ABSTRACT

Chronic kidney disease (CKD) is accompanied by strong cardiovascular risk. In a rather rigid picture of lipoprotein biology, low-density lipoprotein (LDL) is referred to as ‘bad cholesterol’, while high-density lipoprotein (HDL) is referred to as ‘good cholesterol’. However, recent experimental evidence suggests that HDL may be rendered dysfunctional regarding its effects on the vasculature under various clinical conditions such as CKD. Indeed, HDL from the blood of CKD patients has been shown to transform into a particle which promotes endothelial dysfunction, endothelial proinflammatory activation and, thereby, sets the conditions for the development of atherosclerotic disease. Notably, pharmaceutical interventions to raise serum HDL-cholesterol levels have not been proven beneficial so far. Collectively, these findings indicate that HDL cholesterol levels do not represent a valuable marker for indicating the vascular properties of HDL.

INTRODUCTION

Chronic kidney disease (CKD) is a clinical condition associated with a high burden of cardiovascular disease (CVD). Moreover, a reduced glomerular filtration rate has been identified as a strong CV risk factor [1–3], and even mild kidney dysfunction (e.g. microalbuminuria) substantially increases the risk of CV events [4–6].

Low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) play important roles in the regulation of vascular integrity. High serum LDL-cholesterol levels have been identified as a CV risk factor in the general population as well as in patients with CKD, and the development of LDL-lowering therapies represents a successful strategy to prevent CV events in patients at high risk. In contrast, several observational trials documented an inverse association between serum levels of HDL cholesterol and CV morbidity and mortality in the general population [7, 8], but HDL-raising therapies have not been proven beneficial so far. This observation is of interest, because HDL from healthy subjects exerts several protective effects on the vascular system. However, recent evidence suggests that the vascular effects of HDL can be heterogeneous under different clinical conditions [9, 10].

The aim of the present review was to describe the vascular effects of HDL and to highlight on how the vasoprotective properties of HDL are modified in patients with CKD.

HDL METABOLISM AND REVERSE CHOLESTEROL TRANSPORT

The vasoprotective properties of HDL have been mainly attributed to its role in the reverse cholesterol transport. HDL and its main protein component apolipoprotein A-I (apoA-I) accept free cholesterol (FC) and phospholipids (PLs) from peripheral tissues in order to transport them to the liver, where they are biliary excreted. This process of the reverse cholesterol transport is depicted in Figure 1.

Notably, in a recent trial, the cholesterol efflux of macrophages to apolipoprotein B-depleted serum (i.e. serum without LDL) has been reported to inversely correlate with the intima-media thickness, i.e. a surrogate parameter of subclinical atherosclerosis [11]. Moreover, the authors could demonstrate that a higher cholesterol efflux was associated with lower risk of coronary artery disease [11]. Interestingly, Yamamoto et al. recently demonstrated that the cholesterol efflux capacity of HDL from patients with CKD stage 5D is markedly reduced when compared with HDL from healthy subjects [12].
VASOPROTECTIVE PROPERTIES OF HDL

Besides its role in the reverse cholesterol transport, experimental evidence has accumulated showing that HDL directly interacts with cells of the vascular system (mainly with endothelial cells, ECs) and, thereby, exerts a plethora of vasoprotective effects, which are summarized below.

HDL and endothelial nitric oxide production

Nitric oxide (NO) plays an important role in the regulation of vascular integrity. In vascular ECs, HDL from healthy subjects promotes the formation of NO by modulating the activity of endothelial NO synthase (eNOS), the endothelial NO producing enzyme. The pathways mediating endothelial NO production in response to HDL from healthy subjects are summarized in Figure 2.

The scavenger receptor BI was the first receptor on the surface of ECs identified to mediate NO production in response to HDL [13]. Being co-localized with eNOS in endothelial caveolae, this receptor induces the activation of phosphoinositide 3-kinase (PI3K) in ECs. Subsequently, PI3K promotes the phosphorylation of Akt (protein kinase B) at serine 473 residue, which then phosphorylates eNOS at serine 1177 residue. Serine 1177 represents an eNOS-activating phosphorylation site, which has been identified as an important regulation site of eNOS enzymatic activity [14,15]. Furthermore, it has been documented that activation of PI3K also induces the phosphorylation of the mitogen-associated protein kinases (MAPKs) extracellular signal-regulated kinases 1/2 (ERK1/2) in ECs. Similarly, ERK1/2 phosphorylates eNOS at serine 1177 residue and activates eNOS to produce NO [16]. Furthermore, sphingosine-1 phosphate, a major lipid constituent of HDL, also induces endothelial NO production via interaction with its sphingosine-1 phosphate receptor 3 (S1P3). Thereby, interaction of HDL with the endothelial S1P3 receptor activates eNOS via PI3K-dependent phosphorylation of Akt at serine 473 residue and via direct phosphatidylinositol-specific phospholipase C-dependent phosphorylation of eNOS at serine 1177 residue [17, 18].

Recently, the ATP binding cassette transporter ABCG-1, which plays an important role in the transport of PLs and FC from macrophages to HDL, was identified to be also involved in the regulation of HDL-mediated NO production in ECs. ABCG-1 ameliorates endothelial dysfunction by preventing uncoupling of eNOS. This transporter mediates the export of 7-ketocholesterol from ECs, which reduces the production of endothelial reactive oxygen species (ROS) and, thereby, stabilizes eNOS conformation in order to maintain its enzymatic activity [19]. Notably, the clinical relevance of these findings...
has been proven in hypercholesterolaemic patients, in whom infusion of reconstituted HDL improved endothelial function in an NO-dependent manner as determined by flow-mediated vasodilation [20].

HDL also exerts potent anti-thrombotic, anti-inflammatory and anti-apoptotic effects and it promotes the repair of endothelial lesions as summarized in Figure 3.

**Antithrombotic properties of HDL**

Abnormally activated ECs secrete several procoagulatory factors, which on the one hand induce the activation of plasmatic coagulation pathways and on the other hand promote platelet activation. In contrast to these factors, HDL from healthy subjects exerts antithrombotic effects on ECs and platelets. HDL prevents abnormal endothelial activation by reducing the expression of the tissue factor and P/E-selectin on ECs. In addition, HDL diminishes the generation of thrombin via up-regulation of anticoagulatory factors such as activated protein C, protein S as well as thrombomodulin. Finally, HDL inhibits platelet activation by decreasing the secretion of platelet-activating factor and thromboxane A2 [21].

Importantly, the antithrombotic activity of HDL is broadly modulated via its effect on endothelial NO production. NO is known to preserve blood flow, reduce endothelial activation and inhibit platelet activation. Thus, NO generation in response to HDL does not only affect the vascular tone, but also HDL’s antithrombotic properties.

**Anti-inflammatory effects of HDL**

The expression of adhesion molecules and the secretion of proinflammatory and chemotactic cytokines by the activated endothelium represent a prerequisite for the endothelial

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**FIGURE 2:** Effects of HDL on endothelial NO production. Interaction of HDL with spingosine-1 phosphate receptor 3 (S1P3) activates phosphorylation at Serine1177 of eNOS via phosphorylation of protein kinase B (Akt). Moreover, HDL induces NO production via scavenger receptor B-I, cSrc and activation of phosphoinositide 3 kinase (PI3K), which activates phosphorylation of eNOS via Akt and extracellular signal-regulated kinases 1/2 (ERK1/2). HDL promotes the export of 7-ketocholesterol via ATP-binding cassette transporter (ABCG-1) which reduces endothelial production of ROS and, thereby, prevents uncoupling of eNOS.
adhesion and transmigration of mononuclear cells into the subendothelial layer and, thereby, for the formation of atherosclerotic lesions and plaques. It has been previously shown that HDL from healthy subjects reduces the expression of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 on ECs [22, 23]. Moreover, HDL attenuates the expression of CD11b on mononuclear cells and consequently reduces their adhesion to activated ECs [24]. Notably, the anti-inflammatory effects of HDL are at least partially mediated via the interaction between HDL and scavenger receptor BI as well as S1P receptors leading to an enhanced NO production [25].

**Anti-apoptotic action of HDL**

Apoptosis, the programmed cell death of ECs, is a crucial part of atherogenesis. A broad variety of stimuli such as TNFα, oxLDL, IL-1β and ROS circulate in the blood of patients with CV risk factors and may induce ECs to undergo apoptosis [26]. It has been documented that HDL features an intrinsic anti-apoptotic activity. In response to several stimuli, the constituents of HDL apo-AI as well as sphingosine-1 phosphate reduce endothelial caspase-3 activity, maintain the mitochondrial membrane potential and reduce the formation of ROS by the mitochondrial respiratory chain [27–30].

**HDL and endothelial repair mechanisms**

Several repair mechanisms may counteract the formation of endothelial lesions. The proliferation and migration of ECs into de-endothelialized areas play an important role in preventing the formation of atherosclerotic lesions [31]. Moreover, despite an ongoing controversy about their phenotypic and ontogenetic definition, endothelial progenitor cells (EPCs) have been documented under several conditions to promote the repair of endothelial lesions presumably by paracrine effects [32, 33]. Clinical studies documented an association between the number of circulating EPCs and CV outcome [34, 35].

HDL induces both the migration and the proliferation of ECs [36–38]. Interestingly, these effects of HDL are also mediated via scavenger receptor BI and S1P receptors [39]. Moreover, HDL stimulates phosphorylation of Akt in human peripheral blood mononuclear cells, their differentiation into EPCs as well as their migratory and tube-forming activity in a scavenger receptor BI, PI3K and eNOS-dependent manner [40–42]. In vivo, HDL increases the capillary density and blood flow recovery in the hind limb ischaemia model. Furthermore, the re-endothelialized area after perivascular carotid injury was reduced in apoA-I−/− mice when compared with apoA-I+/+ mice. In these experiments, the effect of HDL on endothelial repair was blunted in scavenger receptor BI-deficient mice [39].
These findings clearly indicate that HDL from healthy subjects not only protects endothelial integrity but also potently promotes endothelial repair mechanisms.

**HDL IN PATIENTS WITH CKD**

CKD is accompanied by increased oxidative stress as well as a proinflammatory microenvironment. Moreover, a plethora of uraemic toxins, which are normally excreted by the urine, accumulate in the serum of CKD patients. These factors can structurally modify the HDL particle in several ways:

(i) they can alter the composition of the proteome and lipidome of HDL,
(ii) they can induce posttranslational modifications of HDL’s protein cargo and
(iii) they can non-covalently interact with the HDL particle.

Recent studies could identify fundamental structural alterations of the HDL particle from the blood of patients with CKD. Moreover, they could provide a link between phenotypical changes and impaired vascular function of HDL (Figure 4).

**Changes in the composition of HDL**

Analyses of the protein moiety (i.e. proteome) of the HDL particle have made it possible to identify several proteins associated with the vascular function of HDL [43]. Two recent studies provided novel insights into the protein composition of HDL in patients with CKD [44, 45]. Using the proteomics of HDL, Holzer et al. found the proinflammatory acute phase protein serum amyloid A (SAA) to accumulate in the HDL particle of CKD stage 5D patients. They have shown an inverse association between the accumulation of SAA, albumin, lipoprotein-associated phospholipase A2 and apolipoprotein C-III in the HDL particle and its ability to promote reverse cholesterol transport in macrophages. Moreover, the authors detected a reduced PL and an increased triglyceride and lysospholipid content in HDL of CKD patients. Subsequently, Weichhart et al. identified 49 HDL-associated proteins. They detected proteins involved in the lipid metabolism as well as in the inflammatory response and could confirm a pronounced accumulation of the pro-inflammatory protein SAA in the HDL particle of CKD stage 5D patients. Moreover, the authors have demonstrated that SAA accumulation leads to a proinflammatory phenotype of HDL. While HDL from healthy subjects reduced the secretion of IL-12p40 and IL-10 by monocytes in response to a Toll-like receptor-2 (TLR-2) agonist, HDL from CKD patients

**Figure 4:** Remodelling of HDL from patients with CKD. Changes of the lipid composition, the protein composition and other modifications of HDL in CKD and their functional consequences. Lp-PLA2, lipoprotein-associated phospholipase A2; SDMA, symmetric dimethylarginine.
significantly promoted the cytokine secretion mainly via accumulation of SAA in the HDL particle.

Notably, several years before, Kalantar-Zadeh et al. [46] had already reported that CKD impairs the anti-inflammatory properties of HDL and provided a link between HDL function and mortality. In a subsequent study, the authors have also shown that the ability of HDL from CKD patients to prevent the oxidation of LDL was significantly reduced when compared with HDL from healthy subjects, and that this was accompanied by a reduced activity of antioxidative enzymes such as paraoxonase and glutathione peroxidase [47].

**Posttranslational modifications of HDL**

Besides changes in the protein cargo, posttranslational modifications of proteins are widely associated with their function. Oxidation of lipoproteins, in particular LDL, is crucially involved in the pathogenesis of atherosclerosis. The oxidation of apoA-I, the main structural protein of HDL, mediated by myeloperoxidase in a proinflammatory environment has been demonstrated to impair the capacity of HDL to promote cholesterol efflux [48]. Although CKD is associated with a high load of oxidative stress, a recent study failed to detect higher degrees of oxidation of apoA-I in patients with CKD [45]. Interestingly, the authors even detected lower oxidation of apoA-I when compared with apoA-I of healthy subjects, maybe as a result of yet not identified counter-regulatory mechanisms.

Besides oxidation, carbamylation of lipoproteins represents another posttranslational modification of proteins, which is present in patients with CKD. Urea-derived cyanate induces the carbamylation of lysine residues leading to the formation of e-carbamyllysine (homocitrulline) [49]. Additionally, myeloperoxidase-catalyzed lipoprotein carbamylation was identified as an alternative and quantitatively dominant mechanism for cyanate formation and protein carbamylation [50]. Intriguingly, the degree of protein carbamylation may predict CV events in patients with CVD [50]. Accordingly, two recent studies could demonstrate that carbamylation of HDL induces cholesterol accumulation and lipid-droplet formation in macrophages as well as a loss of HDL’s anti-inflammatory and antioxidative properties [51, 52].

**Association of SDMA with HDL**

Recently, our group could identify the non-covalent association of symmetric dimethylarginine (SDMA) with the HDL particle of CKD patients [53]. In earlier studies, SDMA has been demonstrated to be an excellent predictor of CV events in CKD patients, but its vascular function remained unclear [54, 55]. We could demonstrate that HDL from patients with incipient CKD not only lost its stimulatory effect on endothelial NO production, but even potently inhibited endothelial NO bioavailability. Moreover, we could reveal that HDL from CKD patients increased arterial blood pressure in vivo. We found the accumulation of SDMA in the HDL particle of CKD patients to be responsible for these deleterious vascular effects of uraemic HDL. Additionally, we could show that HDL from CKD patients as well as HDL supplemented with SDMA exerts its effects on ECs via interaction with TLR-2. Finally, we have demonstrated that this noxious HDL promotes endothelial pro-inflammatory activation and suppresses the repair of endothelial lesions.

**Perspectives**

In summary, the results of these studies collectively indicate that, in CKD patients, HDL fundamentally transforms from a vasoprotective particle into a noxious particle, which may play a crucial role in the pathogenesis of atherosclerotic disease in these patients. Although these findings clearly suggest a causal role for HDL in the development of atherosclerosis and endothelial dysfunction in CKD, a proof of concept in humans is still pending.

**HDL-targeted therapies**

Currently, HDL-targeting therapies are in the focus of clinical CV research. However, raising serum HDL-cholesterol levels in patients without CKD by using niacin in addition to statins has not significantly reduced the number of patients reaching the primary CV endpoint [56].

Inhibition of cholesterol-ester transfer protein (CETP) has been recognized as a potent approach to elevate serum HDL-cholesterol levels. Treating patients with the CETP inhibitor torcetrapib increased serum HDL-cholesterol levels by ~72% after 12 months of treatment [57]. Surprisingly, torcetrapib treatment significantly increased systolic blood pressure possibly contributing to the higher rate of CV events in these patients. The effect of torcetrapib has been mainly attributed to an off-target effect leading to an increased secretion of aldosterone. Therefore, other CETP inhibitors without off-target effects such as dalcetrapib have been evaluated. Although dalcetrapib reliably increased HDL-cholesterol levels, the investigators of the DAL-HEART programme could not document a significant reduction of the primary CV endpoint when compared with placebo-treated subjects [58]. Moreover, dalcetrapib treatment did not improve flow-mediated vasodilation as a surrogate for the endothelial NO bioavailability [59].

Collectively, these results clearly indicate that simply raising HDL in serum does not automatically entail a restoration of HDL’s atheroprotective properties. Although these aforementioned studies have not been performed in patients with CKD, it remains more than questionable as to whether an HDL-increasing therapy would be beneficial in CKD patients. This holds particularly true if fundamental changes in the composition and function of HDL are present in CKD patients.

**Future directions for research**

Even though the findings of recent studies have substantially improved our understanding of HDL and its role in CKD-associated CVD, HDL biology in CKD remains a broad and open field for research. It is something of a chicken-and-egg situation, since it is mainly unclear as to whether the progression of CKD promotes changes in HDL vascular function or if dysfunctional HDL itself has an impact on the
progression of CKD. Moreover, future research should evaluate how the detrimental remodelling of HDL in CKD can be prevented by pharmacological or non-pharmacological interventions in order to reduce the high CV risk in patients with CKD. Certainly, the current knowledge of HDL’s functionality clearly indicates that quantitative measurements of serum HDL-cholesterol levels do not provide valuable information on the biological function of HDL at all. New efficient biosays to examine the qualitative properties of HDL should be developed and implemented in routine laboratory analyses.

CONFLICT OF INTEREST STATEMENT
None declared.

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