

# Apolipoprotein A-I/HDL Infusion Therapy for Plaque Stabilization-Regression: A Novel Therapeutic Approach

P.K. Shah\*

*From the Atherosclerosis Research Center of the Division of Cardiology, Burns and Allen Research Institute and the Department of Medicine, Cedars Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, California, USA*

**Abstract:** LDL-lowering therapies, predominantly involving statins, have been shown to significantly reduce cardiovascular events in asymptomatic subjects as well as in subjects with clinically established atherosclerotic cardiovascular disease. However, despite statin therapy, significant number of cardiovascular events continue to occur indicating the need for additional targets for atherosclerosis management. A number of pre-clinical studies have suggested that several HDL based therapies have the potential to stabilize or regress atherosclerosis consistent with epidemiologic evidence of an inverse relationship between coronary heart disease and HDL cholesterol levels. One such therapeutic approach involves direct infusion of HDL or HDL like molecules for rapid remodeling and stabilization of atherosclerosis. Pre-clinical and proof of concept type preliminary clinical studies suggest the feasibility and potential efficacy of this emerging new therapeutic paradigm.

**Key Words:** Atherosclerosis, HDL, Reverse cholesterol transport.

## INTRODUCTION

Cardiovascular disease is the leading cause of mortality, morbidity and health care expenditure in much of the developed world [1]. With increasing urbanization and prevalence of obesity, metabolic syndrome and diabetes, it is expected that cardiovascular disease will continue to be global challenge for the foreseeable future [1]. Current management of atherosclerosis includes adoption of a healthy life-style, use of anti-thrombotic agents, vaso-active drugs, lipid-modifying drugs and mechanical revascularization-oriented procedures. One of the mainstays of medical therapy involves cholesterol modification primarily targeting LDL-cholesterol with statins. Although pleiotropic effects of statins have been demonstrated, in the clinical setting, the primary benefit of these agents appear to be attributable to LDL-cholesterol lowering through inhibition of HMG Co A reductase, a key enzyme in cholesterol biosynthesis [1, 2]. Statin trials have demonstrated benefit in terms of reduction of major adverse cardiovascular events but despite such progress, nearly 3/4ths of adverse cardiovascular events continue to occur in spite of statin therapy thus demonstrating that the "Glass is more empty than full". Recent emphasis on even more aggressive reduction of LDL-cholesterol with high dose statins to levels as low as 70mg/dl, have only provided a modest incremental clinical benefit (Table 1) [3-5]. Clinical studies utilizing imaging techniques to monitor the progress of atherosclerotic lesions have generally shown that atherosclerosis progression often continues despite LDL-C lowering and even with aggressive LDL-C lowering, halting progression rather than

**Table 1. % Event Rates in PROVE-IT TRIAL**

Follow-up	Atorvastatin (80 mg)	Pravastatin (40 mg)	% Risk Reduction
1 Month	1.9	2.2	17
3 Months	6.3	7.7	18
6 Months	12.2	14.1	14
2.5 years	22.4	26.3	16

actual regression is the rule [3]. These stark realities continue to highlight the need for additional approaches beyond LDL-C lowering for atherosclerosis management [6,7]. In this regard, HDL is now becoming the focus of attention.

## WHY FOCUS ON HDL AS A THERAPEUTIC STRATEGY

Several epidemiologic studies have demonstrated an inverse relationship between HDL-cholesterol levels (HDL-C) or apolipoprotein A-I (major structural protein of HDL) and coronary heart disease (CHD) [6-11]. A 1% increase in HDL-C level is associated with a 2-3 % decrease in CHD risk. In fact low HDL-C levels are a common risk factor for CHD in men with a prevalence ranging from 30-50%. As the prevalence and incidence of obesity and diabetes continue to increase, low HDL-C state, a common finding in such conditions, is likely to increase even further. The inverse relationship between HDL-C and CHD does not by itself distinguish HDL-C as a risk factor or a risk marker, however a considerable body of evidence suggests that HDL has direct atheroprotective effects. In this regard the favorable effects of infu-

\*Address correspondence to this author at the Division of Cardiology, Suite 5531, Cedars Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, California 90048, USA; Tel: 310-423-3884; Fax: 310-423-0144; Email: shahp@cshs.org

sion of crude homologous HDL/VHDL in rabbits and transgenic over-expression of apolipoprotein A-I in hyperlipidemic mice provided the first proof of concept in favor of a direct anti-atherogenic effect of HDL [12-15]. Some of the large scale clinical trials have generally supported the benefits of increasing HDL-C although drugs in clinical use that increase HDL-C levels, such as statins, primarily lower LDL-C and have only a modest HDL-C raising (5-10% increase) effect or as in the case of Niacin and Fibrates, increase HDL-C to a greater degree (15-35% increase) but have other favorable effects on non-HDL lipoproteins and non-lipid risk factors [16]. Thus the clinical trial data to date does not unambiguously prove the HDL hypothesis.

**PLEOTROPHIC FUNCTIONS OF HDL**

**Stimulation of Reverse Cholesterol Transport**

HDL is a heterogeneous group of lipoprotein particles with variable size and composition. Apolipoprotein A-I is the major structural protein (70%) of HDL and plays a critical role in the biological functions of HDL. HDL plays a central role in the transport of cholesterol from peripheral tissues to the liver for eventual elimination through the bil-

iary system into the gut and feces (Fig. 1). This ability to stimulate reverse cholesterol or lipid transport is believed to be an important contributor to the athero-protective effects of HDL, since by this action, HDL can remove cholesterol deposits from the foam cells in the atherosclerotic lesion.. The initial step in the reverse cholesterol transport involves the translocation of free cholesterol and phospholipids from peripheral cells (such as macrophages and foam cells) to lipid poor apo A-I, synthesized in the liver and intestine, creating nascent HDL like particles. This step is dependent on the function of ATP binding cassette transporter A-I (ABCA-1), a key transmembrane protein that acts as a flippase or floppase. Loss of function mutations of ABCA-1 are responsible for Tangier’s disease which is associated with severe HDL deficiency and widespread cellular cholesterol deposits. Subsequently the free cholesterol in the HDL is esterified by lecithin cholesterol acyl transferase (LCAT) leading to remodeling of HDL into HDL-3 and eventually into larger HDL-2 particles [6,7,17,18]. The esterified and free cholesterol on HDL particles is delivered to the liver and steroidogenic tissues, adrenals and gonads, through selective uptake *via* the scavenger receptor B-1 (SR-B1).. However most

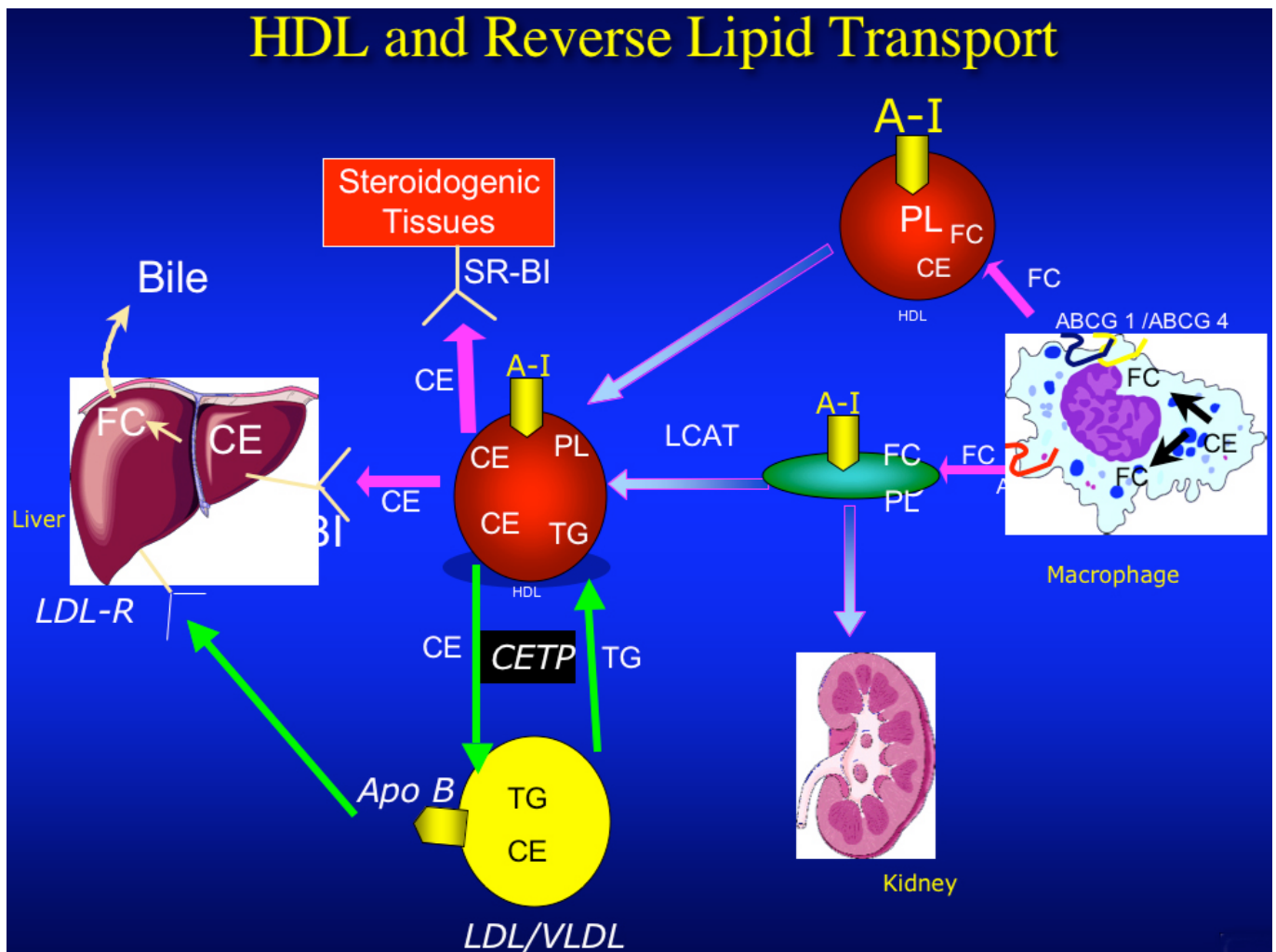


Fig. (1).

of the HDL cholesterol ester is exchanged for triglycerides from VLDL/LDL and this exchange is catalyzed by cholesterol ester transfer protein (CETP). The cholesterol ester acquired by VLDL/LDL from the HDL is delivered to the liver through the low density lipoprotein (LDL) receptor pathway. In the liver, cholesterol secretion into the bile is regulated by key enzymes that include CYP, ABCG5/G8 and excess cholesterol is thus excreted as biliary sterols for eventual excretion into the gut and feces [17,18]. In the gut absorption of intestinal cholesterol is in turn regulated by ABCG5/G8 which tend to eliminate cholesterol from enterocytes into the gut [17]. Recent studies have also shown that cholesterol efflux from macrophages to mature HDL (HDL2 and HDL3) particles, which are poor acceptors for ABCA-I, can occur through the activity of ABCG 1, which unlike ABCA-I does not efflux cholesterol to lipid poor apo A-I [19].

Accumulation of apo B containing atherogenic lipoproteins in the arterial wall with subsequent oxidative modification, induction of inflammation, immune activation, angiogenesis, endothelial dysfunction and arterial remodeling result in atherosclerosis with eventual plaque rupture or erosion leading to arterial thrombosis and serious clinical events

[20]. The beneficial effects of HDL and apolipoprotein A-I in athero-thrombotic vascular disease have been largely attributed to the stimulation of reverse cholesterol transport from arterial wall to liver; however, other biological actions may also play an important role in the athero-protective effects of HDL. These include: anti-inflammatory effects, antioxidant effects, anti-thrombotic effects, pro-fibrinolytic effects and the ability to improve endothelial function [6-8,17-19, 21-26] (Fig. 2). The precise mechanisms for the antioxidant /anti-inflammatory actions of HDL are incompletely understood but may, at least in part, depend on enzymes carried by HDL (such as paraoxonase, platelet activating factor acetyl hydrolase) and the sphingolipid pathway [6,7,17,18, 21]. Recent studies have suggested that increased eNOS activity resulting from HDL and SRB-1 interaction may mediate the favorable effects of HDL on endothelial function [22].

**HDL AS A TARGET FOR ATHEROSCLEROSIS MANAGEMENT**

Based on the epidemiologic data and the biological functions of HDL and apolipoprotein A-I, increasing HDL levels and or enhancing HDL mediated biological actions have

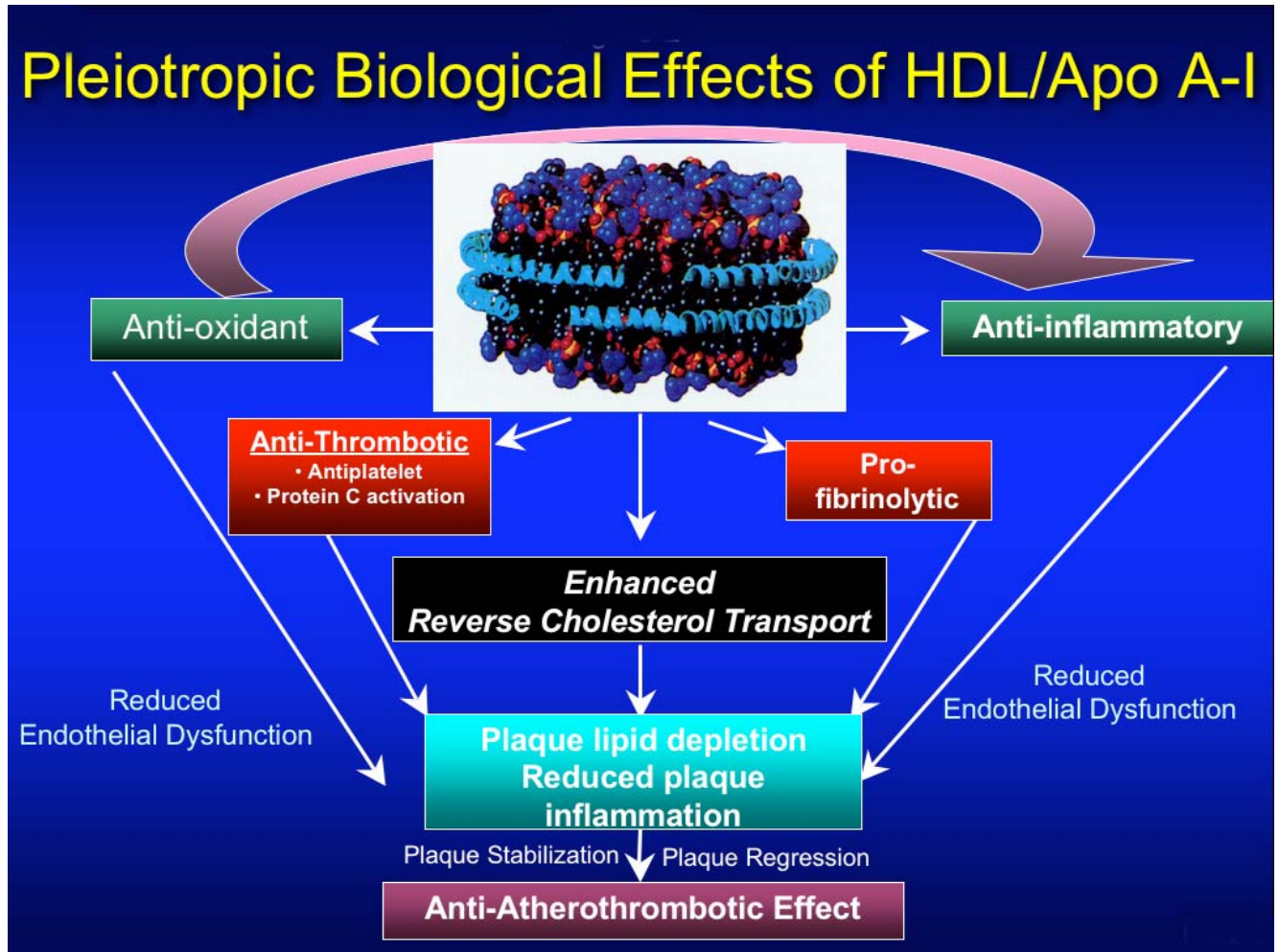


Fig. (2).

become an important focus for potential use in the management of athero-thrombotic vascular disease. A number of different approaches for exploiting the favorable vascular effects of HDL are in various stages of development and testing as summarized in Fig. (3) [6,7,17,18,21]. One such strategy involves direct intravenous infusion of apo A-I or synthetic HDL like particle (apo A-I complexed to phospholipids). Intravenous infusion offers the potential for rapid stabilization/remodeling/regression of atherosclerotic plaque with the expectation of reduction in cardiovascular events.

#### PRECLINICAL STUDIES OF HDL INFUSION (TABLE 2)

The potential for the use of an artificial or synthetic form of HDL for atherosclerosis was first suggested by Orekhov *et al.* in a prescient letter to the *Lancet* in 1984 in which the authors described favorable cholesterol depleting effects of artificial HDL in cell culture experiments [27]. However it was Badimon *et al.* who first reported favorable in-vivo effects of homologous HDL infusion in cholesterol fed rabbits [12,13]. A crude preparation of homologous HDL/VHDL fraction was isolated from rabbits using plasmapheresis and it was then infused intravenously in a second group of cholesterol fed rabbits. Badimon first demonstrated that HDL/VHDL infusion (40mg/week for 8 weeks) could reduce progression of fatty streak lesions in cholesterol-fed rabbits [12]. Badimon also showed that in rabbits with pre-existing fatty streak lesions induced by cholesterol feeding for 8

weeks, four weekly injections of HDL/VHDL (50mg/week) appeared to induce regression of fatty streak like lesions [13]. Miyazaki *et al.* later reported inhibition of progression of atherosclerosis in cholesterol-fed rabbits using purified homologous rabbit-plasma derived apolipoproteinA-I complexed with phospholipids [28]. However unlike Badimon *et al.*, Miyazaki did not observe regression of pre-existing lesions using two different dosing regimens [28]. Interestingly, inhibition of atherosclerosis progression using 1 mg on alternate days for 60 days [cumulative dose of 30 mg] was comparable to that observed with weekly injections of 40mg/injection for 8 weeks (cumulative dose of 320 mg) [28].

Since 1992, our laboratory has been exploring the athero-protective effects of a reconstituted form of HDL made from a complex of recombinant apolipoprotein A-I Milano and phospholipids. Apolipoprotein A-I Milano was the first naturally occurring mutant of apo A-I identified in a small group of individuals in the lakeside town of Limone sul Garda by Sirtori and Francheschini [29,30]. The mutation is characterized by Arginine 173 to Cysteine 173 substitution leading to the formation of homodimers and heterodimers with wild type apolipoprotein A-II in the carriers [30]. There are about 40-45 individuals with this mutation all possibly descendants of a couple traced through church records to 1780 [Giovanni Pomaroli and Rosa Giovanelli]. All carriers are heterozygotes for the mutant allele, have very low levels of HDL cholesterol and apo A-I along with elevated triglycerides and yet appear to have family history of longevity without the

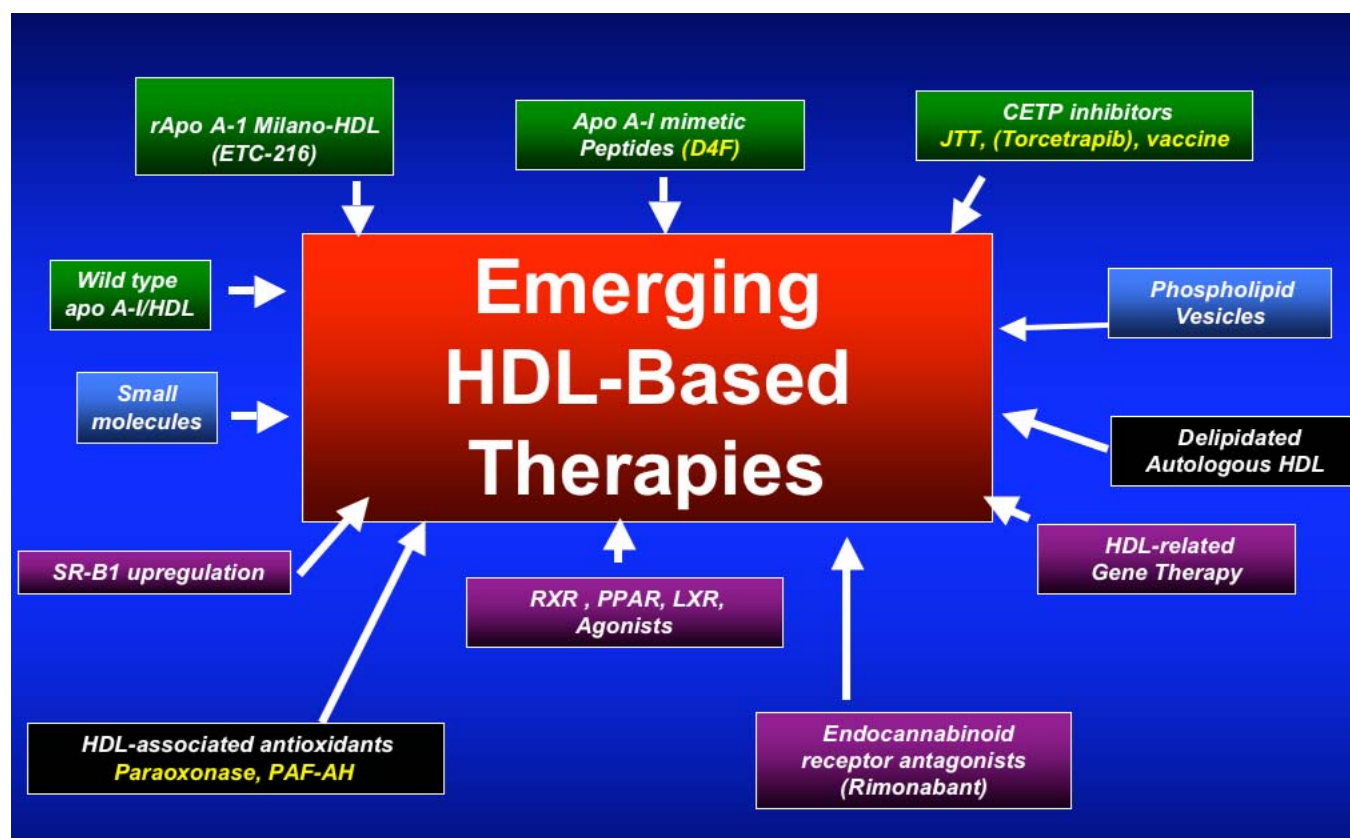


Fig. (3).

Table 2. Studies of HDL/Apo A-I Infusion

Agent	Model	Main Effects	Reference
Homologous plasma derived Rabbit HDL/VHDL	Cholesterol-fed rabbits	Reduced progression, possible regression	Badimon <i>et al.</i> [12,13]
r-Apo A-I milano pc- complex	Cholesterol -fed rabbits with balloon injury	Markedly reduced plaque and macrophages	Ameli, Nilsson, Shah <i>et al.</i> [34]
r-Apo A-I milano pc- complex	Cholesterol -fed rabbits with carotid cuff	Reduced plaque and macrophages	Soma <i>et al.</i> [35]
Purified rabbit Apo A-I	Cholesterol-fed rabbits	Reduced progression (no regression)	Miyazaki <i>et al.</i> [28]
r-Apo A-I milano pc- complex	Apo E null mice	Inhibited progression and regression at high doses Marked reduction in plaque macrophage and lipid content	Shah <i>et al.</i> [36]
r-Apo A-I milano pc- complex	Apo E null mice, single high dose infusion	Rapid Plaque lipid and macrophage depletion within 48 hours	Shah <i>et al.</i> [37]
r-Apo A-I milano pc- complex	Cholesterol-fed rabbit and carotid injury I	Local infusion reduces fatty streaks, lipid and macrophage content	Chiesa <i>et al.</i> [38]
r-Apo A-I milano pc- complex	Apo E null mice and rabbits	Marked improvement in endothelial function	Kaul and Shah <i>et al.</i> [23]
R-Pro-Apo A-I PC Complex	Humans	Stimulation of fecal cholesterol excretion	Carlson [39] Eriksson <i>et al.</i> [40]
Plasma derived Human HDL	Humans	Stimulation of reverse cholesterol transport	Nanjee <i>et al.</i> [41]
Plasma derived Human HDL	Hyperlipidemic humans	Improved endothelial function	Spieker <i>et al.</i> [44]
r-Apo A-I milano pc- complex (ETC-216)	Acute coronary syndrome patients	Rapid coronary atheroma regression in 5 weeks	Nissen <i>et al.</i> [45]

expected excess of cardiovascular disease [29-31]. These observations have suggested the possibility that apolipoprotein A-I Milano may have improved functionality compared to the wild type apolipoprotein A-I [32,33]. In fact increased efflux promoting capacity for apo A-I Milano compared to wild type apo A-I has been shown [32]. Similarly, Bielicki *et al.* have shown that apo A-I Milano has greater antioxidant effect compared to wild type apo A-I or apo A-I Paris, another thiol containing naturally occurring mutant of apo A-I [33]. Our laboratory first reported the marked atheroprotective effects of intravenous injections of a reconstituted form of HDL made from recombinant apolipoprotein A-I Milano complexed to a phospholipids carrier (5 intravenous injections given on alternate days at 40mg/injection) in the iliofemoral arteries of hyperlipidemic rabbits in whom a mature atherosclerotic lesion was induced by cholesterol feeding and balloon injury [33]. In addition to a nearly 70% reduction in atheroma volume compared to controls, we also demonstrated marked macrophage depletion from arterial lesions in apolipoprotein A-I Milano treated rabbits demonstrating a potent anti-inflammatory effect on the vessel wall. These results were achieved without a significant reduction in circulating total cholesterol levels. Subsequently, Soma *et al.* demonstrated similar results in a model of carotid arterial injury in the cholesterol-fed rabbit [35]. Over the subsequent years, our laboratory further demonstrated that recombinant apolipoprotein A-I Milano containing synthetic HDL infusion also inhibited progression of atherosclerosis, promoted regression of atherosclerosis, stimulated lipid and macro-

phage depletion from atherosclerotic lesions and reversed endothelial dysfunction in apo E null mice without a significant change in circulating cholesterol levels [36,23]. Furthermore, we also demonstrated that a single large intravenous infusion of recombinant apolipoprotein A-I Milano (400mg/kg) induced a rapid (within 1 hour) and marked increase in cholesterol efflux in apo E null mice resulting in significant lipid and macrophage depletion out of advanced atherosclerotic lesions within 48 hours [37]. These results were further substantiated later in the carotid injury model in the cholesterol-fed rabbit by Chiesa *et al.* using local infusion of recombinant apo A-I Milano [38]. These series of promising experimental results from our laboratory and those from Milan formed the basis for the eventual launch of clinical studies of recombinant A-I Milano (ETC-216) by Esperion Therapeutics Inc, Ann Arbor, Michigan in 2001.

#### CLINICAL STUDIES OF HDL INFUSION (TABLE 2)

The first clinical studies of HDL infusion in man were reported by Carlson [39,40] The investigators infused recombinant pro-apolipoprotein A-I (precursor of mature apolipoprotein A-I) complexed to soybean phospholipids liposomes (UCB SA, Pharma Sector) in subjects with low HDL or heterozygous familial hypercholesterolemia [40,41]. In 4 patients with familial hypercholesterolemia, fecal sterol excretion was measured for 9 days preceding and for 9 days following a single intravenous HDL infusion (4 gm pro-apo A-I in 200ml over 20 minutes). The HDL infusion was asso-

ciated with increased fecal sterol excretion in all 4 subjects suggesting stimulation of reverse cholesterol transport [40]. Other investigators have also demonstrated that infusion of plasma derived human apo A-I-phospholipid discs or recombinant pro-apo A-I-liposomes resulted in stimulation of various steps in the reverse cholesterol transport pathway in humans [41-43]. Intravenous infusion of plasma derived human apolipoprotein A-I phospholipids complex (40MG/KG over 4 hours) was also shown to rapidly normalize endothelium dependant vasodilation in hypercholesterolemic subjects through increased bio-availability of nitric oxide [44]. Despite these encouraging reports, at the present time, there are no reports of any clinical studies involving the use of wild type apo A-I containing HDL on atherosclerosis in humans.

Based on experimental studies reported from our laboratory and from the Milan team, human trials of recombinant apo A-I Milano phospholipids complex (ETC-216) were initiated in 2001 [45]. Following an initial Phase 1 trial in normal subjects where safety was established, a small randomized double blind placebo controlled trial was conducted in several centers in the US in patients with acute coronary syndromes [45]. In this trial, 47 patients out of a larger initially screened cohort of patients with acute coronary syndrome were randomly allocated to receive once weekly intravenous infusions of ETC-216 at 15mg/kg [n=21] or ETC-

216 at 45 mg/kg [n=15] or saline as placebo [n=11] for 5 weeks beginning within 2 weeks of initial clinical presentation. Intravascular ultrasound (IVUS) was used to measure atheroma volume and thickness in a single coronary artery showing angiographic evidence of 20-50 % narrowing involving at least a 30 mm segment, before the start of infusions and at the conclusion of the study 5 weeks later. The primary efficacy end-point of the trial was a change in % atheroma volume in the ETC-216 group (both doses groups combined) and the secondary efficacy variable was the change in total atheroma volume. The mean % atheroma volume decreased by 1.1 % in the combined ETC-216 group which was significantly ( $p=0.02$ ) different from the mean 0.14% increase observed in the placebo group. The absolute reduction in atheroma volume averaged  $14.1 \text{ mm}^3$  in ETC-216 group reflecting a 4.2% decrease from baseline pre-treatment values [45]. Previous angiographic trials have shown minimal plaque regression, typically <1% over 18-36 months of intensive LDL-lowering therapy [46]. In contrast, this study demonstrated greater plaque regression – nearly 10-fold compared to high-dose atorvastatin in REVERSAL on a much more accelerated time course-5 weeks compared to 18 months in REVERSAL [3]. The infusion was generally well tolerated and no serious side effects were observed. Although this study was quite small, used saline instead of

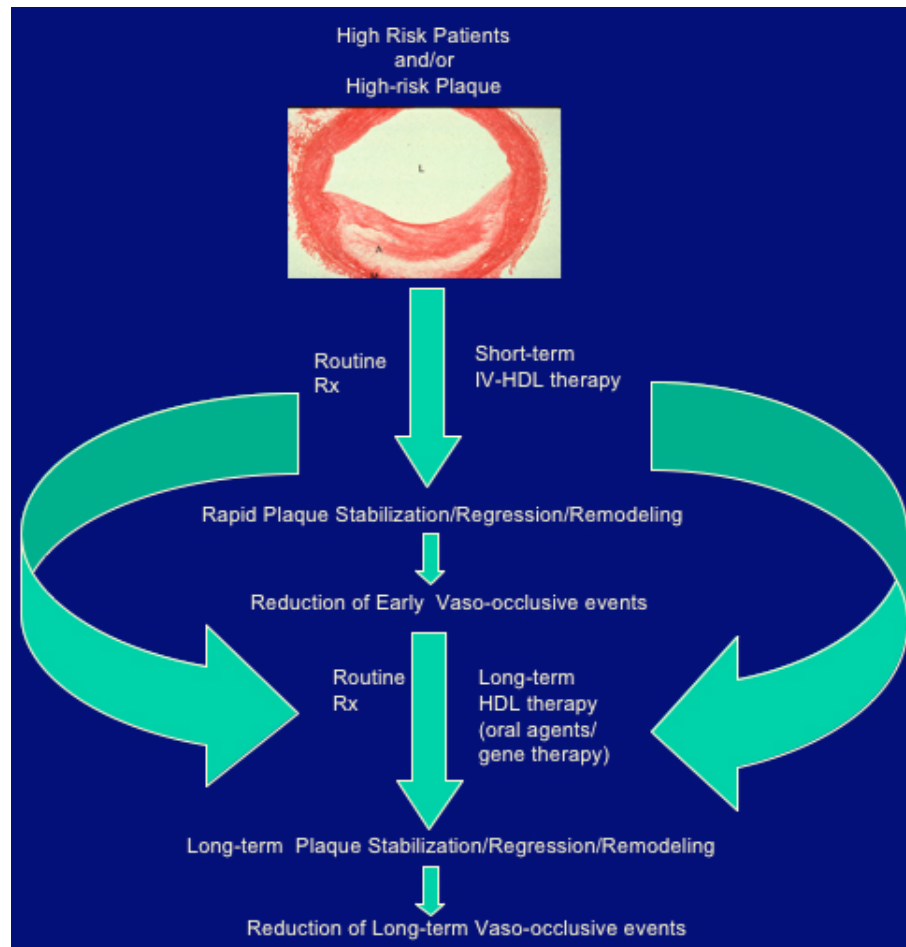


Fig. (4).

phospholipid as a placebo, was underpowered to detect a difference from placebo and failed to show a clear dose response, the findings were consistent with the preclinical results reported by us and our colleagues in Milan. These preliminary proof of concept observations have raised the tantalizing possibility that rapid remodeling /regression or stabilization of atherosclerosis with short-term infusions of recombinant apo A-I Milano (ETC-216) may be feasible warranting further investigations of this promising approach. However, widespread and chronic use of this therapy is likely to pose challenges both in terms of logistics as well as the cost. However, we believe that intravenous HDL infusion therapy, will have an important role as part of a multistep HDL-based therapeutic strategy for atherosclerosis.

#### PERSPECTIVES ON A NEW THERAPEUTIC PARADIGM (FIG. 4)

Infusion of HDL has been shown to reduce atherosclerosis and favorably modify plaque composition to a potentially more stable phenotype in animal models and preliminary small proof of concept studies in humans provide support for this novel therapeutic paradigm. However a number of issues relevant to human application of this therapeutic paradigm need to be addressed. These include demonstration of the consistency and durability of effect on atherosclerosis, dose requirements for optimal benefit, the most effective frequency of infusion needed, delineation of the relative efficacies of different forms of infusible HDL (wild type Apo A-I vs Apo Milano containing HDL, plasma derived versus recombinant HDL, delipidated autologous HDL) and other competing HDL-based therapies (oral agents, phospholipids, gene transfer) and assessment of long term safety and cost-effectiveness. Over the next several years these issues are likely to be addressed with continued development and testing of this promising approach. It is quite possible that short term infusion of apo A-I /HDL will be used for rapid induction of atherosclerosis stabilization/regression and such an effect would then be sustained by either less frequent repeat infusions and or orally effective HDL raising or HDL mimetics or conceivably even HDL-related gene therapy [6,7].

#### REFERENCES

[1] Bonow RO, Smaha LA, Smith SC, Mensah GA, Lenfant C. World Heart Day 2002: the international burden of cardiovascular disease: responding to the emerging global epidemic. *Circulation* 2002; 106: 1602-05.

[2] Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; 360: 7-22.

[3] Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, *et al.*, Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* 2004; 291: 1071-80.

[4] Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004; 350: 1495-1504.

[5] LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, *et al.* Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med* 2005; 352: 1425-35.

[6] Shah PK, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part I. *Circulation* 2001; 104: 2376-83.

[7] Shah PK, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part II. *Circulation* 2001; 104: 2498-502.

[8] Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischemic heart-disease. *Lancet* 1975; 1: 16-19.

[9] Maciejko JJ, Holmes DR, Kottke BA, Zinsmeister AR, Dinh DM, Mao SJJ. Apolipoprotein A-I as a marker of angiographically assessed coronary artery disease. *N Engl J Med* 1983; 309: 385-89.

[10] Gordon DJ, Rifkind BM. High-density lipoprotein: the clinical implications of recent studies. *N Engl J Med* 1989; 321: 1311-16.

[11] Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med* 1991; 325: 373-81.

[12] Badimon JJ, Badimon L, Galvez A, Dische R, Fuster V. High density lipoprotein plasma fraction inhibits fatty streaks in cholesterol-fed rabbits. *Lab Invest* 1989; 60: 455-61.

[13] Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J Clin Invest* 1990; 85: 1234-41.

[14] Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. *Nature* 1991; 353: 265-67.

[15] Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. *Proc Natl Acad Sci U S A* 1994; 91: 9607-11.

[16] Chapman MJ, Assmann G, Fruchart JC, Shepherd J, Sirtori C; European Consensus Panel on HDL-C. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid—a position paper developed by the European Consensus Panel on HDL-C. *Curr Med Res Opin* 2004; 20: 1253-68.

[17] Linsel-Nitschke P, Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat Rev Drug Discov* 2005; 4: 205.

[18] Duffy D, Rader DJ. Drugs in development: targeting high density lipoprotein metabolism and reverse cholesterol transport. *Curr Opin Cardiol* 2005; 20: 301-306.

[19] Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc. Natl Acad Sci USA* 2004; 101: 9774-79.

[20] Shah PK. Insights into the molecular mechanisms of plaque rupture and thrombosis. *Indian Heart J* 2005; 57: 21-30.

[21] Nicholls SJ, Rye KA, Barter PJ. High-density lipoproteins as therapeutic targets. *Curr Opin Lipidol* 2005; 16: 345-49.

[22] Yuhanna IS, Zhu Y, Cox BE, Hahner LD, Osborne-Lawrence S, Lu P, *et al.* High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med* 2001; 7: 853-57.

[23] Kaul S, Coin B, Hedayiti A, Yano J, Cercek B, Chyu KY, *et al.* Rapid reversal of endothelial dysfunction in hypercholesterolemic apolipoprotein E-null mice by recombinant apolipoprotein A-I[Milano]-phospholipid complex. *J Am Coll Cardiol* 2004; 44: 1311-19.

[24] Griffin JH, Kojima K, Banka CL, Curtiss LK, Fernandez JA. High-density lipoprotein enhancement of anticoagulant activities of plasma protein S and activated protein C. *J Clin Invest* 1999; 103[2]: 219-27.

[25] Naqvi TZ, Shah PK, Ivey PA, Molloy MD, Thomas AM, Panicker S, *et al.* Evidence that high-density lipoprotein cholesterol is an independent predictor of acute platelet-dependent thrombus formation. *Am J Cardiol* 1999; 8: 1011-17.

[26] Li D, Weng S, Yang B, Zander DS, Saldeen T, Nichols WW, *et al.* Inhibition of arterial thrombus formation by ApoA1 Milano. *Arterioscler Thromb Vasc Biol* 1999; 19: 378-83.

[27] Orekhov AN, Misharin AY, Tertov VV, Khashimov KA, Pokrovsky SN, Repin VS, *et al.* Artificial HDL as an anti-atherosclerotic drug. *Lancet* 1984; 2: 1149-50.

[28] Miyazaki A, Sakuma S, Morikawa W, Takiue T, Miake F, Terano T, *et al.* Intravenous injection of rabbit apolipoprotein A-I inhibits the progression of atherosclerosis in cholesterol-fed rabbits. *Arterioscler Thromb Vasc Biol* 1995; 15: 1882-88.

[29] Franceschini G, Sirtori CR, Capurso A, Weisgraber KH, Mahley RW. A-I<sub>Milano</sub> apoprotein: decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and with-

- out clinical atherosclerosis in an Italian family. *J Clin Invest* 1980; 66: 892-00.
- [30] Weisgraber KH, Bersot TP, Mahley RW, Franceschini G, Sirtori CR. A-Milano apolipoprotein. Isolation and characterization of a cysteine-containing variant of the A-I apolipoprotein from human high density lipoproteins. *J Clin Invest* 1980; 66: 901-07.
- [31] Sirtori CR, Calabresi L, Franceschini G, Baldassarre D, Amato M, Johansson J, *et al.* Cardiovascular status of carriers of the apolipoprotein A-I[Milano] mutant: the Limone sul Garda study. *Circulation* 2001; 103: 1949-54.
- [32] Franceschini G, Calabresi L, Chiesa L, Parolini C, Sirtori CR, Canavesi M, *et al.* Increased Cholesterol Efflux Potential of Sera From ApoA-I<sub>Milano</sub> Carriers and Transgenic Mice. *Arterioscler Thromb Vasc Biol.* 1999; 19: 1257-62.
- [33] Bielicki JK, Oda MN. Apolipoprotein A-I Milano and Apolipoprotein A-I Paris exhibit an antioxidant activity distinct from that of wild type Apolipoprotein A-I. *Biochemistry* 2002; 41: 20889-2096.
- [34] Ameli S, Hultgardh-Nilsson A, Regnstrom J, Calara F, Yano J, Cercek B, *et al.* Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 1996; 16: 1074-79.
- [35] Soma MR, Donetti E, Parolini C, Sirtori CR, Fumagalli R, Franceschini G. Recombinant apolipoprotein A-IMilano dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits. *Circ Res* 1995; 76: 405-11.
- [36] Shah PK, Nilsson J, Kaul S, Fishbein MC, Ageland H, Hamsten A, *et al.* Effects of recombinant apolipoprotein A-I[Milano] on aortic atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 1998; 97: 780-85.
- [37] Shah PK, Yano J, Reyes O, Chyu KY, Kaul S, Bisgaier CL, *et al.* High-dose recombinant apolipoprotein A-I[milano] mobilizes tissue cholesterol and rapidly reduces plaque lipid and macrophage content in apolipoprotein e-deficient mice. Potential implications for acute plaque stabilization. *Circulation* 2001; 103: 3047-50.
- [38] Chiesa G, Monteggia E, Marchesi M, Lorenzon P, Laucello M, Lorusso V, *et al.* Recombinant apolipoprotein A-I[Milano] infusion into rabbit carotid artery rapidly removes lipid from fatty streaks. *Circ Res* 2002; 90: 974-80.
- [39] Carlson LA. Effect of a single infusion of recombinant propolipoprotein A-I liposomes [synthetic HDL] on plasma lipoproteins in patients with low high density lipoprotein cholesterol. *Nutrition Metabolism Cardiovasc Dis* 1995; 5: 85-91
- [40] Eriksson M., Carlson LA, Mietinnen TA, Angelin B. Stimulation of fecal steroid excretion after infusion of recombinant proapolipoprotein A-I: Potential reverse cholesterol transport in humans. *Circulation* 1999; 100: 594-98.
- [41] Nanjee MN, Cooke CJ, Garvin R, Semeria F, Lewis G, Olszewski WL, *et al.* Intravenous apo A-I/lecithin discs increase pre-beta HDL concentration in tissue fluid and stimulate reverse cholesterol transport in humans. *J Lipid Re* 2001; 42: 1586-93.
- [42] Kujiraoka T, Nanjee MN, Oka T, Ito M, Nagano M, Cooke CJ, *et al.* Effects of intravenous apolipoprotein A-I phosphatidyl choline discs on LCAT, PLTP, and CETP in plasma and peripheral lymph in humans. *Arterioscl Thromb Vasc Biol* 2003; 23: 1653-59.
- [43] Kujiraoka T, Hattori H, Ito M, Nanjee MN, Ishihara M, Nagano M, *et al.* Effects of intravenous apolipoprotein A-I /phosphatidyl discs on paraoxonase and platelet activating factor acetylhydrolase in human plasma and tissue fluid. *Atherosclerosis* 2004; 176: 57-62.
- [44] Spieker LE, Sudano I, Hurlimann D, Lerch PG, Lang MG, Binggeli C, *et al.* High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation* 2002; 105: 1399-1402.
- [45] Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, *et al.* Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 2003; 290: 2292-2300.
- [46] Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, *et al.*, Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001; 345: 1583-92.