An apolipoprotein A-I mimetic dose-dependently increases the formation of preβ₁ HDL in human plasma

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Abstract Preβ₁ HDL is the initial plasma acceptor of cell-derived cholesterol in reverse cholesterol transport. Recently, small amphipathic peptides composed of D-amino acids have been shown to mimic apolipoprotein A-I (apoA-I) as a precursor for HDL formation. ApoA-I mimetic peptides have been proposed to stimulate the formation of preβ₁ HDL and increase reverse cholesterol transport in apoE-null mice. The existence of a monoclonal antibody (MAb 55201) and a corresponding ELISA method that is selective for the detection of the preβ₁ subclass of HDL provides a means of establishing a correlation between apoA-I mimetic dose and preβ₁ HDL formation in human plasma. Using this preβ₁ HDL ELISA, we demonstrate marked apoA-I mimetic dose-dependent preβ₁ HDL formation in human plasma. These results correlated with increases in band density of the plasma preβ₁ HDL, when observed by Western blotting, as a function of increased apoA-I mimetic concentration. Increased preβ₁ HDL formation was observed after as little as 1 min and was maximal within 1 h. Together, these data suggest that a high-throughput preβ₁ HDL ELISA provides a way to quantitatively measure a key component of the reverse cholesterol transport pathway in human plasma, thus providing a possible method for the identification of apoA-I mimetic molecules.—Troutt, J. S., W. E. Alborn, M. K. Mosior, J. Dai, A. T. Murphy, T. P. Beyer, Y. Zhang, G. Cao, and R. J. Konrad. An apolipoprotein A-I mimetic dose-dependently increases the formation of preβ₁ HDL in human plasma. J. Lipid Res. 2008. 49: 581–587.

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The ability of HDL to promote and facilitate reverse cholesterol transport is an important mechanism by which HDL protects against atherosclerosis (1–7). Furthermore, several epidemiological studies have shown a strong inverse relationship between plasma HDL-cholesterol concentrations and clinical coronary heart disease (1–7). As the initial plasma acceptor of cholesterol from cell membranes (8), preβ₁ HDL is of particular interest as a biomarker for therapies targeting the antiatherosclerotic capabilities of HDL. This HDL subclass exhibits preβ₁ mobility in nondenaturing gel electrophoresis and is postulated to consist of two to three apolipoprotein A-I (apoA-I) molecules with some phospholipids and a small amount of unesterified cholesterol (9). As preβ₁ HDL takes on unesterified cholesterol from cell membranes, it exhibits unique epitopes not exposed in spheroidal α-HDL (10–12).

As the precursor and major protein moiety of HDL, apoA-I is thought to promote reverse cholesterol transport. Additionally, it has been shown that infusion and overexpression of apoA-I significantly reduces atherosclerosis in animal models (13–17). Recently, small, amphipathic helical apoA-I mimetic peptides composed of D-amino acids have shown similar antiatherogenic properties. Moreover, a specific apoA-I mimetic peptide, D4F, has shown improved HDL-mediated efflux and reverse cholesterol transport from macrophages, in conjunction with causing the formation of preβ₁ HDL in apoE-null mice (18–20). It is thought that this particular D-amino acid peptide mimics the amphipathic helix of apoA-I, thus allowing it to bind lipids and interact physiologically by mechanisms similar to those of the full-length apoA-I protein (21).

Researchers from Daiichi Pure Chemicals recently developed a monoclonal mouse anti-human preβ₁ HDL antibody (MAb 55201) that is highly specific for apoA-I in the preβ₁ HDL conformation. Additionally, Daiichi now provides an ELISA kit that allows for the capture of human plasma preβ₁ HDL using MAb 55201 and detection via conjugated polyclonal goat anti-human apoA-I antibody (10, 11). As a research tool, this ELISA may provide the chance to better understand the activity of potential D-amino acid peptide therapies that have been shown to mimic the functionality of apoA-I in reverse cholesterol transport. In light of this possibility, we used this ELISA method to investigate the apoA-I mimetic dose-dependent formation of preβ₁ HDL in human plasma.

Abbreviations: apoA-I, apolipoprotein A-I; PK, pharmacokinetic.

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MATERIALS AND METHODS

Sample preparation

Freshly obtained human EDTA plasma from normal healthy volunteers was incubated with the apoA-I mimetic peptide D4F (21) or other similar peptides at concentrations ranging from 0 to 500 μg/ml. Human apoA-I (Academy Bio-Medical) diluted to 100–500 μg/ml in plasma was prepared as a positive control, whereas untreated plasma and vehicle (PBS) in plasma provided baseline negative controls. The resulting treated plasma samples and controls were incubated at 37°C for 0–4 h, followed by a 1:10 dilution of the plasma into a solution of 50% sucrose in MilliQ water to stabilize preβ1 HDL. During this step, the samples were diluted to 1:9 sample-50% sucrose solution. Afterward, samples were stored at −20°C before subsequent analysis. Figure 1 shows an overall flow diagram for the subsequent analyses described in more detail below.

Western blotting analysis of human plasma samples

Human plasma samples treated as above were diluted 1:2 with sample buffer (60% sucrose, 0.1% bromophenol blue) and resolved by nondenaturing, nonreducing one-dimensional 12% polyacrylamide gel electrophoresis at 175 V for ~90 min. Colored molecular weight markers (Invitrogen) were run on each gel. Afterward, the gels were transferred to nitrocellulose for 2 h at 100 V. After transfer, the membrane was blocked with TBS-casein (Pierce) and washed three times (10 min each) with TBST (10 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% Tween 20). The membrane was then probed with 2 μg/ml polyclonal HRP-labeled goat anti-human apoA-I antibody (Academy Bio-Medical) for 1 h at room temperature. Afterward, the blots were washed as above with TBST followed by an additional 10 min wash with TBS. After washing, the blots were developed with ECL reagent (Amersham), and after drying, they were exposed to Bio-Max X-ray film (Kodak).

ELISA determination of preβ1 HDL levels

Stored samples (1:10 in 50% sucrose) were diluted an additional 1:100 in dilution buffer (1% BSA in PBS) for a final dilution of 1:1,000. A standard curve was established by reconstitution of lyophilized apoA-I standard in dilution buffer to 200 ng/ml, followed by serial 1:2 dilutions down to 1.5 ng/ml. Next, preβ1 HDL ELISA kits (Daiichi Pure Chemicals, Inc.) were used to measure preβ1 HDL as described previously (10, 11) with minor modifications. Briefly, 50 μl of sample and standard were added in duplicate to the wells of the MAb 55201-precoated, preblocked 96-well plate. Wells were incubated for 1 h at room temperature, followed by three washes with 100 μl of wash solution (0.1% BSA-PBS). Bound preβ1 HDL was detected with HRP-labeled polyclonal goat anti-apoA-I antibody for 1 h at room temperature followed by four additional washes with BSA-PBS. After washing, wells were incubated with 50 μl of substrate (o-phenylenediamine in citrate buffer) for ~15 min and measured for absorbance at 492 nm using a Spectromax 96-well plate reader. Raw absorbance data were entered into Sigma Plot for subsequent standard curve modeling and sample preβ1 HDL concentration determinations. This ELISA method is highly specific for apoA-I in the preβ1 HDL conformation and has been shown to correlate extremely well with preβ1 HDL levels as measured by two-dimensional gel electrophoresis (10, 11).

Pharmacokinetic analysis of apoA-I mimetic peptides, including preparation and extraction of plasma standards, samples, controls, and liquid chromatography-tandem mass spectrometry

Plasma standards were prepared from 1 to 10,000 ng/ml. For sample analysis, a 100 μl aliquot of each plasma sample, standard, and control plasma was used. A 50 μl aliquot of an internal standard solution (125 ng/ml in water) was added to each sample aliquot. The plasma samples were then transferred to a Waters (Milford, MA) Sep-Pak C18 microelution solid-phase extraction
plate. The plasma samples, standards, and controls were washed three times with 400 μl of water before being eluted with 180 μl of 0.1% trifluoroacetic acid in 40:60 water-acetonitrile. The eluate was dried under nitrogen and then reconstituted with 80 μl of 0.1% acetic acid.

Reconstituted samples (20 μl) were injected onto a 35 × 2.1 mm Capcell PAK UG 300A liquid chromatography column packed with 5 μM C18 resin (Phenomenex, Torrance, CA). The mobile phases used for chromatographic separation consisted of 0.1% acetic acid (0.5:500, v/v, acetic acid-water) (mobile phase A) and 1% acetic acid in acetic acid-water-methanol-acetonitrile (5:50:325:125, v/v/v/v) (mobile phase B). The analytes and internal standard were eluted from the column using a flow rate of 150 μl/min and a programmed binary gradient starting at 10% mobile phase B for 0.5 min, then a linear ramp to 90% mobile phase B at 2.5 min. The column was maintained at 90% mobile phase B for 3.0 min and then returned to 10% mobile phase B. The cycle time from one injection to the next was 5.0 min. The liquid chromatography column was coupled to a TSQ Quantum tandem mass spectrometer (Thermo Electron, San Jose, CA). Analytes were ionized using positive ion electrospray and detected using selected reaction monitoring. Quantitative determination was performed by regression of the calibration curves of the ELISA. Data were plotted using version 2.98 of the program FigP (Biosoft, St. Louis, MO). Statistical analysis was performed using the same program.

**RESULTS**

Preβ₁ HDL has been hypothesized to contain two to three molecules of apoA-I protein. To better understand the apoA-I content of preβ₁ HDL, apoA-I or D4F was incubated with human plasma and samples were analyzed by one-dimensional, nondenaturing, nonreducing Western blotting. These results were also compared with those obtained after incubating apoA-I with buffer alone. Figure 2 shows the results from this series of experiments. When D4F was added to plasma, the formation of a preβ₁ HDL band at ~67 kDa was observed. In contrast, when apoA-I was added to buffer only, a somewhat lower band was observed, and when apoA-I was added to plasma, a broader intermediate band was observed. Because the molecular mass of human apoA-I is ~29 kDa, these results suggested that plasma preβ₁ HDL contains two molecules of apoA-I per particle, with some additional lipid, and that D4F increased preβ₁ HDL, rather than simply displacing apoA-I from HDL to cause an increase in lipid-free apoA-I.

Next, we used one-dimensional, nondenaturing, nonreducing Western blotting to examine the effect of the apoA-I mimetic D4F on preβ₁ HDL formation. As Fig. 3 demonstrates, under baseline conditions, preβ₁ HDL was unable to be visualized in human plasma by direct Western blotting. The apoA-I mimetic D4F, however, dose-dependently increased the density of a 67 kDa preβ₁ HDL band when it was incubated with human plasma. The intensity of this band dramatically increased in response to increasing D4F concentrations. These data suggested that HDL remodeling was taking place as a dose-dependent response to the apoA-I mimic peptide.
These results indicated that one-dimensional, non-denaturing, nonreducing Western blotting could be used to assess pre\(\beta\)\(_1\) HDL levels in human plasma in response to the addition of apoA-I or an apoA-I mimic such as D4F. Because Western blotting is limited by its relatively low throughput, we investigated whether a novel Daiichi ELISA kit could accurately measure pre\(\beta\)\(_1\) HDL levels. This ELISA uses a unique capture antibody that recognizes an epitope of apoA-I that is only exposed when apoA-I is in the pre\(\beta\)\(_1\) HDL state. Figure 4A shows the results from these experiments, in which the ELISA detected increases in pre\(\beta\)\(_1\) HDL concentration after apoA-I mimic incubation. Untreated plasma and vehicle baseline controls demonstrated a pre\(\beta\)\(_1\) HDL concentration of 3 ± 1 µg/ml after a 1 h incubation at 37°C. Incubation of the same plasma with 31 µg/ml of the apoA-I mimic increased the plasma pre\(\beta\)\(_1\) HDL concentration to 25 ± 6 µg/ml. Moreover, further increases in mimic concentration produced dramatic increases in the formation of pre\(\beta\)\(_1\) HDL (Fig. 4B). Figure 4C demonstrates that these ELISA data correlated well with the Western blotting data, suggesting that the ELISA provides a robust method for quantitating human pre\(\beta\)\(_1\) HDL levels.

In light of the fact that paraoxonase is inhibited by EDTA and that D4F has been proposed to work in part by increasing cholesterol-containing particles with pre\(\beta\) mobility enriched in paraoxonase (19), an additional dose curve experiment with D4F was performed with heparinized plasma compared with EDTA plasma. The results observed with heparinized plasma were similar to those obtained with EDTA plasma across the range of D4F concentrations tested, with 500 µg/ml D4F resulting in a level of pre\(\beta\)\(_1\) HDL of 111 ± 2 µg/ml in EDTA plasma versus 112 ± 11 µg/ml in heparinized plasma. Pre\(\beta\)\(_1\) HDL concentrations across the rest of the dose curve of concentrations of D4F were also similar for EDTA and heparinized plasma (data not shown). Furthermore, in light of a previous report that a level of D4F of 0.322 µg/ml or less was able to generate pre\(\beta\) HDL in apoE knockout mice (19), we performed an additional experiment to examine the dose effect of D4F from 0 to 1 µg/ml. At these levels of D4F, the ELISA was unable to detect a significant effect on pre\(\beta\)\(_1\) HDL levels, with levels being 5 ± 1 µg/ml at doses of 0, 250, 500, and 1,000 ng/ml D4F.

We next investigated the time course of D4F-induced increases of pre\(\beta\)\(_1\) HDL in human plasma. Figure 5 shows the results of these experiments, in which human plasma was treated with 500 µg/ml D4F peptide for 0–4 h. D4F-induced pre\(\beta\)\(_1\) HDL formation occurred quickly (as early as 1 min after treatment of the plasma with the peptide), with a plateau reached after ~1 h, indicating that D4F-induced pre\(\beta\)\(_1\) HDL formation is rapid and sustained.

To correlate D4F-induced pre\(\beta\)\(_1\) HDL increases as measured by ELISA and the function and composition of HDL particles in vitro and in vivo, we first intravenously injected 250 µl of a solution containing 3 mg/ml D4F peptide into C57BL6/J mice (weighing ~25 g) and measured the pharmacokinetic (PK) concentration, which was ~0.5 mg/ml. Estimating that plasma constituted 50% of the volume of the blood, this PK level was consistent with almost complete recovery, indicating that the peptide was likely localized to the plasma. Next, we identified a D-amino acid apoA-I mimetic peptide similar to D4F with regard to its ability to increase pre\(\beta\)\(_1\) HDL formation in human serum (data not shown), injected a comparable amount into C57BL6/J mice, and measured the effect on HDL fractions in vivo using capillary isotachophoresis (23). The response to peptide administration showed marked differences between individual HDL peaks. Specifically, the fHDL peak (corresponding to...
mature HDL) showed a significant dose-dependent decrease, whereas the slower migrating sHDL and chylomicron peaks (which contain preβ1 HDL) showed significant dose-dependent increases (data not shown), suggesting that the peptide was remodeling mature α HDL into preβ HDL.

Next we attempted to use the ELISA to measure increases in preβ1 HDL in the mice. Unfortunately, however, it was observed that the ELISA was not able to recognize rodent preβ1 HDL (data not shown). As a result, an additional series of experiments was performed with the D-amino acid peptide described above. The peptide was again spiked into human plasma, resulting in a dose curve on the preβ1 HDL ELISA similar to that of D4F (data not shown). Next, the peptide or vehicle was added in vitro into mouse plasma at a concentration of 300 µg/ml or was}

**Fig. 4.** D4F increases plasma preβ1 HDL as assessed by ELISA, with results correlating to those obtained via Western blotting. A: After the Western blotting analysis shown in Fig. 3, the same samples (preserved in 50% sucrose) were analyzed using a preβ1 HDL ELISA after an additional dilution of 1:100 in 1% BSA-PBS (final dilution = 1:1,000). The ELISA standard curve was established by reconstitution of lyophilized apoA-I standard in dilution buffer at a concentration of 200 ng/ml, followed by serial 1:2 dilutions (closed circles). At a 1:1,000 dilution, all samples (open circles) were well within the standard curve of the ELISA. Results are representative of two independent experiments. A492, absorbance at 492 nm. Results indicate ± SEM. B: Plasma preβ1 HDL concentrations were plotted as a function of apoA-I mimetic concentration. An increase in preβ1 HDL formation was observed in response to increasing concentrations of D4F. Results are representative of two independent experiments. C: Direct comparison between the Western blotting results from Fig. 3 and the ELISA results from A and B. D4F ELISA results are expressed as means ± SEM and represent n = 4 from two independent experiments.

**Fig. 5.** Time course of D4F-induced increases in plasma preβ1 HDL. Human plasma was incubated with 500 µg/ml D4F for 0–4 h. Afterward, the reaction was stopped by diluting the samples 1:10 in 50% sucrose. Samples were diluted an additional 1:100 in 1% BSA-PBS (final dilution = 1:1,000) and analyzed as described for Fig. 4. A direct comparison between the Western blotting results and the ELISA results is shown. D4F ELISA results are expressed as means ± SEM and represent n = 4 from two independent experiments. A492, absorbance at 492 nm.
injected into mice (n = 5 for each group) in amounts (20 or 30 mg/kg) that gave corresponding PK concentrations of ~200–300 µg/ml in the recovered plasma from the injected animals. Subsequently, these plasma samples were assayed for their ability to increase cAMP-dependent, ABCA1-mediated cholesterol efflux using RAW cells with a final plasma concentration of 2.5% (v/v). Results from this series of experiments indicated that at levels consistent with those required for preβ1 HDL formation in vitro in human plasma, there were significant increases in cAMP-dependent, ABCA1-mediated cholesterol efflux. Specifically, a 48 ± 25% increase was observed with plasma from mice treated with 20 mg/kg, an 82 ± 19% increase was observed with plasma from mice treated with 30 mg/kg, and a 45 ± 21% increase was observed in the spiked plasma (all efflux results expressed as means ± SD). Paraoxonase activity was also measured in the 30 mg/kg and control groups and was found not to be increased significantly by administration of the peptide. Control plasma had 32 ± 1 U/ml paraoxonase activity, whereas plasma from peptide-treated animals had 33 ± 1 U/ml paraoxonase activity.

In light of the fact that the ELISA method was unable to recognize mouse preβ1 HDL, we also performed additional experiments to measure preβ HDL formation in vivo. C57BL6/J mice were injected with D4F peptide (~5 mg/kg), and plasma samples were collected at time points ranging from 10 to 60 min. Preβ HDL was then measured via one-dimensional electrophoresis (22) followed by Western blotting with rabbit anti-mouse apoA-I antibody. Results are representative of two independent experiments.

**DISCUSSION**

Our results demonstrate that a preβ1 HDL ELISA method can provide insight into the mechanism of action of apoA-I mimetic peptides. Using the highly selective MAb 55201 capture antibody in this sensitive and robust ELISA format, we were able to observe dose-dependent increases in human plasma preβ1 HDL after treatment of human plasma with the D4F apoA-I mimetic peptide. These increases corresponded well with the increased density of the preβ1 HDL band observed via one-dimensional nondenaturing, nonreducing Western blotting.

Our findings with human plasma are in agreement with those described by Navab et al. (19), which linked D4F administration to preβ HDL formation in apoE-null mice via two-dimensional gel electrophoresis methods. Compared with two-dimensional gel electrophoresis and the earlier gel filtration (8, 9, 19), however, the Daiichi ELISA offers increased throughput. The ELISA also has a relatively broad dynamic range.

Because cardiovascular disease remains a leading cause of mortality worldwide, it is likely that increased efforts will be made toward the development of HDL-modulating therapies. As it has been shown that overexpression and direct infusion of apoA-I have antiatherogenic effects in numerous animal models, treatment with apoA-I analogs presents an enticing possibility as a pharmacological approach to modulating HDL. The large size of apoA-I combined with the high doses that would be required, however, necessitate the need to explore alternative options, such as apoA-I mimic peptides.

These mimetics are currently being examined by numerous researchers as possible therapeutic agents (2, 24). The apoA-I mimetic peptides are composed of D-amino acids and are much smaller than native and full-length recombinant apoA-I, thus likely providing more favorable
possibilities with regard to administration, synthesis, and production cost. For any of these compounds to ultimately become a drug, however, it will be very helpful to have a high-throughput assay that provides the potential for determining whether apoA-I mimetic peptides can associate with preexisting HDL particles in plasma to form preβ1 HDL. Such an assay could also be adapted as a high-throughput screen to identify new apoA-I mimetic peptides. Based on our results in this study, we believe that preβ1 HDL measurement by ELISA will provide this type of information by using a practical, high-throughput method capable of measuring the ability of apoA-I mimetic peptides to induce the formation of preβ1 HDL.

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REFERENCES

1. Kwiterovich, P. O., Jr. 1998. The antiatherogenic role of high-density lipoprotein cholesterol. Am. J. Cardiol. 82: 13Q–21Q.
2. Forrester, J. S., and P. K. Shah. 2006. Emerging strategies for increasing high-density lipoprotein. Am. J. Cardiol. 98: 1542–1549.
3. Shah, P. K., S. Kaul, J. Nilsson, and B. Cercek. 2001. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming. I. Circulation. 104: 2376–2383.
4. Shah, P. K., S. Kaul, J. Nilsson, and B. Cercek. 2001. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming. II. Circulation. 104: 2498–2502.
5. Assmann, G., and A. M. Gotto, Jr. 2004. HDL cholesterol and protective factors in atherosclerosis. Circulation. 109: 8–14.
6. Spady, K. 1999. Reverse cholesterol transport and atherosclerosis regression. Circulation. 100: 576–578.
7. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am. J. Med. 62: 707–714.
8. Castro, G. R., and G. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-B-migrating high-density lipoprotein. Biochemistry. 27: 25–29.
9. O’Connor, P. M., J. M. Naya-Vigne, P. N. Duchateau, B. Y. Ishida, M. Mazur, S. A. Schoenhaus, B. R. Zysow, M. J. Malloy, S. T. Kunitake, and J. P. Kane. 1997. Measurement of prebeta-1 HDL in human plasma by an ultrafiltration-isotope dilution technique. Anal. Biochem. 251: 234–240.
10. Miyazaki, O., J. Kobayashi, I. Fukamachi, T. Miida, H. Bujo, and Y. Saisto. 2000. A new sandwich enzyme immunoassay for measurement of plasma pre-B1-HDL levels. J. Lipid Res. 41: 2083–2088.
11. Miida, T., O. Miyazaki, Y. Nakamura, S. Hirayama, O. Hanyu, I. Fukamanchi, and M. Okado. 2003. Analytical performance of a sandwich enzyme immunoassay for pre-B1-HDL in stabilized plasma. J. Lipid Res. 44: 643–649.
12. Fielding, P. E., M. Kawano, A. L. Catapano, A. Zoppo, S. Marcorina, and C. J. Fielding. 1994. Unique epitope of apolipoprotein A-I expressed in pre-B1 high-density lipoprotein and its role in the catalyzed efflux of cellular cholesterol. Biochemistry. 33: 6981–6985.
13. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein Al. Nature. 353: 265–267.
14. Zhang, Y., I. Zanotti, M. P. Reilly, J. M. Glick, G. H. Rothblat, and D. J. Rader. 2003. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. Circulation. 108: 661–663.
15. Duverger, N., H. Kruth, F. Emmanuel, J. Caillaud, C. Viglietta, G. Castro, A. Tailleux, C. Fiervet, J. C. Fruchart, L. M. Houldebine, et al. 1996. Inhibition of atherosclerosis development in cholesterol-fed human apolipoprotein A-I transgenic rabbits. Circulation. 94: 713–717.
16. Shah, P. K., J. Nilsson, S. Kaul, M. C. Fishbein, H. Ageland, A. Hamsten, J. Johansson, F. Karpe, and B. Cercek. 1998. Effects of recombinant apolipoprotein A1-Milano on aortic atherosclerosis in apolipoprotein E-deficient mice. Circulation. 97: 780–785.
17. Shah, P. K., J. Yano, O. Reyes, K. Y. Chyu, S. Kaul, G. L. Bisgaier, S. Drake, and B. Cercek. 2001. High-dose recombinant apolipoprotein A1-Milano mobilizes tissue cholesterol and rapidly reduces plaque lipid and macrophage content in apolipoprotein E-deficient mice: potential implications for acute plaque stabilization. Circulation. 103: 3047–3050.
18. Navab, M., G. M. Anantharamaiyah, S. Hama, D. W. Garber, M. Chaddha, G. Hough, R. Lallone, and A. M. Fogelman. 2002. Oral administration of an apo A-I mimetic peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. Circulation. 105: 290–292.
19. Navab, M., G. M. Anantharamaiyah, S. T. Reddy, S. Hama, G. Hough, V. R. Grijalva, A. C. Wagner, J. S. Frank, G. Datta, D. Garber, et al. 2004. Oral D-4F causes formation of pre-B high-density lipoprotein and improves high-density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages in apolipoprotein E-null mice. Circulation. 109: 3125–3129.
20. Li, X., K. Chyu, J. R. Faría Neto, J. Yano, N. Nathwani, C. Ferreira, P. C. Dimayuco, B. Cercek, S. Kaul, and P. K. Shah. 2004. Differential effects of apolipoprotein A-I mimetic peptide on evolving and established atherosclerosis in apolipoprotein E-null mice. Circulation. 110: 1701–1705.
21. Datta, G., M. Chaddha, S. Hama, M. Navab, A. M. Fogelman, D. W. Garber, V. K. Mishra, R. M. Epand, R. F. Epand, S. Lund-Katz, et al. 2001. Effects of increasing hydrophobicity on the physical-chemical and biological properties of a class A amphipathic helical peptide. J. Lipid Res. 42: 1096–1104.
22. DeMattos, R. B., L. L. Rudel, and D. L. Williams. 2001. Biochemical analysis of cell-derived apoE3 particles active in stimulating neurite outgrowth. J. Lipid Res. 42: 976–987.
23. Schmitz, G., C. Mollers, and V. Richter. 1997. Analytical capillary isothachophoresis of human serum lipoproteins. Electrophoresis. 18: 1807–1813.
24. Shah, P. K., and K. Chyu. 2005. Apolipoprotein A-I mimetic peptides: potential role in atherosclerosis management. Trends Cardiovasc. Med. 15: 291–296.