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Invited commentary

Reversal of atherosclerosis with apolipoprotein A1: Back to basics

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The recognition that statin-induced LDL-cholesterol (LDL-c) lowering established a maximum of 25–30% relative risk reduction in cardiovascular (CV) events [1] fuelled the quest for novel strategies to further reduce the CV disease burden. The potent inverse relationship between HDL-cholesterol (HDL-c) and CV risk set the stage for HDL-c raising therapies as the next-in-line strategy in CV risk management. The rationale of this complementary strategy comprised the cumulative effectiveness of decreasing the likelihood of LDL-c deposition ('forward' transport) by lowering LDL-c, whilst concomitantly enhancing the reverse cholesterol transport ('backward' transport) by increasing HDL-c. The need for removal of cholesterol from the vessel wall is emphasized by observational studies showing that the presence of a lipid-rich necrotic core in the atherosclerotic plaque is a strong predictor for future CV events [2]. Since the beginning of this century, however, the story of HDL-c increase in CV risk management has become increasingly clouded.

In observational studies, the concept that changes in HDL-c concentration predict future CV risk has been challenged. The concentration of HDL-c was shown to be heavily confounded by pro-atherogenic risk factors, comprising of sex, smoking, body mass index, exercise, insulin resistance and systemic inflammation [3]. In fact, the strong inverse relationship between HDL-c concentration and CV risk was markedly attenuated after adjustment for these risk factors, eventually losing significance with CV risk altogether [4]. In support, the genome-wide-association studies [5] confirmed a strong inverse relation between HDL-c concentration and CV disease, whereas genetic changes in HDL-c concentration did not reveal any association with CV disease [6]. These findings led to the concept that HDL-c may rather be a 'marker' than a causal factor in atherogenesis. In parallel, several studies emerged convincingly refuting the usefulness of HDL-c concentration as a marker for the reverse cholesterol transport pathway. Pronounced increases in HDL-c up to 46% following treatment with torcetrapib [7] were not accompanied by changes in faecal sterol excretion. In addition, the tissue cholesterol efflux (TCE) in patients with genetic low HDL-c displayed only a modest 19% reduction in TCE despite a more than 60% reduction in HDL-c concentration [8]. Even in patients without any HDL-c or apolipoprotein A-1 (apoA-1) the majority of TCE was found to be preserved [8], implying the presence of highly efficient backup systems for TCE.

In intervention studies, Briel et al. [9] did not find an association between HDL-c increase and CV disease in a meta-analysis comprising almost 300.000 individuals, despite a highly significant relation between LDL-c decrease and CV risk reduction. Whereas this paper was criticized for including studies with very modest HDL-c changes, these critiques have resided following more recent trials. Torcetrapib increased HDL-c by 72%, yet CV mortality was counterintuitively increased with a hazard ratio of 1.25 [10]. Although this has largely been attributed to compound-toxicity unrelated to CETP-inhibition, the ensuing DAL-OUTCOMES trial [11] reported a 31–40% increase in HDL-c without any change in CV events. In parallel, the AIM-HIGH [12] study as well as the larger

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HPS2-THRIVE trial [13], using nicotinic acid derivatives, both failed to report a significant CV benefit despite a 25% increase in HDL-c. Collectively, these data indicate that changes in HDL-c can no longer serve as a valid surrogate for reverse cholesterol transport capacity or changes in future CV risk.

In the present issue of Atherosclerosis, Tardy et al. [14] report the effect of infusions of CER-001, consisting of full length apoA-1 with sphingomyelin, on reverse cholesterol transport and atherosclerosis regression in mice. The main findings of this study confirm a CER-001 induced increase in cholesterol removal from the vessel wall. After a single infusion of 10 mg/kg CER-001, unesterified cholesterol is rapidly mobilized to the plasma compartment leading to an increased cholesterol excretion as evidenced by a 10% increase in cholesterol concentrations in the liver and the faeces. Multiple infusions (5 or 10 doses in 10 or 20 days, respectively) in LDL-receptor knockout ($LDLr^{-/-}$) mice resulted in a dose-dependent decrease in atherosclerotic plaque burden (−17 and −32% following 5 or 10 doses, respectively). In line, lipid content in the plaque was reduced by 17% (5 doses) and 23% (10 doses). CER-001 in vitro effectively enhanced efflux of oxidized LDL-c from J774 macrophage cells. Combined with the increased cholesterol excretion in the faeces and decreased plaque size, these findings lend further support to the concept that apoA-1-mimetics promote cholesterol removal from lipid-laden macrophages in the vessel wall.

This experimental study fuels the hope for apoA-1-increasing strategies, after the bar had been raised significantly for HDL-c increasing therapies. The evidence for apoA-1 as a beneficial factor to attenuate atherogenesis is by far the strongest in experimental studies, since overexpression of human apoA-1 in a variety of animal models was consistently found to prevent or reverse atherosclerosis [15]. In contrast, the data for other HDL-c modifying strategies have revealed highly variable effects [3]. Furthermore, apoA-1- and HDL-particle number in humans were shown to have a higher predictive value for CV disease compared to HDL-c concentration [4,16]. Altogether, these findings have induced a paradigm shift from strategies aimed at increasing HDL-c concentration towards strategies primarily targeting an increase in HDL-particle number. The most effective way to achieve a marked increase in HDL-particle number is offered by the infusion of either recombinant or reconstituted apoA-1. To date, three compounds have entered more advanced clinical research: reconstituted HDL, consisting of human apoA-1 isolated from donor plasma with phosphatidylcholine (CSL111/112 [17]), apoA-1 − Milano [18] and CER-001 [14]. Early studies in humans with apoA-1-mimetics reported a small, but significant increase in sterol as well as bile-acid excretion in faeces [19,20], supportive of a role of apoA-1 in the whole-body reverse cholesterol transport pathway. Clinical relevance of increased reverse cholesterol transport most likely depends, however, on the successful efflux of cholesterol from lipid-laden macrophages in the atherosclerotic plaque. To date, it has proven extremely challenging to quantify the cholesterol efflux from the vessel wall (macrophages) separately [21], since this flux is minute compared to the whole body tissue cholesterol efflux. In the absence of clinically feasible and validated reverse cholesterol transport assays, an alternative consists of quantifying the dimension of the plaques in patients using imaging techniques such as intravascular ultrasound (IVUS) [22] or magnetic resonance imaging (MRI) [23]. Five weekly infusions of recombinant apoA-1 Milano in a dose of 15 mg/kg ($n = 23$) or 45 mg/kg ($n = 22$) resulted in a 4.2% reduction of coronary atheroma volume measured by IVUS in the combined treatment groups [18], although this did not reach statistical significance compared to placebo infusion ($n = 12$). In a larger randomized controlled trial using reconstituted HDL (CSL-111 40 mg/kg ($n = 105$) versus placebo ($n = 54$)), a similar 3.4% reduction of plaque volume was achieved after 4 weekly infusions, which was also accompanied by a beneficial effect on plaque composition [17]. However, as in the apoA-1-Milano study, the difference between CSL-111 and placebo did not reach statistical significance. Collectively, the positive indices for increased cholesterol excretion and the trends towards reduced coronary atherosclerotic burden support a beneficial effect of apoA-1-mimetics in humans, more or less comparable to the effects reported by Tardy et al. in the present issue [14]. The proof-of-the-pudding is in the eating. Therefore, the results of the CHI-SQUARE study, an IVUS study in 500 patients with acute coronary syndrome receiving either CER-001 or placebo, will hopefully give us a clear picture.

Besides the effect of CER-001 on cholesterol fluxes, the present study [14] also reports a marked anti-inflammatory effect of CER-001, as attested to by an attenuation of the lipopolysaccharide (LPS)-induced release of pro-inflammatory cytokines (IL-6, IL-8, MCP-1, GM-CSF) from human umbilical vein endothelial cells in vitro. In support, CER-001 also reduced the number of plaque macrophages by 80% and VCAM expression by 16% in $LDLr^{-/-}$ mice. These anti-inflammatory properties of apoA-1 are in line with earlier reports, showing that relatively small amounts of exogenous apoA-1 markedly inhibited VCAM-1 expression in the vascular endothelium as well as white blood cell adhesion in the rabbit carotid collar model [24]. In humans, subjects with low HDL-c were also found to have a pro-inflammatory phenotype of circulating white blood cells [25] as well as hyperresponsiveness to a low-dose challenge with LPS [26]. Whether and to what extent these anti-inflammatory effects, or for that matter also the other pleiotropic effects attributed to the HDL-particle, bear any relevance for the CV outcome in patients, remains to be established. It should be taken into account that removal of cholesterol from the plaque also leads to a marked reduction of the inflammatory response, making it more difficult to dissect the role of these various effects separately [27]. Awaiting evidence to support relevant pleiotropic effects of apoA-1 or HDL beyond increasing cholesterol efflux, it may be prudent to focus primarily at decreasing the dimension of atherosclerotic plaques following apoA-1 infusion.

Summary

The study by Tardy et al. demonstrated increased reverse cholesterol transport and decreased atherosclerotic burden after treatment with CER-001 in an atherosclerotic mouse model. Supported by similar results from early clinical human studies with apoA-1-mimetics, these findings suggest that the conceptually attractive hypothesis of increasing cholesterol efflux from the vessel wall to reverse atherosclerosis may still be alive. Results from larger randomized controlled trials, such as the CHI-SQUARE study expected to report in October this year, will hopefully provide definite answers whether apoA-1 is able to truly effectuate regression of atherosclerotic plaques.

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