NEW TOOL TO COMBAT HEART DISEASE

Compound designed to treat bone diseases shows unexpected benefit for removing cholesterol from atherosclerotic lesions.

Although still the leading cause of death in the United States, the mortality rate from coronary heart disease has been decreasing over the past 50 years (Figure 1). The rate of decrease has accelerated since 1980, and it has been estimated that approximately 50% of the change can be attributed to better therapy [E.S. Ford, et al. (2007) New Eng. J. Med., 356, 2388]. One of the most successful approaches to the treatment of heart disease has been the use of the statin class of cholesterol lowering agents. These drugs have proven highly effective at reducing serum total cholesterol and low density lipoprotein (LDL) cholesterol (the “bad” cholesterol”) levels, primarily by blocking cholesterol synthesis. However, these drugs do little to help remove excess cholesterol from the body. Thus levels of high density lipoprotein (HDL) cholesterol (the “good” cholesterol), which result from transport of the lipid from peripheral tissues back to the liver, are usually not raised by statin treatment. Now, Vanderbilt Institute of Chemical Biology (VICB) member Charles Hong, his collaborator Aloke Finn (Division of Cardiology, Emory University, Atlanta, GA), and their laboratories have found a novel approach to remove excess cholesterol from peripheral tissues and reduce atherosclerotic lesions in a mouse model of cardiovascular disease [(2011) O. Saeed et al., Arterioscler. Thromb. Vasc. Biol., published online Nov. 17, DOI: 10.1161/ATVBAHA.111.240101].

Atherosclerosis is characterized by the formation of hardened, lipid-laden plaques in arterial walls. Macrophages play a major role in plaque formation, as they accumulate cholesterol and cholesteryl esters to form enlarged foam cells, a major plaque component. The Finn lab discovered a type of macrophage, which they designated M(Hb), that was notable for its resistance to foam cell formation. Compared to other macrophages, M(Hb) cells had antioxidant properties, attributable to a reduced level of intracellular iron. This low oxidant tone promoted the expression of the ABCA1 and ABCG1 transporters, which are primarily responsible for export of cholesterol out of the macrophage. The low iron content of M(Hb) cells

was the result of high levels of expression of another transporter, the free iron exporter ferroportin (FPN). These results suggested that conditions that favor expression of FPN should reduce macrophage oxidant tone and promote cholesterol efflux from the cells.

Intracellular FPN levels are regulated by the liver hormone, hepcidin. Binding of hepcidin to FPN leads to its degradation. Hepcidin expression is, in turn, regulated by bone morphogenic protein (BMP), a growth factor originally discovered through its ability to promote bone growth. BMP has been the subject of intense investigation in the Hong lab for many years, and they have discovered highly specific inhibitors of BMP signaling, among them LDN 193189 (LDN, Figure 2). Thus, the Hong and Finn labs hypothesized that use of LDN to block BMP-induced hepcidin expression should lead to increased macrophage FPN levels, and ultimately increased cholesterol efflux (Figure 3).

The Finn and Hong labs tested their hypothesis using mice bearing a genetic deletion of ApoE (ApoE⁻/⁻), a protein required for the formation of lipoproteins. When these mice are fed a high cholesterol diet, they develop severe atherosclerosis. The investigators found that treatment of cholesterol-fed ApoE⁻/⁻ mice for ten weeks with LDN, led to a reduction in the size and severity of atherosclerotic plaques at multiple arterial sites. They also found increased expression of the cholesterol transporter ABCA1 in the plaques, consistent with their prediction that blocking BMP signaling would indirectly lead to increased cholesterol transport.
A four day treatment with LDN led to decreased hepcidin mRNA levels in the livers of ApoE–/– mice. Macrophages isolated from the treated mice revealed increased expression of FPN accompanied by decreased intracellular iron levels and reduced production of hydrogen peroxide. These macrophages also showed increased levels of the mRNAs for ABCA1 and ABCG1, as well as higher levels of the ABCA1 protein. Incubation of these macrophages with oxidized LDL resulted in similar uptake of total cholesterol and free cholesterol as was observed in macrophages from untreated mice. However, macrophages from LDN-treated mice accumulated much lower levels of cholesterylesters. Furthermore, incubation of the lipid-laden macrophages with ApoA1, an apolipoprotein that facilitates removal of cholesterol from cells, led to much lower cholesterol levels in macrophages from LDN-pretreated mice than from control mice. All of the effects of LDN on macrophage function were reversed if the mice were treated with hepcidin in addition to LDN, confirming that LDN’s action was through blocking BMP-mediated hepcidin expression.

A ten week LDN treatment caused no significant difference in weight gain, total cholesterol, triglycerides, hematocrit, or cardiac ejection volume, suggesting low toxicity. Whereas plasma iron levels were higher in LDN-treated mice, liver iron levels were not different from those of controls. The results support the hypothesis that controlling macrophage iron levels through suppression of BMP signaling leads to increased macrophage cholesterol efflux and reduced atherosclerotic cardiovascular disease. However, the authors note that hepcidin is the primary hormonal regulator of iron metabolism. Thus, long term suppression of hepcidin expression could lead to iron-related toxicity that must yet be explored.