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Rapid Change in Plaque Size, Composition, and Molecular Footprint After Recombinant Apolipoprotein A-I$_{Milano}$ (ETC-216) Administration

Magnetic Resonance Imaging Study in an Experimental Model of Atherosclerosis

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Objectives This study sought to assess the effect of short-term apolipoprotein (apo) A-I$_{Milano}$ administration on plaque size and on suspected markers of plaque vulnerability.

Background Long-term lipid-lowering interventions can regress and stabilize atherosclerotic plaques. However, the majority of recurrent events occur early after the first episode. Interventions able to acutely induce plaque regression and stabilization are lacking. Regression of human coronary lesions after 5 weeks of treatment with apoA-I$_{Milano}$ administration has been shown. However, there are no data regarding its effect on plaque vulnerability.

Methods Advanced aortic lesions were induced in New Zealand White rabbits (n = 40). Plaque size was assessed by magnetic resonance imaging (MRI) at the end of atherosclerosis induction. Animals were randomized to placebo or apoA-I$_{Milano}$ phospholipids (ETC-216), 2 infusions 4 days apart. After the last dose, another MRI study was performed and aortas were processed for cellular composition and gene protein expression of markers associated with plaque instability.

Results Pre-treatment MRI showed similar plaque size in both groups, whereas post-treatment MRI showed 6% smaller plaques in apoA-I$_{Milano}$–treated animals compared with placebo ($p = 0.026$). The apoA-I$_{Milano}$ treatment induced a 5% plaque regression ($p = 0.003$ vs. pre-treatment), whereas the placebo showed no significant effect. Plaque regression by apoA-I$_{Milano}$ was associated with a reduction in plaque macrophage density and a significant down-regulation in gene and protein expression of tissue factor, monocyte chemoattractant protein-1, and cyclooxygenase-2, as well as marked decrease in gelatinolytic activity. Conversely, cyclooxygenase-1 was significantly up-regulated.

Conclusions Acute plaque regression observed after short-term apoA-I$_{Milano}$ administration was associated with a significant reduction in suspected makers of plaque vulnerability in an experimental model of atherosclerosis. (J Am Coll Cardiol 2008;51:1104–9) © 2008 by the American College of Cardiology Foundation

Lipid-lowering interventions have shown that atherosclerosis is not necessarily a constant cumulative process, as it is feasible to stop or even regress it (1–3). Initial studies showed that atherosclerosis progression can be halted by low-density-lipoprotein cholesterol lowering (2). More recent evidence has suggested that increasing high-density-lipoprotein (HDL) cholesterol can further regress atherosclerotic lesions (3) The latter observation, added to other pre-clinical (4,5) and clinical work (6), set up the basis for the HDL-raising approaches in the treatment of atherosclerotic disease.

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Acute complications of atherothrombosis are mostly secondary to plaque disruption with superimposed thrombus formation. Several post-mortem studies have strongly sug-

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gested the importance of plaque composition, rather than its stenotic severity, in the clinical manifestations of atherosclerosis. An increased macrophage density combined with the presence of high gelatinolytic activity is among the pathological features associated with plaque vulnerability. In addition, it is known that certain components are clearly associated with high-risk plaques, especially because on plaque rupture the local environment is highly pro-coagulant.

Statins have been shown to reduce cardiovascular events and even to induce plaque stabilization (7), but these plaque-stabilizing effects were always seen after long-term treatments. However, the majority of recurrent cardiovascular events take place within the first weeks after the initial episode (8).

Apolipoprotein (apo) A-I_{Milano} is a mutant form of apoA-I associated with low incidence of cardiovascular disease. Recombinant-ApoA-I_{Milano} (rApoA-I_M) has been tested in numerous pre-clinical studies showing a reduced plaque size and lower lipid and macrophage plaque content compared with control subjects (9,10). It has also been tested in humans, showing a plaque reduction after 5 weekly injections (6). What remains to be determined is whether this rapid plaque shrinkage is associated with plaque stabilization.

In this study we have aimed to study the effects of rApoA-I_M not only on plaque size but also on its composition and activity. In a model of advanced human-like atherosclerotic lesions, short-term rApoA-I_M administration induced an acute and significant regression of the lesions. Plaque regression was associated with cellular and molecular changes, suggesting a plaque stabilizing effect.

Methods

See the Online Appendix for an expanded methods section.

The atherosclerotic plaques were induced in the abdominal aortas of New Zealand White rabbits (n = 40). At the end of the atherosclerosis induction, all animals underwent a pre-treatment magnetic resonance imaging (MRI) study for plaque size quantification. Animals were then randomized to receive 2 intravenous injections (75 mg/kg), 4 days apart, of rApoA-I_M phospholipids (ETC-216, n = 22) (Pfizer, Groton, Connecticut) or equal volume of placebo (n = 18). Four days after the last dose, a second MRI study was performed. Subsequently, rabbits were euthanized and aortas were processed for further analyses.

The study protocol was approved by an institutional research committee, and animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

On MRI, sequential transverse images (3-mm thickness) of the 5 cm of abdominal aorta immediately distal to the celiac trunk were obtained. The initial and final images were matched for anatomical position as previously described (11). Cross-sectional areas of the lumen and vessel wall were determined by a validated semiautomatic quantification method (12).

The mean values for each rabbit were considered for statistical analysis. A secondary analysis included the effect of treatments in the most diseased lesion, which was defined as the largest plaque in 3 consecutive segments on initial MRI study.

Histological sections were stained with Masson trichrome-elastin stain, rabbit alveolar macrophage (RAM)-11 (macrophages), and alpha-actin (vascular smooth muscle cells). Tissue factor (TF), monocyte chemoattractant protein (MCP)-1, cyclooxygenase (COX)-1, and COX-2 antigen expression were analyzed by Western blot analysis. Polymerase chain reaction was used to assess messenger ribonucleic acid expression of the same markers. Gelatinase activity of matrix metalloproteinase (MMP)-2 was assessed in protein extracts from atherosclerotic plaques by zymography.

Abbreviations and Acronyms

apo = apolipoprotein
CETP = cholesteryl ester transfer protein
COX = cyclooxygenase
ETC-216 = recombinant apolipoprotein A-I_{Milano} and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes
HDL = high-density lipoprotein
MCP = monocyte chemoattractant protein
MMP = matrix metalloproteinase
MRI = magnetic resonance imaging
rApoA-I_M = recombinant apolipoprotein A-I_{Milano}
TF = tissue factor

Statistical analysis. Continuous variables are expressed as mean ± standard deviation. Statistical comparisons of means were made by Student paired and unpaired *t* tests for normally distributed variables. For nonnormally distributed variables, Wilcoxon and Mann-Whitney *U* tests were applied appropriately. A value of $p < 0.05$ (2-tailed) was considered statistically significant. The investigators had full access to the data and take responsibility for its integrity. All investigators have read and agree to the article as written.

Results

Circulating levels of rApoA-I_M peaked 30 min after ETC-216 infusion. Thereafter there was a slow reduction in circulating rApoA-I_M levels. The increase in rApoA-I_M levels was still significant up to 48 h post-administration (Online Appendix).

Effect of rApoA-I_{Milano} on vessel wall. Results of the effect of the treatments on vessel wall measurements assessed by MRI are presented in Table 1. Before treatments, there were no differences in plaque size between the 2 groups. Post-treatment MRI showed that plaque size was 6.2% smaller in animals receiving rApoA-I_M compared with placebo ($p = 0.026$). The administration of rApoA-I_M resulted in a 5% plaque regression ($p = 0.003$ vs. initial MRI), whereas a nonsignificant effect was observed in the placebo group. Figure 1 shows an example of plaque regression after rApoA-I_M administration. A similar effect was observed on the most diseased lesion: a statistically significant 6%