New Insights Into the Role of HDL as an Anti-inflammatory Agent in the Prevention of Cardiovascular Disease

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Several known functions of high-density lipoproteins (HDLs) may contribute to their ability to protect against atherosclerosis. The best known of these functions is the ability to promote cholesterol efflux from cells in a process that may minimize the accumulation of foam cells in the artery wall. However, HDLs have additional properties, including antioxidant, anti-inflammatory, and antithrombotic effects, that may also be anti-atherogenic. Recent in vivo studies in several animal models have demonstrated that HDLs can inhibit acute and chronic vascular inflammation. The fact that these effects can be achieved with very low doses of reconstituted discoidal HDL or even lipid-free apolipoprotein A-I suggests that they may reflect activity of a minor, highly active HDL subpopulation. These results have potentially important clinical implications in regard to managing the acute vascular inflammation states that accompany acute coronary syndrome and acute ischemic stroke.

Introduction

In many large-scale prospective studies, an inverse relationship between the concentration of cholesterol in high-density lipoproteins (HDLs) and the development of premature coronary heart disease has been observed [1,2]. Furthermore, animal studies provide robust evidence that HDLs protect against the development of atherosclerosis [3–6], although the precise mechanism of this protection is uncertain. HDLs have several properties with the potential to inhibit development of atherosclerosis. The best known of these relates to their ability to promote cholesterol efflux from macrophages in the artery wall [7]. However, HDLs have additional activities, some that appear unrelated to their role in plasma cholesterol transport.

HDLs possess antioxidant properties [8]. They also promote the maintenance of normal, endothelium-dependent vasoreactivity [9,10] and inhibit endothelial cell apoptosis in response to a number of stimuli [11–13]. HDLs have antithrombotic effects both in vitro and in vivo [14–16]. They help repair damaged endothelium by stimulating migration of adjacent healthy endothelial cells [17] and by promoting recruitment of endothelial progenitor cells from plasma [18••]. Lastly—and relevant to the topic of this review—HDLs also have potent anti-inflammatory properties.

Anti-inflammatory Properties of HDL

In both in vitro and in vivo studies, anti-inflammatory properties of HDLs have been documented with native HDLs isolated from plasma and with reconstituted HDLs (rHDLs) containing a single apolipoprotein (apo) complexed with phospholipids.

In vitro studies

HDLs inhibit the cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin in endothelial cells growing in tissue culture in a concentration-dependent manner [19,20]. One process responsible for this inhibition involves an HDL-mediated inhibition of endothelial cell sphingosine kinase [21]. The HDL-mediated inhibition of VCAM-1 and E-selectin protein expression is paralleled by significant reductions in their steady-state mRNA levels [19], suggesting that the lipoproteins may suppress gene transcription. The extent to which HDLs inhibit endothelial cell VCAM-1 expression varies markedly with HDLs isolated from different people (Fig. 1) [22]. Why this variability exists is not known.

The anti-inflammatory properties of HDLs in vitro are apparent with native HDLs isolated from plasma

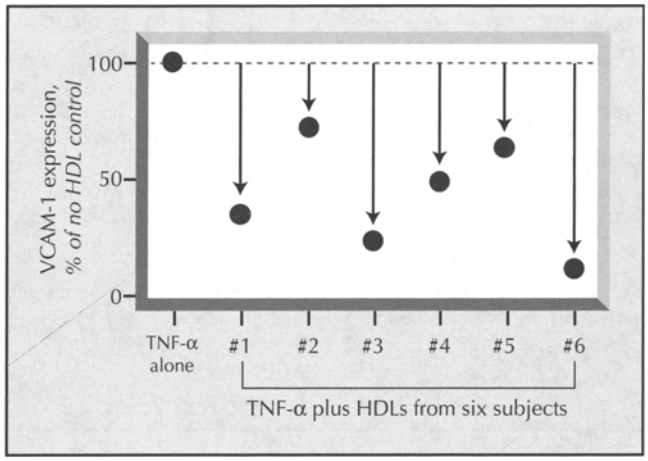

Figure 1. Inhibition of cytokine-induced endothelial cell vascular cell adhesion molecule-1 (VCAM-1) expression by high-density lipoprotein (HDL) isolated from six human subjects [22]. Human umbilical vein endothelial cells were preincubated for 1 hour with HDLs isolated from each of six subjects before being activated with tumor necrosis factor (TNF)-α and incubated for another 4.5 hours. Expression of VCAM-1 was quantified by flow cytometry. Values are expressed relative to the samples that were preincubated in the absence of HDL before being activated with TNF-α.

and with rHDLs consisting of complexes of apoA-I (the main protein constituent of HDLs) and phosphatidylcholine. The effects are dependent on the presence [23] and composition [24] of phospholipids in the particles. Along with inhibiting adhesion molecules, HDLs also inhibit the binding of monocytes [25] and neutrophils [26] to endothelial cells growing in culture.

The HDL-mediated inhibition of adhesion molecule expression in cytokine-activated endothelial cells growing in tissue culture can be achieved by pre-incubating the cells with the HDLs. HDLs do not have to be present at the time they are activated by tumor necrosis factor (TNF)-α [27], indicating that the inhibition is not the consequence of HDLs binding to and interfering with the activity of TNF-α. Indeed, this inhibition persists even if the HDLs are removed from the cells up to 8 hours before TNF-α activation [27]. This suggests that HDLs have the capacity to modify endothelial cells in such a way as to make them resistant to subsequent cytokine activation.

Mechanism by which HDLs inhibit endothelial inflammation

Evidence exists that HDLs inhibit the nuclear factor-kappa B (NF-κB) signaling pathway. Xia et al. [21] reported that HDLs reduce the nuclear translocation of NF-κB in activated endothelial cells by about 50%. The results of this study indicated that the reduction in NF-κB translocation was secondary to an inhibition of endothelial cell sphingosine kinase and, thus, a reduction in the generation of sphingosine-1-phosphate, which is known to promote nuclear translocation of NF-κB. Indeed, the inhibition of NF-κB translocation by HDLs was overcome by adding exogenous sphingosine-1-phosphate to the incubation mixture [21].

Park et al. [28], who found that pretreatment of endothelial cells with HDLs inhibited the DNA binding of both NF-κB and the transcription factor, activator protein-1, confirmed the ability of HDLs to inhibit activation of NF-κB. These investigators also suggested that HDLs may act by modulating cellular kinase activity, although the mechanism was not elucidated. In addition, Schmidt et al. [29] found that HDL-associated sphingosylphosphorylcholine and lysosulfatide initiate the signaling cascade–involving activation of phosphoinositide 3-kinase and Akt, with a consequent attenuation of TNF-α–induced E-selectin gene expression. Because this reduction strongly correlates with repression in nuclear levels of NF-κB, these authors concluded that NF-κB is likely a direct or secondary downstream target of phosphorylated Akt. However, it should be noted that the precise mechanism by which phosphorylated Akt mediates cytosolic retention of NF-κB is not yet known.

In vivo studies

There is growing evidence that the anti-inflammatory properties of HDLs also operate in vivo, although, until recently, this has been demonstrated mainly in a setting of hypercholesterolemia and atherosclerosis. For example, intravenous infusion of rHDLs reduces the in vivo expression of endothelial cell adhesion molecules induced by insertion of carotid periarterial cuffs in cholesterol-fed, apoE knockout mice [30]. In another study of apoE knockout mice, the increase in HDL concentration that accompanied overexpression of the human apoA-I gene reduced macrophage accumulation in the aortic root by more than threefold [31]. This was associated with a reduced in vivo oxidation of β-very-low-density lipoprotein, lower ICAM-1 and VCAM-1 expression, and diminished ex vivo leukocyte adhesion. In another study conducted in rabbits in which aortic atherosclerosis was induced by a balloon injury followed by 17 weeks of a high-cholesterol diet, as little as two intravenous injections of relatively small amounts of HDL given during the last week of the study markedly inhibited the extent of inflammation in the aortic wall [32••].

Several in vivo studies have also documented the ability of HDLs to inhibit acute vascular inflammation in the absence of hypercholesterolemia and atherosclerosis. For example, in studies of experimental stroke in rats, pretreatment with rHDLs significantly and substantially reduced the brain necrotic area in a process possibly related to an rHDL-induced reduction in reactive oxygen species levels [33••]. Furthermore, in a study of hemorrhagic shock in rats, the resulting multiple organ dysfunction syndrome was largely abolished by a single injection of human HDLs given 90 minutes after the hemorrhage and 1 minute before resuscitation [34]. In that model, injection of HDLs prevented the severe

disruption of tissue architecture and extensive cellular infiltration into the affected tissues. In a porcine model, injection of rHDLs has also been shown to inhibit the development of a local inflammatory infiltrate following the subcutaneous administration of interleukin-1 [35]. In other studies, Levkau et al. [36] reported that infusion of human HDLs significantly increased the myocardial uptake of the perfusion tracer 99mTc-MIBI. This increase in myocardial perfusion was abolished in endothelial nitric oxide (NO) synthase–deficient mice. It was concluded that HDLs exert direct NO-mediated vasodilatory effects on the coronary circulation; thus, the ability of HDLs to increase the NO activity in the coronary arteries may contribute to a reduction in arterial inflammation at the site of atheroma formation.

A potential role of HDL in combating the tissue damage arising from generalized states of inflammation has also been reported. McDonald et al. [37] found that pretreatment of rats with rHDLs greatly attenuated the multi-organ tissue injury and renal dysfunction resulting from endotoxic shock. Treatment with rHDLs had no effect on the hypotension and increase in circulating levels of TNF-α induced by lipopolysaccharide. However, they did decrease the expression of P-selectin and ICAM-1, suggesting that in the setting of endotoxic shock, the anti-inflammatory properties of HDLs result from direct inhibition of adhesion molecule expression. The ability of HDLs to bind and neutralize lipopolysaccharide, inhibit lipid peroxidation, and stimulate endothelial NO synthase are also likely to contribute to these beneficial effects.

Infusion of rHDLs is also beneficial in models of gastrointestinal inflammation. Cuzzocrea et al. [38] administered rHDLs to rats as either an 80-mg/kg infusion 30 minutes prior to splanchnic artery occlusion shock or as daily 40-mg/kg intravenous infusions in the setting of dinitrobenzene sulfonic acid–induced colitis. In each model, rHDLs reduced the infiltration of inflammatory cells into the vessel wall and the degree of histological injury. The infusions also delayed the onset of clinical signs. In another study of acute intestinal inflammation, Vowinkel et al. [39] observed that apoA-IV, the third most abundant apolipoprotein in HDL, had profound anti-inflammatory properties in a mouse model of acute colitis. In this study, daily intraperitoneal injections of apoA-IV delayed the onset and reduced the severity and extent of colonic inflammation. This was associated with lower scores of clinical activity and less tissue myeloperoxidase activity. Studies of the colonic microvasculature revealed that administration of apoA-IV inhibited the up-regulation of P-selectin and subsequent interactions between leukocytes and platelets. Demonstrating that acute colitis following administration of dextran sulfate sodium was much more severe in apoA-IV knockout mice than in wild-type mice—and that this was reversed in the apoA-IV–deficient mice by injecting exogenous apoA-IV—provided further evidence of an anti-inflammatory role of apoA-IV [39].

In other in vivo studies, Nicholls et al. [40••] reported that infusing rHDLs containing 25-mg apoA-I into normocholesterolemic rabbits on three consecutive days markedly inhibited the infiltration of neutrophils into the carotid arterial wall in response to application of a nonocclusive Silastic (Dow Corning Corp., Midland, Michigan) periarterial collar. In this study, apoA-I also inhibited the collar-induced increase in reactive oxygen species in the vascular wall, as well as expression of adhesion molecules and chemokines on the endothelial surface. Because this benefit was seen in animals with low systemic cholesterol levels, the effects of HDLs in this setting were determined unlikely to be secondary to enhanced cholesterol efflux.

In subsequent studies in which carotid arteries were examined 24 hours after insertion of the collar, intravenous infusions of lipid-free apoA-I were found to inhibit acute arterial inflammation as effectively as rHDLs, as judged by infiltration of neutrophils into the vessel wall and expression of VCAM-1, ICAM-1, and myeloperoxidase [41]. Furthermore, these anti-inflammatory effects were maximal following a single intravenous injection of lipid-free apoA-I, whether given 24 hours before or at the time of collar insertion [41]. The anti-inflammatory effects of apoA-I were apparent even when it was administered several hours after insertion of the collar (Fig. 2). Perhaps an even more striking observation from these studies was that the anti-inflammatory effects of apoA-I were preserved when it was administered at a dose as low as 2 mg/kg, an amount that would have increased the plasma apoA-I pool at the time of injection by no more than 10% [41]. Therefore, it follows that the observed anti-inflammatory effects of apoA-I cannot be explained simply in terms of an increase in the concentration of plasma HDL. Rather, it indicates that when lipid-free apoA-I is infused into animals, it assumes anti-inflammatory properties that are much greater than those of the bulk, endogenous HDL.

It is interesting to note that lipid-free apoA-I does not inhibit endothelial cell inflammation when added to incubations in vitro [23], suggesting that the apoA-I was incorporated in vivo into particles with a high degree of anti-inflammatory activity. Presumably, in the in vivo setting, apoA-I becomes rapidly lipidated after being injected into the animals to form particles that may initially resemble the discoidal rHDLs, which have been shown to be highly anti-inflammatory when added in vitro to incubations of endothelial cells.

Another potentially protective effect of HDLs relates to their role in promoting endothelial repair. Disruption of the endothelial monolayer integrity is an important contributing factor in vascular disorders, and its repair plays a fundamental role in the ultimate outcome. HDLs can enhance endothelial repair by at least two distinct mechanisms. First, in studies conducted in vitro in a model of endothelial injury, HDLs were shown to stimulate endothelial cell migration in a NO-independent manner via scavenger receptor B type I (SR-BI)–mediated activation

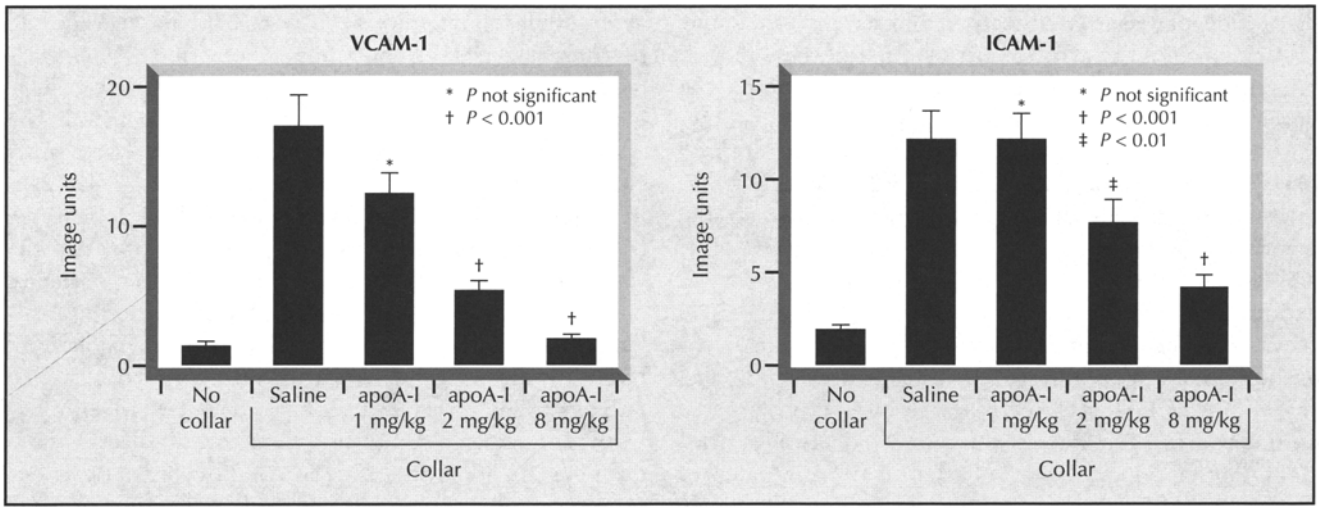

Figure 2. Effects of infusing lipid-free apolipoprotein (apo) A-1 on vascular inflammation in rabbits. Normocholesterolemic New Zealand White rabbits were infused with either saline or 1, 2, or 8 mg/kg of lipid-free apoA-I (n = 5/group) at the time of a nonocclusive collar insertion to induce vascular inflammation in a carotid artery. The animals were sacrificed 24 hours after collar insertion. Carotid arteries were isolated and expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) were quantitated [41]; the results are expressed as the mean ± standard errors of the mean.

of Rac GTPase [17]. This process depends on the activation of Src kinases, phosphatidylinositol 3-kinase, and p44/42 mitogen-activated protein kinases. Paralleling the in vitro findings, re-endothelialization of carotid arteries after perivascular electric injury was blunted in apoA-I knockout mice; reconstitution of apoA-I expression in these animals promoted restoration of the endothelium but only in mice expressing SR-B1 [17].

Other studies conducted in mice have shown that endothelial progenitor cell engraftment into damaged endothelium is also enhanced by HDL infusion [18••]. The number of endothelial progenitor cells was significantly increased in the aortic endothelium after damage induced by lipopolysaccharide and also in the aortic endothelium of apoE-deficient (-/-) mice. When apoE(-/-) mice were given a single intravenous infusion of a relatively small amount of rHDLs containing human apoA-I complexed with phospholipids, the number of endothelial progenitor cells in the aortic endothelium more than doubled. These findings suggest an additional protective function of HDLs in promoting endothelial repair.

HDLs and Inflammation in Humans

In humans, a relationship between plasma concentrations of HDL cholesterol and soluble cell adhesion molecules has been reported. In a study of subjects with a wide range of HDL cholesterol concentrations, the plasma levels of soluble ICAM-1 (sICAM-1) and soluble E-selectin (sE-selectin) (but not soluble VCAM-1) were significantly higher in subjects with low HDL levels compared with subjects with average or high HDL levels [42]. Furthermore, the concentration of HDL cholesterol correlated inversely with both sICAM-1 and sE-selectin in the low HDL subjects, but not in those with normal or elevated HDL levels. It was also reported that the fenofibrate treatment–induced increase in HDL levels is associated with a significant reduction in the plasma concentrations of sICAM-1 and sE-selectin [42]. However, it is unclear whether the reduction in sICAM-1 and sE-selectin results from the increased HDL level or due to a direct anti-inflammatory effect of the fibrate on the artery wall.

Another human study investigated the influence of altering the fatty acid composition of a high-fat meal on the anti-inflammatory properties of HDLs [43]. In this study, 14 healthy human subjects consumed, on two occasions, an isocaloric meal enriched with either a polyunsaturated or saturated fat. The effects of postprandial HDL on endothelial cell expression of ICAM-1 and VCAM-1 were determined. HDLs collected 6 hours after the saturated fat meal were significantly less effective than HDLs isolated from fasting plasma in terms of their ability to inhibit expression of both ICAM-1 and VCAM-1 in activated endothelial cells, whereas HDL collected 6 hours after the polyunsaturated fat meal had an inhibitory activity significantly greater than that of HDL collected from fasting plasma. This study concluded that the anti-inflammatory potential of HDL is reduced by consumption of a meal containing saturated fat but enhanced following consumption of polyunsaturated fat [43].

Infusion of human apoA-I into human recipients results in LDLs becoming resistant to oxidation and being less effective in inducing monocyte chemotactic activity in a human artery wall co-culture [44]. There is also circumstantial evidence that HDLs have direct anti-inflammatory effects in vivo in humans. In one study, a single intravenous infusion of rHDLs into hypercholesterolemic humans normalized endothelium-dependent vasodilation,

possibly by increasing NO bioavailability [9]. In a second human study, a single injection of rHDLs corrected the endothelial dysfunction associated with low HDL levels in ATP-binding cassette-A1 heterozygotes [10].

In another human study, people with coronary artery disease received five weekly intravenous injections of a preparation of rHDLs containing a variant of apoA-I (apoA-I Milano) complexed with a phospholipid [45]. This resulted in a significant reduction in the atheroma burden in the coronary arteries, as assessed by intravascular ultrasound. Although the study included only a small number of subjects, the result was consistent with a profound protective action of HDLs via a mechanism that may well have included an inhibition of vascular inflammation.

Clinical Implications

The benefits observed following infusion of rHDLs into animals and humans raises the possibility that such preparations may have a place in managing conditions associated with acute vascular inflammation. It is worth noting that the anti-inflammatory effects of infusing rHDLs (and also lipid-free apoA-I) into rabbits that had been fitted with periarterial carotid collars were achieved with remarkably small amounts of apoA-I. Each infusion contained only 2 to 8 mg/kg of apoA-I. This amount, when given to a 3-kg rabbit (plasma volume of about 120 mL), would have increased the plasma apoA-I concentration in the immediate postinfusion period by 0.05 to 0.2 mg/mL, an increase of only 10% to 40%. Furthermore, with a fractional catabolic rate for rabbit plasma apoA-I of 0.8 pools/day [46], the level returned to baseline within a matter of hours. Yet, despite what was only a minor and transient increase in the concentration of plasma HDLs, the infusions resulted in an almost complete inhibition of collar-induced acute arterial inflammation. This suggests that the infused rHDLs and apoA-I had anti-inflammatory effects that extended well beyond those resulting from a simple increase in the concentration of plasma HDL. The explanation for this finding is not known but the therapeutic implications are considerable. If rHDLs or apoA-I are to be used therapeutically to minimize tissue damage in states of acute vascular inflammation such as occurs in acute coronary syndromes, stroke, and ischemia reperfusion injury, the amounts required may be much less than what is currently regarded as an appropriate amount.

Conclusions

Anti-inflammatory properties of HDL have now been demonstrated in vitro and in vivo in a number of animal models, with circumstantial evidence of an in vivo effect in humans. The effects resulting from infusions of very small amounts of discoidal rHDLs and lipid-free apoA-I raise the possibility that a minor, highly active subpopulation of HDLs with profound anti-inflammatory effects exists. Developing new strategies to combat the vascular inflammation that accompanies acute coronary syndromes and acute ischemic stroke may assist in possibly exploiting these findings.

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