was capable of reducing atherosclerosis. Of note, they established that consumption of the whole fruit tissue is effective without any need for peptide isolation and purification.

Chattopadhyay et al. looked for evidence for the site of action of the 6F peptide. In preliminary experiments, 6F incorporated into the diet in transgenic tomato tissue was detectable in the intestine but not in the plasma. If the absence of the peptide in plasma (or levels below detection) holds up on more extended studies, it carries important implications for the action of the mimetic peptide, at least of 6F. It strongly suggests the peptide’s anti-atherogenic effects are unlikely to involve the direct association with HDL, the direct stimulation of reverse cholesterol transport, or the direct modification of the phenotype of lesional macrophages.

How then may this peptide work? In an important paper by Van Lenten and colleagues (8), it was shown that the active mimetic peptides have a very high affinity for oxidized lipids, oxidized fatty acids, oxidized phospholipids, and oxidized sterols. Accordingly, Chattopadhyay and colleagues have assessed the concentrations of oxidized fatty acids, HETEs, prostaglandins, and lysophosphatidic acid (LPA) in the plasma and intestine. Studies reviewed previously (6) indicated that the oxidized metabolites of arachidonic acid were much higher in the enterocyte than in the hepatocyte but were proportionately reduced in both organs by D4F treatment. In the present paper, the feeding of transgenic tomatoes during the atherosclerosis experiment in *Ldlr*−/− mice reduced plasma SAA, total cholesterol, and triglycerides, and unsaturated but not saturated LPA levels. Plasma lysophosphatidic acid may have been derived by the phospholipolysis (phospholipase D) of the lysolecithin in the oxidized LDL. Interestingly, feeding both transgenic tomatoes and empty vector tomatoes reduced plasma levels of 5-HETE and 15-HETE, suggesting that the wild-type tomatoes had some limited but significant antioxidative activity. It remains to be established whether there is a synergistic influence of tomatoes and 6F peptide in transgenic tomatoes, though the action of the synthetic 6F added to the diet suggests that this may not be necessary. Along with the decline in many plasma biomarkers of oxidation associated with transgenic tomato treatment, an increase in HDL cholesterol and paraoxonase was also noted. Several of these biomarkers were significantly positively correlated (e.g., SAA, 15-HETE, unsaturated LPA levels) and negatively correlated (e.g., HDL-C and paraoxonase) with the percent of the aorta covered with lesions. Consistent with the intestine being the site of action of the mimetic peptides, unsaturated LPA levels in segments of the small intestine were also decreased in mice fed the transgenic tomatoes and the levels were positively correlated with aortic atherosclerosis.

As novel and exciting as are these observations in the Chattopadhyay et al. paper, many questions are outstanding. We do not have the space to explore these in detail. Some are mentioned in the article, and here we highlight and add some additional questions. The detailed physical and biological properties of 6F are yet to be explored, especially in comparison to 4F. Whether the changes in plasma HDL-C are attributable to a selective influence on the intestinal contribution to HDL biosynthesis (about 30% in mice) should be investigated. SAA is predominantly a hepatic product and whether it is simply a biomarker or mediator of the changes in lesions needs study. Chait and colleagues (9) have shown that SAA may be involved in the retention of lipoproteins in the vessel wall and the reduction in SAA levels may have contributed to the reduction in atherosclerosis directly. The affinity of 4F for LPA has only been studied with LPA 20:4. This needs to be extended both to other Acyl LPAs and to 6F. The role of the intestinal microbiome in producing the high oxidative burden in the small intestine requires further analysis.

In summary, one could postulate that the peptide in the intestine may serve as a reservoir for trapping oxidative

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**Fig. 1.** The reduction in the oxidative intermediates in the intestine mediated by the apoA-I mimetic peptides may lead to decreased levels of circulating proinflammatory oxidative intermediates resulting in decreased proinflammatory responses in the arterial vessel wall and other tissue such as the liver.
intermediates, so that the total circulating pool may be reduced. This reduction in circulating pool of oxidative intermediates would be expected to reduce the activation of the vessel wall and the generation of SAA in the liver. This formulation would not require the peptide to enter the plasma and circulate. These conjectures are reflected in the oversimplified diagram of the effect of the peptides (Fig. 1).

REFERENCES


