Inhibition of Apolipoprotein(a) Synthesis by Farnesoid X Receptor and Fibroblast Growth Factor 15/19
A Step Toward Selective Lipoprotein(a) Therapeutics

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Lipoprotein(a), or Lp(a), is a plasma lipoprotein that accumulates in concentrations ranging from undetectable to as high as 200 mg/dL, mostly depending on the synthesis rate of its unique protein, apo(a), which consists of multiple repetitions of kringle IV, type 2 of plasminogen. Additionally, plasma levels of Lp(a) are inversely proportional to the size of the apo(a) isoform, which is genetically determined. Considering that cholesterol represents about one fifth of the particle, Lp(a) can be equivalent to an extra 40 mg/dL of low-density lipoprotein (LDL) cholesterol in some individuals. The complex is the result of a covalent association between apoB100 and the function-free apo(a). The size of this lipoprotein ranges from that of a large high-density lipoprotein (HDL) to that of a small remnant depending on its triglyceride content. It is an apoB-containing lipoprotein and therefore viewed as an atherogenic particle. Because apo(a) does not form Lp(a) in murine plasma, development of a transgenic mouse model has required the concomitant expression of human apo(a) and apoB100. Apo(a) binds via a single disulfide bridge to the carboxyl-terminus of apoB100 and thus cannot associate with chylomicrons, which only contain apoB48. Also, apo(a) preferentially binds apoB100 of LDL-sized particles, although the linkage between the 2 proteins is likely to take place on the surface of hepatocytes, which produce exclusively VLDL-sized particles. On the clearance front, Lp(a) does not bind to the LDL receptor, and only modest increases in Lp(a) levels are seen in patients with familial hypercholesterolemia. This means that cholesterol-lowering agents acting more or less directly via upregulation of the LDL receptor (statins, resins, and cholesterol absorption inhibitors) have modest to no effects or may even raise plasma Lp(a) levels. Other agents, such as fibrates and niacins, share similar lipid-modulating effects (decreasing triglycerides, increasing HDL cholesterol) but show diverging effects on Lp(a) levels, which may be increased modestly by fenofibrate and decreased significantly by niacin. The mechanism of action of niacin on Lp(a) is not defined, but is likely related to reduced secretion of apoB-containing lipoproteins by the liver. This notion is confirmed by emerging therapies that directly regulate hepatic apoB output, such the apoB antisense mipomersen and the liver-specific thyromimetic eprotirome, both of which lower Lp(a) levels.

Because production of apo(a) is the critical step in the assembly and plasma accumulation of Lp(a), a logical approach to targeting this lipoprotein pharmacologically would be via inhibition of the synthesis of this apparently unnecessary protein. No available drugs are known to act on this mechanism. Even though Lp(a) was discovered nearly 50 years ago, the debate on whether it represents a risk factor for cardiovascular disease is still ongoing, but consensus is finally mounting up for its role in the disease process, necessity of screening, and recommendation of therapeutic targeting. Because of the absence of agents that exclusively and significantly modulate Lp(a) levels, there is no clinical trial information on the importance of lowering Lp(a) levels to reduce cardiovascular events. However, Maher et al in 1995 published evidence showing that the excess cardiovascular risk attributable to elevated Lp(a) levels was nearly eliminated by lowering LDL below 100 mg/dL. This was interpreted by many practitioners as a recommendation to use Lp(a) simply as a marker to decide the level of LDL lowering necessary to control risk. It is undeniable, however, that some high-risk patients in practice present with extremely elevated Lp(a) levels as the most prominent risk factor, and it is not known whether aggressive reduction of Lp(a) would be of benefit in these individuals. Recent population data have given what many consider the final confirmation of the importance of apo(a) and Lp(a) in cardiovascular risk attribution. Therefore, more emphasis is currently being placed on determining Lp(a) levels as part of cardiovascular risk profiling, often leading to management of inappropriately elevated levels (>50 mg/dL of particle mass). Niacin, the only commonly used lipid-lowering agent that reduces Lp(a) levels, is currently being questioned as an appropriate agent for high-risk subjects given the negative results of the Atherosclerosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health (AIM-HIGH) trial, where it failed to convey additional risk reduction to coronary artery disease patients already taking statin, alone or with ezetimibe. In this National Institutes of Health cosponsored trial, niacin was used with the intent of increasing HDL cholesterol levels in subjects at LDL goal, and therefore the lack of clinical effects is currently being attributed to either an ineffective HDL modulation by niacin or the general futility of raising HDL in high-risk individuals with LDL already at goal. Because niacin also reduced Lp(a) levels by 25%, it may
be inferred that this maneuver also fails to produce benefits. It has to be noted, however, that in the AIM-HIGH trial the median Lp(a) level was low (<20 mg/dL).

In the study by Chennamsetty et al19 published in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, evidence is presented for a negative regulation of apo(a) synthesis that could eventually be exploited for development of therapeutics to reduce Lp(a) levels. In a recent report, the same group of investigators showed that bile acid strongly inhibits apo(a) synthesis.20 Patients with biliary obstruction and plasma bile acid levels 10 times above normal had Lp(a) levels either undetectable or inappropriately low for the apo(a) isoform. Lp(a) levels increased by nearly 10-fold on average after correction of the biliary obstruction. It should be noted that a similar observation was previously made with cholic acid treatment. We encourage an evaluation of Lp(a) levels in a similar setting using human proteins in the mouse model, these results are novel and relevant to development of therapeutics, as they forecast the possibility that natural molecules such as bile acids or novel FXR and FGF19 agonistic agents may be used to decrease apo(a) synthesis and regulate plasma Lp(a) levels. However, a more informed expectation for the clinical applicability of such an intervention would obviously derive from human studies. Since FXR agonists have been tested and may be marketed for treatment of subjects with primary biliary cirrhosis (http://clinicaltrials.gov/ct2/show/NCT00550862), this patient population is unlikely to yield information on Lp(a), as its levels should be depressed by the condition and further suppressed by treatment. We encourage an evaluation of Lp(a) level changes in existing clinical trial data sets of subjects taking either bile acids for treatment or prevention of cholelithiasis, or bile acid binding resins for lipid or glucose management.

Sources of Funding
The authors were partially supported by National Institutes of Health grants HL106845, HL 57986 (to Sergio Fazio), and HL086988 and HL105375 (to MacRae F. Linton).

Disclosures
None.

References
3. Linton MF, Farnese RV Jr, Chiesa G, Grass DS, Chin P, Hammer RE, Hobbs HH, Young SG. Transgenic mice expressing human apo(a) reduced the synthesis of this protein by nearly 90%. This effect was repeated by cholic acid treatment, thus suggesting a role for farnesoid X receptor (FXR), a transcription factor that binds and gets activated by bile acids. Indeed, the effect of cholic acid on apo(a) synthesis was not noted in FXR null mice. The authors were able to identify the direct repeat 1 element (nucleotides −826 to −814) of the apo(a) gene promoter as a negative FXR response element. The inhibitory effect of FXR was attributable to competitive direct repeat 1 binding with hepatocyte nuclear factor 4α, which instead promotes transcription of the apo(a) gene. Although impressive, the results also showed that the inhibitory effect of cholic acid on apo(a) synthesis could not be attributed exclusively to FXR competition with hepatocyte nuclear factor 4α at the direct repeat 1 element. Because FXR also transactivates mouse fibroblast growth factor (FGF)15 and its human ortholog, FGF19, an intestinal protein that signals transcriptional repression of bile acid biosynthesis,22 the authors conducted another series of experiments to complete this promising story.

In the current paper, the investigators show that FGF19 binds to fibroblast growth factor receptor 4 and activates a signaling cascade involving the MAPK/ERK1/2 pathway leading to suppression of apo(a) transcription via nuclear displacement of Elk-1 and binding to Ets-1, a negative control element (−1630 to −1615) in the human apo(a) gene promoter. FGF19 treatment of primary hepatocytes from human apo(a) transgenic mice reduced accumulation of apo(a) in the medium by nearly 50%. Injection of FGF19 in mice produced a similar effect on liver mRNA and plasma levels of apo(a). An acute knockdown of fibroblast growth factor receptor 4, the receptor for FGF19, significantly reduced the inhibitory effect on apo(a) transcription. After showing that FGF19 causes phosphorylation of ERK1/2, both cloning experiments and in silico analysis suggested the Ets-1 element as the binding site for an inhibitory effect. Elk-1 was then an obvious choice, because this nuclear factor is a substrate for ERK1/2 and is known to bind Ets-1. Proof of this involvement comes from a chromatin precipitation experiment showing binding of Elk-1 to the Ets-1 element of the apo(a) promoter after incubation with FGF19.

Although obtained in an artificial setting using human proteins in the mouse model, these results are novel and relevant to development of therapeutics, as they forecast the possibility that natural molecules such as bile acids or novel FXR and FGF19 agonistic agents may be used to decrease apo(a) synthesis and regulate plasma Lp(a) levels. However, a more informed expectation for the clinical applicability of such an intervention would obviously derive from human studies. Since FXR agonists have been tested and may be marketed for treatment of subjects with primary biliary cirrhosis (http://clinicaltrials.gov/ct2/show/NCT00550862), this patient population is unlikely to yield information on Lp(a), as its levels should be depressed by the condition and further suppressed by treatment. We encourage an evaluation of Lp(a) level changes in existing clinical trial data sets of subjects taking either bile acids for treatment or prevention of cholelithiasis, or bile acid binding resins for lipid or glucose management.


**KEY WORDS:** intravascular ultrasound/Doppler
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doi: 10.1161/ATVBAHA.112.245571
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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