Anti-Inflammatory and Cardioprotective Activities of Synthetic High-Density Lipoprotein Containing Apolipoprotein A-I Mimetic Peptides

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ABSTRACT

Apolipoprotein A-I (apoA-I) mimetic peptides may represent an alternative to apoA-I for large-scale production of synthetic high-density lipoproteins (sHDL) as a therapeutic agent. In this study, the cardioprotective activity of sHDL made with either L37pA peptide or its d-stereoisomer, D37pA, was compared to sHDL made with apoA-I. The peptides were reconstituted with palmitoyl-oleoyl-phosphatidylcholines, which yielded sHDL particles comparable to apoA-I sHDL in diameter, molecular weight, and α-helical content. Pretreatment of endothelial cells with either peptide sHDL reduced tumor necrosis factor α-stimulated vascular cell adhesion molecule-1 expression to the same extent as apoA-I sHDL. In an isolated rat heart model of ischemia/reperfusion (I/R) injury, L37pA and D37pA sHDL significantly reduced postischemic cardiac contractile dysfunction compared to the saline control, as indicated by a 48.7 ± 6.4% (L37pA; P < 0.001) and 53.0 ± 9.1% (D37pA; P < 0.001) increase of left ventricular-developed pressure (LVDP) after reperfusion and by a 49.7 ± 3.4% (L37pA; P < 0.001) and 49.6 ± 2.6% (D37pA; P < 0.001) decrease of creatine kinase (CK) release. These effects were similar to the 51.3 ± 3.0% (P < 0.001) increase of LVDP and 51.3 ± 3.0 (P < 0.001) reduction of CK release induced by apoA-I sHDL. Consistent with their cardioprotective effects, all three types of sHDL particles mediated an approximate 20% (P < 0.001) reduction of cardiac tumor necrosis factor α (TNFα) content and stimulated an approximate 35% (P < 0.05) increase in postischemic release of prostacyclin. In summary, L37pA and D37pA peptides can form sHDL particles that retain a similar level of protective activity as apoA-I sHDL on the endothelium and the heart; thus, apoA-I mimetic peptides may be useful therapeutic agents for the prevention of cardiac I/R injury.

Plasma-derived and synthetic high density lipoproteins (HDL) exert a diverse set of atheroprotective effects, ranging from stimulation of reverse cholesterol transport to prevention of endothelial dysfunction and reduction of oxidative stress (Calabresi et al., 2006). Early studies in cholesterol-fed rabbits showed that only a few injections of homologous plasma-derived HDL can induce regression of established lesions (Badimon et al., 1990), thus providing a rationale for a new therapeutic approach aimed at atherosclerosis regression through the administration of synthetic HDL (sHDL), a complex of purified apolipoprotein A-I (apoA-I) with phospholipids. Indeed, a number of preclinical investigations in different animal models of human atherosclerosis have demonstrated that sHDL treatment can bring about significant changes in arterial lipid and cell dynamics, leading to both plaque stabilization and regression (Calabresi et al., 2006). sHDL-induced cholesterol mobilization from the arterial wall is likely to play an integral role in atherosclerosis stabilization/regression, as indicated by the enhanced cholesterol ef

ABBREVIATIONS: HDL, high-density lipoprotein; sHDL, synthetic HDL; I/R, ischemia/reperfusion; apoA-I, apolipoprotein A-I; POPC, palmitoyl-oleoyl-phosphatidylcholine; CD, circular dichroism; LVDP, left ventricular-developed pressure; CPP, coronary perfusion pressure; HUVEC, human umbilical vein endothelial cells; VCAM, vascular cell adhesion molecule; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; K-H, Krebs-Henseleit; CK, creatine kinase; PGI2, prostacyclin; AUC, area under the curve; NF-κB, nuclear factor-κB; S1P, sphingosine-1-phosphate.
flux-promoting capacity of plasma (Shah et al., 2001), and by the rapid and striking increase of unesterified cholesterol in the plasma HDL fraction (Chiesa et al., 2002) in HDL-treated animals. The consistent positive findings in preclinical studies have provided the basis for the clinical development of HDL containing the apoA-Milano variant (Nissen et al., 2003) or wild-type apoA-I (Tardif et al., 2007), as a novel treatment for promoting atherosclerosis regression in coronary patients.

The growing interest in HDL as a therapeutic agent has prompted us to also test its cardioprotective effect against myocardial ischemia/reperfusion (I/R) injury in isolated rat hearts (Rossoni et al., 2004). Increasing doses of HDL were given either immediately before ischemia or during the 1st min of reperfusion. In both instances, treatment caused a dose-dependent improvement in the recovery of left ventricle contractile capacity at reperfusion, associated with a reduction of myocyte necrosis (Rossoni et al., 2004). The two constituents of HDL, apoA-I, and phosphatidylcholine, given separately, did not show any improvement in postischemic cardiac function (Rossoni et al., 2004). To establish that HDL can exert a cardioprotective effect against I/R injury in vivo, HDL made with the apoA-Milano variant was also examined in rabbits undergoing ligation and reopening of the left anterior descending artery (Marchesi et al., 2004). Rabbits treated intravenously with HDL indeed developed significantly smaller infarcts than vehicle-treated animals (Marchesi et al., 2004). Because most of the pathogenic mechanisms responsible for I/R injury are shared by all organs, it is not surprising that HDL was also found to be protective against cerebral, renal, and intestinal I/R injury (Thiemermann et al., 2004). Because previo.

Materials and Methods

Preparation of sHDL. ApoA-I was purified from human blood plasma (Calabresi et al., 1997); the L37pA peptide (DWLKAFLDK-VAEKLKEAP-F-DWLKAFLDYKVAEKLKEAP) was synthesized by a solid-phase procedure using a Fmoc (9-fluorenlymethoxycarbonyl) protocol on a Biosyn 9600 peptide synthesizer with t-amino acids. The D37pA peptide was synthesized with the same sequence as L37pA but with d-amino acids. All peptides were purified to greater than 98% homogeneity by reverse-phase high-performance liquid chromatography on an Aquapore RP-300 column (Remaley et al., 2003).

sHDL containing palmitoyl-oleoyl-phosphatidylcholine (POPC; Sigma-Aldrich Chemie, Steinheim, Germany) and either apoA-I, L37pA, or D37pA were prepared by the cholate dialysis technique (Calabresi et al., 1997), with a starting POPC/protein weight ratio of 2.5:1. Protein, peptide, and phospholipid concentrations were measured as described previously (Calabresi et al., 1997). sHDL preparations were dialyzed against sterilized saline immediately before use, and their concentrations were expressed as protein (peptide) concentration.

Characterization of sHDL. sHDL particle dimension and molecular weight were measured by fast liquid protein chromatography. sHDL were applied to a Superose 6 10/30 column (GE Healthcare Biosciences, Uppsala, Sweden), equilibrated with phosphate buffer (150 mM NaCl, 1 mM NaH2PO4, H2O, 1.6 mM Na2HPO4, 2H2O, 1.4 mM EDTA, and 3 mM Na2SO4), eluted at a flow rate of 0.5 ml/min. Protein levels were monitored by absorption at 280 nm. sHDL particle dimension and molecular weight were calculated based on the retention coefficients of ferritin (12.2 nm, 440 kDa), aldolase (9.6 nm, 232 kDa), and albumin (7.1 nm, 67 kDa) (GE Healthcare Biosciences). The number of apoA-I or 37pA molecules per sHDL particle was determined by cross-linking with dimethyl suberimidate (Calabresi et al., 1994). Circular dichroism (CD) spectra were recorded with a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan) at the constant temperature of 25°C. All of the spectra were baseline-corrected.

VCAM-1 Expression in Endothelial Cells. Primary cultures of human umbilical vein endothelial cells (HUVEC) were purchased from PromoCell (Heidelberg, Germany) and cultured as described previously (Calabresi et al., 1997). HUVEC were incubated overnight with sHDL at 1 mg/ml, washed, and then stimulated with tumor necrosis factor α (TNFα; R&D Systems, Minneapolis, MN) at a concentration of 10 ng/ml for 8 h. At the end of the experiment, cells and conditioned media were immediately frozen at −80°C. VCAM-1 concentration in the conditioned media was evaluated on MaxiSorp plates (Nunc GmbH and Co., Wiesbaden, Germany) using a fluo.

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3'. PCR products were separated by electrophoresis on 1% agarose gel, stained with ethidium bromide, visualized by ultraviolet irradiation, and photographed with Polaroid film. Band densities were evaluated with a GS-690 Imaging Densitometer and a Multi-Analyst software (Bio-Rad Laboratories). VCAM-1 band intensities were normalized by their β-actin (for Western blotting) or GAPDH (for RT-PCR) values.

I/R Injury in Isolated Rat Hearts. Male Sprague-Dawley rats (Charles River Italia, Calco, Lecco Province, Italy) were anesthetized with sodium pentobarbital (50 mg/kg i.p.); the hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer (K-H buffer: 118 mM NaCl, 2.8 mM KCl, 1.2 mM KH2PO4, 2.5 mM CaCl2, 1.2 mM MgSO4, 25 mM NaHCO3, and 5.5 mM glucose), and mounted for retrograde perfusion at 37°C through the aorta (Calabresi et al., 2003). Left ventricular developed pressure (LVDP) and coronary perfusion pressure (CPP) were continuously monitored throughout the experiment. Isolated hearts were mounted and perfused with K-H buffer at a flow rate of 15 ml/min for 80 min. Hearts were equilibrated with K-H buffer at a flow rate of 15 ml/min for 30 min. A moderate ischemia was induced by reducing the perfusion flow rate to 1 ml/min for 20 min; the normal flow rate (15 ml/min) was then restored, and perfusion continued for 30 min. CK release was evaluated by measuring CK activity in the coronary effluent collected every 150 s during reperfusion. sHDL (1 mg/ml in K-H buffer) were administered within the 10 min immediately before ischemia. In separate experiments, sHDL were administered during the first 10 min of reperfusion at the concentration of 2 mg/ml protein of buffer.

Hearts were snap-frozen in liquid nitrogen immediately at the end of the experiment and stored at −80°C; cardiac tissue was homogenized and solubilized as described previously (Calabresi et al., 2003). Coronary effluent was collected and immediately frozen at −80°C. The concentration of TNFα in cardiac homogenates and in coronary effluents collected during reperfusion was measured by a commercial ELISA kit specific for rat TNFα (Endogen Inc., Woburn, MA) (Calabresi et al., 2003). The generation of prostacyclin (PG12) by cardiac tissue was assessed by measuring its stable metabolite 6-keto-PGF1α, on coronary effluent aliquots collected in the 5 min immediately before ischemia and during the first 10 min of reperfusion by a commercial ELISA kit (GE Healthcare Biosciences). All investigational animal procedures conformed to the Institute of Laboratory Animal Resources (1996).

Statistical Analyses. Results are reported as mean ± S.E.M., unless otherwise stated. Group differences were determined by analysis of variance; differences with a value of P < 0.05 were considered as statistically significant. For the preischemia treatment I/R experiments, a sample size of four per group has 90% power to detect a difference of 300 mm in LVDP-area under the curve (AUC), assuming a standard deviation of 93 mm (Calabresi et al., 2003) using a t test with a 0.025 significance level. For the postischemia treatment I/R experiments, a sample size of three per group has 90% power to detect a difference of 300 mm in LVDP-AUC, assuming a standard deviation of 65 mm (Calabresi et al., 2003) using a t test with a 0.025 significance level. The AUC calculations were determined for the entire reperfusion time period (30 min) and were measured by the trapezoidal method, with a MicroCal Origin 3.5 program (OriginLab Corp, Northampton, MA).

### Results

**Characterization of sHDL.** The POPC/apoA-I weight ratio in sHDL made with purified apoA-I was 2.28 ± 0.11, and the percentage of lipid-associated apoA-I was 89.6 ± 3.5% of the starting amount. sHDL made with L37pA and D37pA peptides had a final POPC/peptide weight ratio of 3.33 ± 0.25 and 3.43 ± 0.19, respectively, significantly higher than that of apoA-I sHDL (p = 0.013 and 0.009). The percentage association with POPC was 62.5 ± 2.5 and 63.1 ± 4.8%, respectively. Any of the unassociated peptides (4.2 kDa) or phospholipids was removed by gel filtration and not present in the final sHDL preparations.

The various sHDL preparations were comparable in terms of particle diameter and molecular weight, as shown in Table 1. Cross-linking of apoA-I in sHDL particles resulted in two bands corresponding to monomeric (28 kDa) and dimeric (56 kDa) apoA-I; thus, one sHDL particle (189 kDa) contained up to two molecules of apoA-I and 175 molecules of POPC (MPC = 760; Table 1). Cross-linking of the 37pA peptides in sHDL resulted in 10 bands, ranging from a monomer (4.2 kDa) to a decamer (42.5 kDa); thus, a single sHDL particle (194–214 kDa) contained up to 10 molecules of the 37 pA peptide and approximately 198 to 225 molecules of POPC (Table 1). Because apoA-I is approximately 6.5 times larger in molecular weight compared to the peptides, the ratio of the mass of the peptides to phospholipids is approximately 40% lower than that for apoA-I sHDL. Each of the far-UV CD spectra of lipidated apoA-I and L37pA is indicative of a highly α-helical structure, with negative troughs at 208 and 222 nm; as expected, D37pA gave an inverted but completely symmetrical profile to that of L37pA (Fig. 1). The overall percentage of α-helix in all three sHDL preparations was similar (Table 1).

**Anti-Inflammatory Effects of sHDL on Endothelial Cells.** sHDL made with the 37pA peptides were tested for their capacity to inhibit VCAM-1 expression in comparison to apoA-I sHDL. HUVEC were pretreated overnight with sHDL before stimulation with TNFα, and the concentration of soluble VCAM-1 in the conditioned media was then measured by ELISA. Stimulation of HUVEC with TNFα caused a marked increase in the release of soluble VCAM-1 protein in cell culture medium compared to unstimulated cells (47.2 ± 3.4 versus 2.0 ± 0.5 ng/ml; respectively; P < 0.001), consistent with our previous findings (Calabresi et al., 1997). Treatment of HUVEC with apoA-I sHDL before TNFα stimulation caused a significant 67.2% reduction of VCAM-1 concentration in the medium (15.5 ± 6.3 ng/ml, P = 0.002) (Fig. 2). The concentration of VCAM-1 in conditioned medium positively correlated with endothelial expression of VCAM-1 protein, as evaluated by Western blotting analysis of total cell lysate. VCAM-1 was undetectable in unstimulated cells, whereas a marked signal was visible after TNFα stimulation.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td><strong>Characterization of sHDL particles</strong></td>
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<tr>
<td>Molecular weights used for calculations were 28 kDa for apoA-I, 4255 Da for 37pA, and 760 Da for POPC. Percentage of α-helix was calculated at 222 nm.</td>
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<tr>
<td><strong>FPLC Diameter</strong></td>
</tr>
<tr>
<td><strong>No. molecules/particle</strong></td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
</tr>
<tr>
<td><strong>Protein or peptide</strong></td>
</tr>
<tr>
<td><strong>Circular dichroism</strong></td>
</tr>
<tr>
<td><strong>apoA-I</strong></td>
</tr>
<tr>
<td><strong>Circular dichroism</strong></td>
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**FPLC, fast protein liquid chromatography.**
Preincubation with sHDL reduced VCAM-1 protein expression in cells (Fig. 3B). The inhibition occurred at the transcriptional level, as indicated by the sHDL-mediated reduction of VCAM-1 mRNA levels in endothelial cells treated with sHDL before stimulation with TNF α/H9251 (Fig. 3A).

sHDL made with both L37pA and D37pA peptides had a similar anti-inflammatory activity as apoA-I sHDL (Fig. 2). VCAM-1 concentration in the conditioned medium of cells treated with L37pA sHDL was 13.8 ± 2.0 ng/ml, with a 70.8% reduction compared to untreated TNF α-stimulated cells (p = 0.002); likewise, VCAM-1 concentration in the conditioned medium of cells treated with D37pA sHDL was 16.6 ± 7.3 ng/ml, a 64.8% reduction compared to the control (p = 0.002). As demonstrated for apoA-I sHDL, the modulation of VCAM-1 concentration occurred at the transcriptional level (Fig. 3).

**Cardioprotective Effects of sHDL against I/R.** The 20-min low-flow ischemia caused a marked cardiac dysfunction and necrosis at reperfusion, as demonstrated by the partial recovery of LVDP after reperfusion, the elevation of CPP, and the release of CK in the coronary effluent of saline-treated hearts (Table 2). The preischemic administration of 37pA sHDL improved the postischemic LVDP recovery and attenuated the CPP elevation and CK release (Fig. 4). The cardioprotective effect of the three sHDL preparations was compared by calculating the AUC described by LVDP, CPP, and CK during the 30 min of reperfusion. No difference was detected among the cardioprotective effect of sHDL containing L37pA, D37pA, or apoA-I (Table 2). L37pA sHDL and D37pA sHDL improved LVDP recovery by 49.7 and 53.0% and reduced CPP at reperfusion by 42.3 and 40.4%, respectively; CK release was also reduced by 45.4 and 49.6%, respectively. These effects were comparable to the 51.3% increase of LVDP, the 40.4% reduction of CPP, and the 50.3% reduction of CK release induced by apoA-I sHDL. A significant cardioprotection was also observed when L37pA sHDL were given during the first 10 min of reperfusion (Fig. 5); the response was somewhat lower than that of apoA-I sHDL, but the difference between the two preparations did not reach statistical significance (p = 0.091 for LVDP, P = 0.118 for CPP, and P = 0.065 for CK) (Table 2).

The cardioprotective effect of apoA-I sHDL was previously shown to be mediated, at least in part, by an improvement of postischemic prostaglandin release and by the ability of sHDL to bind and remove TNF α from cardiac tissue (Calabresi et al., 2003; Tardif et al., 2007). L37pA sHDL and D37pA sHDL, given either before or after ischemia, significantly and equally reduced cardiac TNF α content compared to saline (Table 3) and, as expected, resulted in a concomitant increase of TNF α concentration in the coronary effluent (Ta-
Table 2

**Effect of sHDL on cardiac function**

Data are expressed as area under the curve estimated accordingly to the trapezoid method from data recorded during the 30 min of reperfusion, mean ± S.E.M. Concentrations of sHDL are expressed as milligram of protein (peptide) per milliliter of buffer.

<table>
<thead>
<tr>
<th></th>
<th>LVDP (mm Hg)</th>
<th>CPP (mm Hg)</th>
<th>CK (U/L)</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>12</td>
<td>905 ± 35</td>
<td>1563 ± 70</td>
</tr>
<tr>
<td>sHDL (1 mg/ml) preischemia</td>
<td>8</td>
<td>1369 ± 65***</td>
<td>932 ± 51***</td>
</tr>
<tr>
<td></td>
<td>L37pA</td>
<td>4</td>
<td>1355 ± 58***</td>
</tr>
<tr>
<td></td>
<td>D37pA</td>
<td>4</td>
<td>1385 ± 83***</td>
</tr>
<tr>
<td>sHDL (2 mg/ml) at reperfusion</td>
<td>6</td>
<td>1508 ± 65***</td>
<td>816 ± 61***</td>
</tr>
<tr>
<td></td>
<td>L37pA</td>
<td>3</td>
<td>1203 ± 67**</td>
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</table>

**P** < 0.01 vs Saline, ***P** < 0.001 vs Saline.

Discussion

HDL has been found in numerous epidemiologic studies to be one of the main antiatherogenic constituents of plasma (Linsel-Nitschke and Tall, 2005; Ader, 2006). The ability of HDL to promote reverse cholesterol transport was believed to largely account for its atheroprotective effect, but recently, HDL has been recognized to also have a multitude of other salutary effects on endothelial cells and inflammatory cells in preventing atherosclerosis (Mineo et al., 2006; Navab et al., 2006). Several epidemiologic studies have also shown that HDL may have a direct cardioprotective effect beyond its ability to prevent atherosclerosis (Berge et al., 1982; Goldbourt et al., 1986; Wang et al., 1998). It is interesting to note that patients with a myocardial infarction have improved cardiac function and a better prognosis if they have elevated HDL at the time of infarction (Berge et al., 1982; Goldbourt et al., 1986; Wang et al., 1998). These findings have prompted this and other recent studies on the use of HDL as a potential therapeutic agent for preventing cardiac IR injury. The main finding from this study is that sHDL made with apoA-I mimetic peptides can also reduce cardiac IR injury, which suggests that such peptides may be useful as therapeutic agents for not only reversing atherosclerosis in patients with acute coronary syndrome (Navab et al., 2006; Sethi et al., 2007) but also in the acute treatment of patients with myocardial infarction.

The mechanism for the cardioprotective effect of apoA-I mimetic peptides is not known, but these peptides have been shown to mediate a wide range of biological effects, such as antioxidant, anti-inflammatory, and provasodilatory effects (Navab et al., 2006; Sethi et al., 2007), all of which have been proposed to explain their ability to block the progression of atherosclerosis. HDL can potentially mediate these various cellular effects either by removing substances from cells, such as lipids, by acting as a donor to cells, or by triggering cell signaling events. Some of these cellular effects may also be relevant to their ability to protect against I/R injury. For example, apoA-I, as well as L37pA and D37pA, can mediate the efflux of excess cholesterol from cells by the ABCA1 transporter (Remaley et al., 2003). Because of the rapidity of the events related to cardiac I/R injury, it is not likely that increased efflux of cholesterol by sHDL is relevant to the protection observed in this study. However, besides cholesterol, other lipids, including oxidized lipids, can also be removed and sequestered by HDL, as well as catalyzed by HDL-associated enzymes such as paraoxonase (Navab et al., 2004). Oxidized lipids rapidly form during reperfusion after ischemia and can have acute effects on vascular cells by a several different signaling pathways (Carney et al., 1996; Buffon et al., 2000; Carden and Granger, 2000). Thus, the antioxidant properties of apoA-I mimetic peptides is one possible explanation for the observed benefit of these peptides on cardiac IR injury.

As was shown for HDL (Calabresi et al., 1994), sHDL made with apoA-I mimetic peptides was able to remove the proinflammatory cytokine TNFα from the heart (Table 3). The production of TNFα during reperfusion is known to mediate some of the detrimental effects of I/R injury on the heart (Meldrum, 1998; Moe et al., 2004). L37pA and D37pA sHDL were shown in this study to be equally effective as apoA-I sHDL in removing TNFα; thus, the anti-inflammatory property of the apoA-I mimetic peptides could also be contributing to the protection of sHDL against cardiac IR injury.

Decreased tissue perfusion after ischemia also plays a central role in the pathogenesis of cardiac injury after a myocardial infarction (Carden and Granger, 2000). Similar to apoA-I sHDL, sHDL made with L37pA and D37pA were equally effective in increasing the concentration of 6-keto-PGF_1α, a stable catabolite of the potent vasodilator PGL_2 (Tanaka et al., 2004). Thus, increased production of PGL_2 and increased vasodilation by 37pA sHDL could also account for the observed decrease in CPP after reperfusion of the rat hearts (Table 2). HDL has been shown previously to increase PGL_2 by several different mechanisms (Mineo et al., 2006). HDL can induce the expression of cyclooxygenase-2 in endothelial (Norata et al., 2004) and smooth muscle cells (Vinals et al., 2004), although this is unlikely the mechanism in this study given the relatively short time frame of the reperfusion study. HDL can also increase PGL_2 by providing cells a source of arachidonate (Pomerantz et al., 1985) for production by cyclooxygenase-1 and by stabilizing PGL_2 against degradation (Pirich et al., 1993), which could both possibly contribute to the observed provasodilatory effect in this study for sHDL made with L37pA and D37pA.

Another novel finding from this study was the observation of the potent vasodilator PGI2, in the coronary effluent (Table 3). When given at reperfusion, sHDL also enhanced postischemic 6-keto-PGF_1α release, with no effect on preischemic effluent concentrations (Table 3). Again, no major difference was detected between sHDL made with the 37pA peptides or apoA-I (Table 3).
that L37pA and D37pA sHDL, like apoAI sHDL, can inhibit the expression of VCAM-1 by endothelial cells (Figs. 2 and 3). VCAM-1 is one of the main adhesion proteins on endothelial cells; thus, blocking the attachment of circulating neutrophils and monocytes to the endothelium by HDL and their subsequent infiltration into cardiac tissue could reduce I/R injury. However, no blood cells were present in the isolated heart perfusion system, thus precluding this as a possible explanation for the experimental results in this study, although this is probably an important in vivo factor for the vascular protection afforded by HDL (Nicholls et al., 2005).

HDL is known to block VCAM-1 expression by blocking the activation of the transcription factor NF-κB (Calabresi et al., 1997; Robbesyn et al., 2003). NF-κB activation can lead to numerous other gene and cellular changes besides VCAM-1, such as increased cytokine production, induction of iNOS, and increased monocyte chemotactic protein-1 (De Martin et al., 2000), all of which all can possibly contribute to cardiac I/R injury and activation of endothelial cells. Inhibition of NF-κB activation in the heart has already been shown to markedly decrease I/R injury (Yeh et al., 2005); thus, the inhibition of NF-κB activation in endothelial cells and possibly in cardiac myocytes could be another mechanism for the cardioprotective effects of sHDL, which warrants future studies.

Finally, HDL is also known to be the main transporter of sphingosine-1-phosphate (S1P) in plasma (Nofer and Assmann, 2005), and S1P has been shown to protect against cardiac I/R injury (Theilmeier et al., 2006). S1P is a potent bioactive lipid that mediates a wide range of effects on endothelial and cardiac cells via specific S1P receptors (Nofer and Assmann, 2005). However, ApoA-I sHDL and 37pA sHDL used in this study were synthesized in the absence of any S1P, so it is unlikely that S1P accounts for any of their...
observed effects, unless sHDL in the perfusate was able to scavenge S1P from the cardiac tissue and then deliver it back to cells in such a way that it still mediated signaling via the S1P receptor. Even if S1P does not account for any of the cardioprotective benefits in this study, it is possible that the addition of S1P during the reconstitution of the apoA-I mimetic peptides with phospholipids could further enhance their cardioprotective effects.

The main approach for treating patients with a myocardial infarction is to restore blood flow as soon as possible with stents or thrombolytic therapy. It would be very valuable if such therapies could be coupled to a drug to also prevent any subsequent I/R injury after cardiac blood flow is restored. Many different agents have been shown to prevent I/R injury in various animal models, but none has been found so far to be useful in clinical trials. Therefore, much further work has to be done to determine whether infusion of sHDL containing apoA-I mimetic peptides will be a useful approach. However, the fact that one can duplicate the benefit of HDL with apoA-I mimetic peptides when given after ischemia does raise the possibility of using these peptides as therapeutic agents. Compared with full-length apoA-I, apoA1 mimetic peptides are relatively short and can be readily synthesized, which would facilitate their manufacturing and the ability to do future structure-function studies to optimize their synthesis and to better understand their mechanism of action. Results from this study showing that lack of stereoselectivity in any of the anti-inflammatory or cardioprotective effects of L37pA and D37pA sHDL have already revealed that the molecular interaction accounting for their biological properties most likely does not involve a classic receptor ligand-type interaction. This is further supported by the fact that the sequence of the 37pA peptide has no homology to apoA-I or to the other apolipoproteins but simply contains an amphipathic helix (Remaley et al., 2003), thus indicating that this is the main protein structural motif necessary for their cardioprotective effect. Finally, the main finding of this study that apoA-I mimetic peptides protect against cardiac I/R and the potential mechanisms uncovered may provide new insights into their atheroprotective properties and to the ongoing work in developing these agents for the treatment of atherosclerosis (Navab et al., 2004; Sethi et al., 2007).

References


TABLE 3

Effect of sHDL on TNFα and 6-keto-PGF1α concentrations

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<tr>
<th></th>
<th>TNFα</th>
<th>6-keto-PGF1α</th>
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<tbody>
<tr>
<td></td>
<td>Cardiac Tissue</td>
<td>Preiscemia</td>
</tr>
<tr>
<td>Saline</td>
<td>83.3±2.0</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>sHDL (1 mg/ml)</td>
<td>86.5±1.8***</td>
<td>9.0±1.4***</td>
</tr>
<tr>
<td></td>
<td>L37pA</td>
<td>67.7±3.5***</td>
</tr>
<tr>
<td></td>
<td>D37pA</td>
<td>64.7±6.7***</td>
</tr>
<tr>
<td>sHDL (2 mg/ml)</td>
<td>67.7±2.8**</td>
<td>9.5±0.8***</td>
</tr>
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</table>

* P < 0.05 vs Saline; ** P < 0.01 vs Saline; *** P < 0.001 vs Saline.


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