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Editorial

HDL cholesterol in cardiovascular diseases: The good, the bad, and the ugly?

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ABSTRACT

Atherosclerotic cardiovascular diseases are the leading cause of death in developed and developing countries. HDL-raising therapeutic modalities (such as cholesterol ester transferase protein (CETP) inhibitors) are being developed to combat these diseases. However, recent setback of two CETP inhibitors (Torcetrapib and Dalcetrapib) has highlighted the importance of measuring qualitative functionality of HDL particles, rather than focusing quantitatively on HDL cholesterol serum concentrations. It has been known that, HDL from patients with coronary artery disease (CAD) (i.e., HDL^{CAD}) limits the anti-inflammatory and endothelial repair properties of normal HDL, due to the activation of lectin-like oxidized LDL receptor-1 (LOX-1), thereby causing failure in endothelial nitric oxide (NO) production. A more recent study (*Immunity 2013; 38: 754–768*) also demonstrates that HDL from patients with chronic kidney dysfunction (CKD) (i.e., HDL^{CKD}), unlike its healthy counterpart (i.e., $HDL^{Healthy}$), promotes superoxide production, reduces NO bioavailability and raises blood pressure via toll-like receptor-2 (TLR-2) activation. This study provides novel insights into understanding why HDL-raising agents failed to demonstrate beneficial effects on cardiovascular mortality in large clinical trials and why CKD accelerates the development of atherosclerosis in CAD patients. Further research is warranted to elucidate whether HDL^{CKD} and HDL^{CAD} participate in other cellular processes in atherosclerosis, such as foam cell formation, the proliferation and migration of smooth muscle cells, and most importantly, plaque destabilization.

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Atherosclerotic cardiovascular diseases are the leading causes of death in developed and developing countries [1]. It is well established that reducing plasma level of LDL (such as therapy with lipid-lowering statins) is beneficial for patients with coronary artery diseases (CAD). Although epidemiological studies indicated that increased plasma level of HDL inversely correlates with the risk for cardiovascular events, recent clinical trials using different therapeutic modalities to raise HDL serum concentrations have failed to demonstrate a beneficial effect on cardiovascular outcomes [2]. Like smoking and lifestyle, chronic kidney dysfunction (CKD), is an important risk factor for CAD. However, the mechanisms by which CKD accelerates the development of vascular disease and magnifies cardiovascular morbidity and mortality remain elusive. In a recent issue of *Immunity*, Speer et al. [2] revealed that HDL from patients with CKD (HDL^{CKD}), in contrast to its healthy counterpart ($HDL^{Healthy}$), promoted endothelial superoxide production, substantially reduced nitric oxide (NO) bioavailability, and subsequently increased arterial blood pressure via toll-like receptor-2 (TLR-2) pathway. This study provides novel insights into understanding why HDL-raising agents failed to demonstrate a beneficial effect on cardiovascular mortality in large clinical trials as well as why CKD accelerates the disease progression of CAD.

"Good" cholesterol is turning "bad"

In vascular cells, HDL is a complex, heterogeneous, and versatile lipoprotein particle that possesses putative anti-inflammatory, anti-apoptotic, anti-oxidative (including prevention of LDL oxidation), anti-thrombotic, endothelial protective as well as reverse cholesterol transport (RCT)-promoting properties [3,4] (Fig. 1). However, the so-called "good" cholesterol is turning bad now, as demonstrated by two recent studies by Besler et al. [5] and Speer et al. [2], respectively. It is now established that HDL becomes dysfunctional, even pro-atherogenic, after being modified, for example, by myeloperoxidase (MPO) [6] and 15-lipoxygenase (15-LPO) [7], two enzymes up-regulated in the atherosclerotic plaques. In a carotid artery electric injury model on nude mice, MPO-oxidized HDL (oxHDL) inhibited re-endothelialization compared to normal HDL *in vivo*. Further evidence shows that oxHDL reduced the proliferation and migration of endothelial cells (EC) [6]. OxHDL also reduced the capacity for cholesterol efflux and some of other anti-atherogenic properties of HDL. A recent study shows that, in non-diabetic subjects with dyslipidemia, oxHDL was independently, significantly and positively correlated with fasting glucose [9]. This finding implicates that high glucose level may contribute to the

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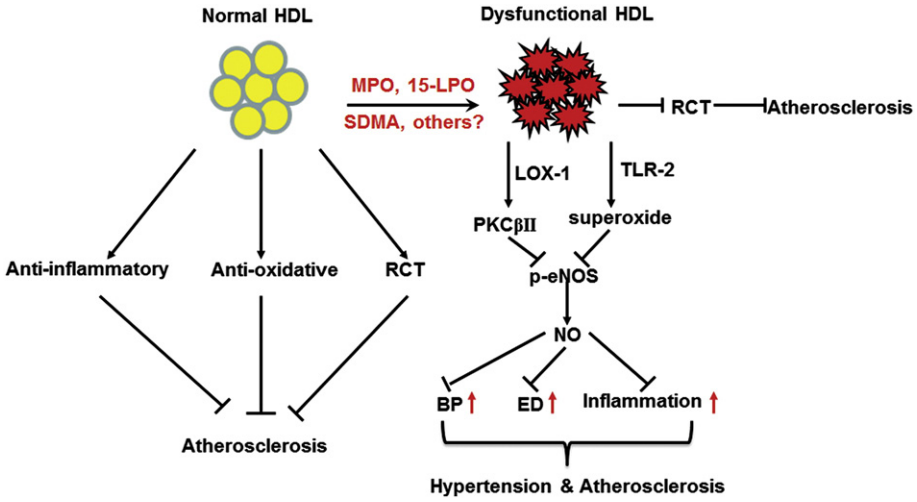

Fig. 1. Schematic representation of the biological functions of normal and dysfunctional HDL. Normal HDL exerts atheroprotective effects by its anti-oxidative, anti-inflammatory, and reverse cholesterol transport (RCT)-promoting properties. However, under pathological conditions, such as coronary artery disease (CAD), chronic kidney disease (CKD), dyslipidemia, diabetes, and chronic ischemic cardiomyopathy, HDL can be modified by myeloperoxidase (MPO), 15-lipoxygenase (15-LPO), symmetric dimethylarginine (SDMA) and other unidentified factors, generating abnormal HDL, which renders this atheroprotective lipoprotein dysfunctional (such as defective RCT function). HDL^{CAD} elicits a lectin-like oxidized LDL receptor-1 (LOX-1)/protein kinase C-βII (PKC-βII) pathway and HDL^{CKD} (or HDL^{SDMA}) initiates a toll-like receptor-2 (TLR-2) pathway on endothelial cells that results in increased superoxide production, reduced nitric oxide (NO) bioavailability and, consequently, increased blood pressure (BP), endothelial dysfunction (ED) and inflammation. These effects act in concert to promote the initiation and progression of cardiovascular diseases, including hypertension and atherosclerosis.

HDL oxidation, and also suggests the measurement of oxHDL may be a clinically useful biomarker of dysfunctional HDL [9].

In addition to oxidation, there are potentially other factors that can modify HDL. For example, to understand the difference between HDL in healthy subjects ($HDL^{Healthy}$) and HDL in patients with CAD or acute coronary syndrome (HDL^{CAD}), Besler et al. [5] evaluated the impact of both lipoproteins on the activation of endothelial NO synthase (eNOS)-dependent signaling pathways. The authors showed that HDL^{CAD} lost the anti-inflammatory effects, and endothelial repair properties because of failure in NO production. Mechanistically, HDL^{CAD} bound more MDA (compared with $HDL^{Healthy}$), probably because of low paraoxonase 1 activity detected in HDL^{CAD}. HDL^{CAD} induced superoxide generation, increased adhesion of monocytes, and reduced eNOS activity because of activation of lectin-like oxidized receptor-1 (LOX-1) and subsequent phosphorylation of PKC-βII (Fig. 1), similar to the effects observed by stimulation with oxidized LDL. In support of this study, Pirillo et al. [8] has recently identified oxHDL as a new ligand for LOX-1, substantiating the crucial role of oxHDL in LOX-1/PKC-βII/eNOS pathway (Fig. 1). These studies reinforce the concept that oxidative modification of HDL (i.e., by MPO and 15-LPO) impairs the atheroprotective functions of HDL and also point to potential therapies aimed at preventing HDL oxidation in diseased settings.

By the same token, to understand the difference between $HDL^{Healthy}$ and HDL^{CKD}, Speer and colleagues [2] first showed that HDL^{CKD} stimulates the generation of reactive oxygen species (ROS) and reduces NO bioavailability, hence, promotes endothelial dysfunction. As reduced endothelial NO production was shown to increase aortic blood pressure (BP), HDL^{CKD} was injected into C57BL/6 J mice. HDL^{CKD} significantly increased BP *in vivo*, but this effect was completely abolished in $eNOS^{-/-}$ mice, suggesting that eNOS-NO pathway mediates the hypertensive effect of HDL^{CKD} on BP. To decipher the molecular mechanisms by which the dysfunctional HDL was generated, the authors identified the non-toxic symmetric dimethylarginine (SDMA), rather than its toxic counterpart asymmetric dimethylarginine (ADMA) as the culprit that converts normal HDL to "notorious" abnormal HDL. This effect was recapitulated by combining HDL with SDMA (HDL^{SDMA}). This aspect is of clinical relevance because SDMA is elevated in the plasma of patients with CKD and it also complements the notion that ADMA is an endogenous inhibitor of eNOS. To study the biological functions of HDL^{CKD}, the investigators employed both *in vitro* and *in vivo* assays to examine whether HDL^{CKD} affects endothelial repair and inflammation. Interestingly, HDL^{CKD} and HDL^{SDMA} inhibited endothelial repair in a carotid artery injury model. Moreover, in contrast to $HDL^{Healthy}$, HDL^{CKD} also elicited TNF-α mediated monocytes adhesion to EC by the activation of vascular cell adhesion molecule-1 (VCAM-1), suggesting that HDL^{CKD} was actually pro-atherogenic *in vivo* by impairing endothelial repair and promoting monocytes adhesion to EC (an early cellular event in endothelial dysfunction). However, these detrimental effects of HDL^{CKD} and HDL^{SDMA} were abolished in TLR-$2^{-/-}$ mice, confirming the pivotal role of TLR-2 in mediating the pro-atherogenic effects of HDL^{CKD}. The authors further provided convincing evidence to suggest that TLR-2, rather than TLR-1 and TLR-6 coreceptor and NF-kB, was responsible for the effect of HDL^{CKD} and HDL^{SDMA}. The investigators also observed that TLR-2 activated NADPH oxidase (Nox) activity to promote superoxide production in EC. This is corroborated by a recent study showing that Nox1 directly interacts with TLR-2 and is essential for TLR-2-dependent ROS production, contributing to enhanced inflammation and migration of in smooth muscle cells (SMC) [9]. It is now well recognized that inflammation and immune effector mechanisms (both innate and adaptive immunity) are implicated in the pathogenesis of atherosclerosis [10]. TLRs are classic pattern recognition receptors which recognize both pathogen-associated molecular patterns and danger-associated molecular patterns. Given the previously recognized role of TLR-2 in atherogenesis (TLR-2 is expressed at sites of disturbed blood flow in hyperlipidemic $LDLR^{-/-}$ mice [11] and genetic deficiency of TLR-2 attenuates atherosclerotic lesions in $LDLR^{-/-}$ mice fed a high-fat diet [12]), the study by Speer et al. [2] represents a leap forward, linking immunity with increased atherosclerosis in patients with CKD.

CETP inhibitors: failures and hopes

HDL is a hot but controversial therapeutic target in the management of cardiovascular diseases. Till now, there are four CETP inhibitors being developed to boost HDL, including Torcetrapib (by Pfizer), Dalcetrapib (by Roche), Anacetrapib (by Merck), Evacetrapib (by Eli Lilly). Of the four inhibitors in clinical trials, none has been on the market; two are halted due to increased cardiovascular mortality (Torcetrapib), and a lack of clinically meaningful efficacy (Dalcetrapib). The failures raise the possibility that HDL level is simply a biomarker indicating the risk

for atherosclerosis but not a viable pharmacological target for clinical interventions [13]. Currently, Anacetrapib and Evacetrapib remain under clinical development. We may know the answer whether CETP inhibitors are effective and safe HDL-boosters for patients with CAD when the phase III REVEAL (ClinicalTrials.gov Identifier: NCT01252953) and ACCELERATE (ClinicalTrials.gov Identifier: NCT01687998) clinical trials are completed [13].

Concluding remarks and future considerations

In summary, current studies suggest that HDL is anti-oxidative, anti-inflammatory, RCT-promoting under physiological state, but its atheroprotective capacities are impaired in patients with diseases, such as CAD [5], CKD [2], dyslipidemia [14], diabetes [15], and chronic ischemic cardiomyopathy [16], as shown in Fig. 1. While the findings of Speer et al. [2] have delineated a key mechanism responsible for the dysfunctionality of HDL in CKD patients, this study has provoked many important and intriguing questions that merit further basic and translational research. For instance, why is the elevation of SDMA, not his "bad" brother ADMA responsible for endothelial dysfunction in CKD? Does statin therapy reduce cardiovascular risk in patients with CKD by reducing SDMA content in HDL^{CKD}? What are the potential effects of HDL^{CKD} and HDL^{CAD} on other cellular processes in atherosclerosis? Such as foam cell formation, the proliferation and migration of SMC, and most importantly, plaque destabilization. Other downstream targets of HDL^{CKD} and HDL^{CAD} need to be identified. Also, other factors (in addition to SDMA, 15-LPO, and MPO) that convert physiologically normal HDL to noxious dysfunctional HDL remain to be identified and studied. It will also be of clinical relevance to explore the possibility of the development of HDL^{CAD} and HDL^{CKD} as diagnostic biomarkers of cardiovascular diseases. Altogether, the present study by Speer et al. [2] is just the tip of the iceberg and provides an intriguing mechanistic starting point to understand the various implications of abnormal HDL in the pathogenesis of atherosclerotic cardiovascular diseases.

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References

[1] Xu S, Ogura S, Chen J, Little PJ, Moss J, Liu P. LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. Cell Mol Life Sci 2013 Aug;70:2859–72.
[2] Speer T, Rohrer L, Blyszczuk P, et al. Abnormal high-density lipoprotein induces endothelial dysfunction via activation of toll-like receptor-2. Immunity 2013;38:754–68.
[3] Tran-Dinh A, Diallo D, Delbosc S, et al. HDL and endothelial protection. Br J Pharmacol 2013;169:493–511.
[4] Prosser HC, Ng MK, Bursill CA. The role of cholesterol efflux in mechanisms of endothelial protection by HDL. Curr Opin Lipidol 2012;23:182–9.
[5] Besler C, Heinrich K, Rohrer L, et al. Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest 2011;121:2693–708.
[6] Pan B, Yu B, Ren H, et al. High-density lipoprotein nitration and chlorination catalyzed by myeloperoxidase impair its effect of promoting endothelial repair. Free Radic Biol Med 2013;60:272–81.
[7] Pirillo A, Uboldi P, Bolego C, Kuhn H, Catapano AL. The 15-lipoxygenase-modified high density lipoproteins 3 fail to inhibit the TNF-alpha-induced inflammatory response in human endothelial cells. J Immunol 2008;181:2821–30.
[8] Pirillo A, Uboldi P, Ferri N, Corsini A, Kuhn H, Catapano AL. Upregulation of lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) expression in human endothelial cells by modified high density lipoproteins. Biochem Biophys Res Commun 2012;428:230–3.
[9] Lee JH, Joo JH, Kim J, et al. Interaction of NADPH oxidase 1 with toll-like receptor 2 induces migration of smooth muscle cells. Cardiovasc Res 2013 Aug 1;99:483–93.
[10] Libby P, Lichtman AH, Hansson GK. Immune effector mechanisms implicated in atherosclerosis: from mice to humans. Immunity 2013;38:1092–104.
[11] Mullick AE, Soldau K, Kiosses WB, Bell III TA, Tobias PS, Curtiss LK. Increased endothelial expression of toll-like receptor 2 at sites of disturbed blood flow exacerbates early atherogenic events. J Exp Med 2008;205:373–83.
[12] Mullick AE, Tobias PS, Curtiss LK. Modulation of atherosclerosis in mice by toll-like receptor 2. J Clin Invest 2005;115:3149–56.
[13] Kypreos KE, Gkizas S, Rallidis LS, Karagiannides I. HDL particle functionality as a primary pharmacological target for HDL-based therapies. Biochem Pharmacol 2013;85:1575–8.
[14] Kotani K, Sakane N, Ueda M, et al. Oxidized high-density lipoprotein is associated with increased plasma glucose in non-diabetic dyslipidemic subjects. Clin Chim Acta 2012;414:125–9.
[15] Pan B, Ma Y, Ren H, et al. Diabetic HDL is dysfunctional in stimulating endothelial cell migration and proliferation due to down regulation of SR-BI expression. PLoS One 2012;7:e48530.
[16] Patel PJ, Khera AV, Wilensky RL, Rader DJ. Anti-oxidative and cholesterol efflux capacities of high-density lipoprotein are reduced in ischaemic cardiomyopathy. Eur J Heart Fail 2013. http://dx.doi.org/10.1093/eurjhf/hft084.