Sphingolipids and Atherosclerosis
A Mechanistic Connection? A Therapeutic Opportunity?
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When inhibitors of biochemical pathways are fed to experimental animals, the goals are to understand physiological or pathophysiological consequences of the inhibited pathway and possibly to obtain evidence for new therapeutic strategies for diseases affected by the pathway. In this issue of Circulation, Park et al1 fed Apoe−/− mice a compound isolated from fungi, myriocin, that inhibits the rate-limiting enzyme in ceramide and sphingolipid biosynthesis, serine palmitoyl transferase (SPT). The study was founded on a series of previous observations that implicated sphingomyelin (SM) and ceramide in lipoprotein metabolism and atherosclerosis, including a study showing that plasma SM is an independent risk factor for coronary artery disease in humans.2 The goal of the study by Park et al1 was to test the effect of myriocin on plasma lipoprotein levels and atherogenesis in a well-defined animal model of atherosclerosis. The authors found that myriocin treatment in Apoe−/− mice was associated with a protective lipoprotein profile—namely, a decrease in βVLDL and LDL, an increase in HDL, and a reduction in atherosclerosis.

What can we learn from these results in terms of mechanism and therapeutic potential? With regard to mechanism, two crucial issues are the specificity of myriocin and the distinction between primary lipoprotein versus primary arterial-wall mechanisms. As the authors point out, myriocin may have effects that are independent of its ability to inhibit SPT, such as an immunosuppressive action.3 Given the role of immunologic processes in atherogenesis,4 one needs to consider the possibility that this effect of the compound could be at least partially responsible for the decrease in atherosclerosis, perhaps working in concert with a protective alteration in the lipoprotein profile. Examples of ways to deal with specificity issues related to compounds are to complement the findings by using genetic manipulation and to use structurally related but inactive enzyme inhibitors as controls. Although SPTLC1/2 gene–targeted mice have not been reported and may not be viable, the authors mention that a myriocin analogue exists that has immunosuppressive effects without inhibiting SPT. Unfortunately, the study did not include a control group of mice fed this analogue. Nevertheless, the authors present some evidence suggesting that immunomodulation is not responsible for the myriocin effect. For example, control and experimental lesions had similar numbers of CD3-positive T cells.

The most important finding in the study was a marked decrease in atherosclerosis in the myriocin-fed mice. When genetic or pharmacological alterations affect atherosclerosis, the key mechanistic issue is to distinguish between primary arterial-wall effects and changes in plasma lipoproteins that secondarily affect the arterial wall. Because myriocin treatment of Western diet–fed Apoe−/− mice resulted in a favorable change in plasma lipoproteins, this distinction in the study by Park et al1 becomes very difficult. To deal with this issue, one is tempted to compare the experimental group with another group with similar plasma lipoproteins. In the present study, Western diet–fed Apoe−/− mice treated with myriocin (labeled “WD+myr”) and Apoe−/− mice on a standard chow diet without myriocin (labeled “STD”) had similar aortic SM content and similar plasma levels of SM, total cholesterol, triglyceride, LDL, and HDL. Moreover, compared with the Chow-fed control group, the fat-fed myriocin group actually had higher levels of βVLDL, which is thought to be the most important atherogenic lipoprotein in Apoe−/− mice.5–7 Despite these lipoprotein comparisons, the myriocin-treated mice still had significantly less atherosclerosis than the Chow-fed control group. These data would suggest that at least part of the antiatherogenic effect of myriocin is independent of plasma lipoproteins and thus attributable to a direct arterial-wall effect. One must be careful in comparing different diet groups, however, because interactions between dietary effects and drug effects may confound the interpretation, resulting in an “apples-versus-oranges” type of comparison.

Despite these uncertainties, inhibition of sphingolipid synthesis is likely to have several independent effects, including those that could alter both lipoprotein metabolism and atherogenesis. For example, Park et al1 point out that the SM content of HDL has been shown to affect lecithin-cholesterol acyltransferase activity in vitro,8 and the SM content of LDL and βVLDL might affect the atherogenicity of these lipoproteins in the arterial wall.9 In particular, our laboratory has shown that SM-rich LDL and βVLDL are more susceptible to an arterial-wall sphingomyelinase called secretory sphingomyelinase (S-SMase), leading to ceramide-mediated aggregation.9 Aggregated subendothelial lipoproteins, because of their size, are better retained in the arterial wall than are monomeric lipoproteins and are readily phagocytosed by macrophages, which leads to massive foam cell formation.9 The finding that aggregated lipoproteins isolated from human atherosclerotic lesions are enriched in ceramide, indicating

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prior action by SMase on these particles, suggests relevance to human atherosclerosis.10 The HDL–lecithin-cholesterol acyltransferase and lipoprotein aggregation findings are only two examples of what is likely to be a multitude of effects of SPT inhibition, particularly when one considers the many biological activities of ceramide and complex sphingolipids in addition to SM. For example, decreases in cellular ceramide levels as a result of SPT inhibition could have marked effects on proliferation and apoptosis of arterial-wall cells.11

The second major goal of in vivo inhibitor studies is to test therapeutic potential. Therapeutic potential in humans is intimately linked to mechanism because certain mechanisms of antiatherogenesis in mice may not be relevant to humans. In particular, lipoprotein metabolism in wild-type and Apoe−/− mice is different from that in humans. In contrast to humans, wild-type C57BL6 mice have high plasma levels of HDL (which largely is attributable to the absence of cholesterol ester transfer protein activity) and low levels of LDL. Apoe−/− mice have high levels of βVLDL but relatively low levels of LDL, and LDL is the lipoprotein most closely associated with atherosclerosis in humans. Perhaps most relevant to the present study, acid SMase−deficient mice have high plasma HDL, whereas acid SMase−deficient humans (types A and B Niemann-Pick disease) have low plasma HDL.12–14

Similarly, the Apoe−/− mouse is an incomplete model of human atherothrombotic vascular disease. Whereas these mice develop both early and advanced lesions that are similar to those in humans, they are not a good model for plaque rupture and thrombosis, which constitute the critical link among atherosclerosis, acute vascular obstruction, and tissue infarction. In this regard, Park et al1 note that myricin-treated mice had a decrease in lesional necrosis. Although these data were not quantified, a similar effect in humans treated mice had a decrease in lesional necrosis. Although infarction. In this regard, Park et al1 note that myriocin–infarction. In this regard, Park et al1 note that myriocin–amidate levels as a result of SPT inhibition could have marked effects on proliferation and apoptosis of arterial-wall cells.11

Notwithstanding the limitations discussed above, the study by Park et al1 is an important step in understanding the associations among sphingolipid metabolism, lipoprotein metabolism, and atherogenesis and in considering how these associations might someday be translated into a novel antiatherogenic class of drugs. If sphingolipid biosynthesis inhibitors were to have a beneficial effect on plasma lipoproteins in humans with a good safety profile, they may have a niche in combination therapy. For example, the recent experience with the cholesterol absorption inhibitor ezetimibe in combination with statins has taught us that LDL lowering can be markedly enhanced by drug combinations with complementary mechanisms of action.17 If sphingolipid biosynthesis inhibitors turn out to have beneficial effects on the arterial wall, the combination with other drugs that lower plasma LDL could be particularly powerful in preventing atherosclerotic vascular disease.