Protection Against Ischemia/Reperfusion Injury by High-Density Lipoprotein and Its Components

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Identification of Apolipoprotein D as a Cardioprotective Gene Using a Mouse Model of Lethal Atherosclerotic Coronary Artery Disease

Apolipoprotein D is expressed in many tissues after injury, such as reperfusion injury after myocardial infarction. Antioxidant activity of apolipoprotein D appears to confer cardioprotective properties after myocardial infarction in a mouse model of severe rapidly progressing coronary atherosclerosis and in a mouse model of ischemia/reperfusion injury.

Mice with homozygous-null mutations in the high-density lipoprotein (HDL) scavenger receptor class B type I (SR-BI) and also with homozygous-null mutations in apolipoprotein E (apoE) experience development of severe occlusive coronary atherosclerosis and have myocardial infarction (MI) starting at approximately 31 days after birth. By 42 days after birth, ≈50% have died from cardiac causes. Tsukamoto et al1 studied myocardial gene expression before MI (21 days after birth) and at 31 and 43 days after birth in these mice (SR-BI−/−/apoE−/−). The expression of the gene for osteopontin increased 416-fold, and the gene for apoD increased 80-fold. Because it had previously been reported that the gene for osteopontin increased post-MI,2,3 the authors concentrated on the study of apoD. In an ischemia/reperfusion injury model, adenoviral expression of apoD in the liver was associated with high plasma apoD levels and reduced MI size. In contrast, deficiency of apoD (in apoD-null mice) was associated with increased MI size. The ability of apoD to protect cultured rat cardiomyocytes from hypoxia/reoxygenation injury correlated with the ability of apoD to inhibit oxidation in an in vitro antioxidant assay, suggesting that the antioxidant properties of apoD are important in mediating its cardioprotective properties.

The authors determined the time course of disease development in the SR-BI+/−/apoE−/− mice and compared gene expression by microarray analysis at 21, 31, or 43 days of age. They then compared 89 genes whose relative transcript levels at 43 days were increased >6-fold in the hearts of the SR-BI+/−/apoE−/− mice to those induced in mouse hearts between 1 and 8 weeks after surgical coronary artery ligation as previously reported in the literature. Of the 89 genes induced >6-fold in the SR-BI+/−/apoE−/− mice at 43 days, 81 were shown to be induced after coronary ligation. The 81 genes included those encoding matricellular proteins, matrix proteases, tissue inhibitors of metalloproteinases, and inflammation-associated and fibrosis-associated proteins. In the coronary ligation experiment, apoD increased early (48 hours) after ligation and was substantially increased in noninfarcted, but not in infarcted, tissue. Four days after the mice were administered adenoviruses without (empty vector) or expressing apoD, the mice were subjected to 60 minutes of coronary ischemia followed by ≈24 hours of reperfusion. The mice receiving apoD adenoviruses had ≈20-fold increase in plasma levels of apoD and had a relative infarct size of 59% compared with 81% for the mice receiving empty vector (P<0.005). Conversely, mice that were genetically null for apoD and that were subjected to the same procedure had a significant increase in the infarct area (76% versus 37% for wild-type; P<0.0001). In vitro primary adult rat ventricular myocytes or neonatal rat ventricular myocytes were subjected to hypoxia followed by reoxygenation in the presence of either bovine serum albumin or human apoD. Only apoD was protective against cell death. The authors noted that osteopontin was also previously reported to protect cultured neonatal rat ventricular myocytes from similar stress.4

Because apoD is structurally a member of the lipocalin family and has a β-barrel structure with an elongated hydrophobic pocket that binds small-molecule ligands such as progesterone, the authors extensively dialedyzed human apoD and found that this did not alter the ability of apoD to protect neonatal rat ventricular myocytes from hypoxia/reoxygenation injury. However, boiling apoD virtually eliminated its protective activity. Based on previous reports of antioxidant activity by apoD, the authors compared human apoD with Trolox (a water-soluble derivative of vitamin E) for ability to inhibit the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt by hydrogen peroxide and myoglobin. They found that 1.2 μmol/L of apoD was equivalent to 314 μmol/L Trolox, indicating that apoD is a potent antioxidant. Extensive dialysis of apoD did not alter its antioxidant properties in this assay, but boiling reduced the antioxidant activity of apoD in this assay by 71%. The authors concluded that a properly folded conformation of apoD is important to its antioxidant properties and the antioxidant properties of apoD are likely important to its cardioprotective activity.

ApoD was previously shown by others to potently reduce hydroperoxyicosatetraenoic acids to their hydroxyl derivatives.
by a mechanism that seems to involve methionine 93 in apoD.\textsuperscript{7,8} Treatment of apoD with hydroperoxyeicosatetraenoic acids resulted in loss of one-third of the methionine residues in apoD and was accompanied by the formation of methionine sulfoxide and resulted in protein aggregation. ApoD is known to be upregulated in the brains of patients with Alzheimer disease, and apoD aggregates were detected in the hippocampus of patients with Alzheimer disease.\textsuperscript{7} In other studies, it was shown that overexpression in mouse brain of human apoD prevented the increase in brain lipid peroxides after oxidant treatment and improved survival; the loss of apoD had the opposite effect.\textsuperscript{9}

ApoD is carried in the plasma mainly on HDLs.\textsuperscript{10} HDL has been reported to show altered activation of endothelial antiapoptotic and proapoptotic pathways depending on whether it is taken from healthy individuals or patients with coronary disease.\textsuperscript{11,12} Remaley et al\textsuperscript{13} reported that peptides mimicking apoA-I (the main protein in HDL) significantly reduced injury in an isolated rat heart model of ischemia/reperfusion injury. Navab et al\textsuperscript{14} reported that an apoA-I mimetic peptide (4F) that is known to bind hydroperoxyeicosatetraenoic acids with extraordinary high affinity reduces systemic inflammation by modulating intestinal lipid metabolism to reduce the content of these oxidized lipids in the small intestine and plasma. Together, these reports suggest that HDL and its associated apolipoproteins and enzymes may play a role in modulating ischemia/reperfusion oxidative injury.

In the studies of Tsukamoto et al\textsuperscript{1} in which apoD was overexpressed by adenoviruses in the liver 4 days before the ischemia/reperfusion injury, no evidence was presented to demonstrate that the \(\pm 20\)-fold increase in plasma apoD resulted in increased apoD in the myocardial tissue subjected to ischemia/reperfusion. Thus, it is possible that at least some of the protective effect of apoD produced in the liver and exported to the plasma was mediated by modulation of the acute phase response that would be expected from the procedure. It would not be surprising if apoD was transported from plasma to myocardium and also directly acted at the site of injury and in adjacent areas. Recently, Hazen et al\textsuperscript{15} demonstrated that human aorta with atherosclerotic plaques contained 100-fold enrichment of apoA-I compared with normal aorta. The apoA-I recovered from aorta was oxidized and crosslinked and, therefore, was different from plasma apoA-I.\textsuperscript{15} As noted, apoD in the brains of patients with Alzheimer disease seems to show similar changes of oxidation and aggregation.\textsuperscript{7,9} Thus, there is emerging evidence that one role for HDL and its associated proteins and enzymes may be to provide protection against oxidative damage to tissues as well as to modulate the acute phase response.\textsuperscript{14}

The Figure provides a schematic representation of the various mechanisms by which HDL and its associated proteins and enzymes may protect tissues from ischemia/reperfusion injury.

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A.M.F., S.T.R., and M.N. are principals for Bruin Pharma, and A.M.F. is an officer for Bruin Pharma.

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