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Effect of chronic kidney disease on macrophage cholesterol efflux

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ABSTRACT

Aims: Patients with chronic kidney disease (CKD) have a high risk to develop atherosclerosis. The capacity of high-density lipoproteins (HDL) or serum to accept cholesterol from macrophages and the capacity of macrophages to export excess cholesterol are critical for the atheroprotective role of reverse cholesterol transport. HDL cholesterol acceptor capacity was reported to be decreased in middle aged hemodialysis patients, but the role of confounding factors remains unclear.
Main methods: We measured the cholesterol acceptor capacity (CAC) of HDL or serum in 12 pediatric and 17 young adult patients with CKD stages 3–5, 14 young adult hemodialysis patients and 15 adult renal transplant recipients without associated diseases and matched controls using THP-1 macrophages. Moreover we studied the cholesterol export capacity (CEC) of patients' monocyte-derived macrophages (HMDMs) to control serum or HDL.
Key findings: In adults with CKD stages 3–5 serum CAC was slightly increased, whereas CEC of HMDMs was unaltered in both, adult and pediatric patients. In hemodialysis patients, however, serum CAC was markedly reduced to 85 ± 11% of control ($p < 0.001$), presumably due to low serum apolipoprotein A-I. Interestingly, CEC of HMDMs from dialysis patients was increased. In transplant patients no alterations were found.
Significance: CKD without hemodialysis does not reduce cholesterol export from macrophages. Hemodialysis patients might benefit from therapies aiming to restore serum CAC by increasing apolipoprotein A-I. The enhanced export of cholesterol by HMDMs from dialysis patients may represent an adaptive response.

1. Introduction

Young adults with chronic kidney disease (CKD) have a high prevalence of atherosclerosis [1]. In CKD patients on maintenance hemodialysis cardiovascular mortality is increased excessively [2]. In CKD development of atherosclerosis starts early, resulting in increased intima-media thickness and arterial stiffness already during childhood [1,3,4].

Among cardiovascular risk factors associated with CKD alterations plasma lipid transport plays an important role [5,6]. Total and low-density lipoprotein (LDL) cholesterol levels often are normal in CKD, but lipoprotein particles are subject to increased carbamylation [7] and sensitive to oxidation [8] thereby exerting proatherogenic effects [9–12].

The contribution of high-density lipoproteins (HDL) to the pathogenesis of atherosclerosis in CKD is less well understood. Its atheroprotective effect is thought to be mainly due to its role in reverse cholesterol transport, where HDL accepts excess cholesterol from the periphery and deliver it to the liver for excretion [13]. Adequate cholesterol efflux from macrophages is essential for preventing foam cell formation, the hallmark of atherosclerotic lesions. In CKD HDL maturation is impaired [14–16]. Plasma HDL levels are often low and HDL lipid composition and proteome are altered [17,18]. The capacity of HDL or serum to accept cellular cholesterol from macrophages is an important determinant of cholesterol efflux. The cholesterol acceptor

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Table 1
Clinical characteristics, serum lipids and apolipoproteins of patients and matched controls.

	CKD stages 3–5 (pediatric)		CKD stages 3–5 (adult)		Hemodialysis		Renal transplantation	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
n	12	12	17	17	14	14	15	15
Gender (m/f)	(9/3)	(10/2)	(6/11)	(6/11)	(8/6)	(8/6)	(7/8)	(7/8)
Age (years)	11.0 ± 4.8	11.1 ± 5.2	34.9 ± 9.3	34.6 ± 8.6	36.0 ± 9.6	36.7 ± 10.2	46.8 ± 5.3	47.7 ± 6.2
BMI (kg/m^2)	17.0 ± 3.3	20.4 ± 4.2	22.3 ± 2.5	22.7 ± 3.5	24.0 ± 3.1	25.3 ± 4.8	26.5 ± 4.17	25.1 ± 3.5
KDOQI (3/4/5) (n)	–	5/5/2	–	6/8/3	–	–/–/14	–	–
GFR	–	29.0 ± 12.0	–	25.0 ± 13.2	–	–	–	64.9 ± 13.1
Creatinine (mg/dl)	0.5 ± 0.2	3.2 ± 1.3***	0.8 ± 0.1	3.8 ± 3.0***	0.9 ± 0.2	7.7 ± 4.1***	0.9 ± 0.2	1.2 ± 0.4**
Dialysis duration (months)	–	–	–	–	–	42.8 ± 31.8	–	–
Cholesterol (mmol/l)	4.06 ± 0.62	4.50 ± 1.18	4.44 ± 0.76	4.82 ± 1.18	4.86 ± 0.98	4.39 ± 1.34	5.50 ± 0.83	5.74 ± 1.33
Triglycerides (mmol/l)	0.73 ± 0.30	2.10 ± 1.14***	0.94 ± 0.56	1.27 ± 0.53	1.04 ± 0.63	1.43 ± 0.78	1.38 ± 0.75	1.74 ± 0.91
HDL-C (mmol/l)	1.25 ± 0.26	0.83 ± 0.34**	1.28 ± 0.41	1.09 ± 0.33	1.37 ± 0.36	0.95 ± 0.24**	1.42 ± 0.08	1.43 ± 0.12
LDL-C (mmol/l)	2.47 ± 0.57	2.70 ± 1.12	2.73 ± 0.82	3.16 ± 1.06	3.01 ± 0.85	2.78 ± 1.23	3.45 ± 0.22	3.51 ± 0.31
Free cholesterol (mmol/l)	1.13 ± 0.20	1.49 ± 0.37**	1.30 ± 0.21	1.44 ± 0.34	1.41 ± 0.29	1.38 ± 0.47	1.54 ± 0.27	1.81 ± 0.48
Phospholipids (mmol/l)	2.32 ± 0.27	2.67 ± 0.57	2.79 ± 0.35	2.72. ± 0.52	2.96 ± 0.46	2.75 ± 0.84	3.10 ± 0.37	3.23 ± 0.57
FFAs (mmol/l)	0.9 ± 0.4	0.8 ± 0.4	0.5 ± 0.2	0.5 ± 0.3	0.4 ± 0.1	1.1 ± 0.8**	0.7 ± 0.3	0.6 ± 0.3
ApoA-I (mg/dl)	126.6 ± 13.5	121.9 ± 32.6	155.4 ± 27.9	145.1 ± 32.0	166.6 ± 29.8	132.4 ± 27.0**	156 ± 20.9	160 ± 37.4
ApoA-II (mg/dl)	29.5 ± 2.6	30.8 ± 13.2	35.5 ± 4.9	34.2 ± 7.8	41.4 ± 7.3	31.0 ± 5.8***	43.7 ± 3.3	39.5 ± 6.8**
ApoB (mg/dl)	70.3 ± 12.0	95.8 ± 30.5*	71.3 ± 22.5	88.0 ± 26.4	76.6 ± 27.1	68.1 ± 18.4	103 ± 20.5	103 ± 31.6
ApoC-II (mg/dl)	3.1 ± 1.5	5.8 ± 3.1*	2.9 ± 1.5	3.8 ± 1.6	3.2 ± 1.5	4.5 ± 2.9	5.2 ± 1.9	5.5 ± 2.7
ApoC-III (mg/dl)	5.7 ± 1.6	8.2 ± 3.6*	7.6 ± 2.5	11.2 ± 7.1	11.1 ± 4.4	17.7 ± 11.0	11.9 ± 3.9	15 ± 7.7
ApoC-II/apo C-III	0.5 ± 0.1	0.9 ± 0.8	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.44 ± 0.02	0.38 ± 0.02
ApoE (mg/dl)	8.3 ± 1.9	11.1 ± 3.5*	7.9 ± 1.6	8.9 ± 1.9	8.9 ± 2.8	14.1 ± 14.0	11.1 ± 1.8.	12.2 ± 3.1

Data are given as means ± SD.
* $p < 0.05$ *vs.* controls (unpaired t-test).
** $p < 0.01$ *vs.* controls (unpaired t-test).
*** $p < 0.001$ *vs.* control (unpaired t-test).

capacity (CAC) of HDL is inversely correlated with carotid intima-media thickness and likelihood of coronary artery disease [17]. CAC of serum or HDL was found to be decreased in patients with coronary heart disease [18], familial hypercholesterolemia [19], or diabetes mellitus [18], but enhanced in patients with android obesity and insulin resistance [20], or primary hypertriglyceridemia [21]. Results in middle-aged patients on maintenance hemodialysis suggest reduced CAC of isolated HDL [22,23].

In addition to CAC of HDL, cholesterol efflux capacity (CEC) depends on the capacity of macrophages to export excess cholesterol. Human monocyte-derived macrophage (HMDM) CEC was unaltered in patients with low plasma HDLs or familial hypercholesterolemia [19,24].

We analyzed the effects of CKD stages 3–5, of CKD requiring maintenance hemodialysis and of renal transplantation 1) on the capacity of serum or HDL to accept cholesterol from macrophages and 2) on the capacity of HMDMs isolated from CKD patients to export cellular cholesterol to control serum or HDL.

2. Materials and methods

2.1. Patients

We recruited 12 pediatric and 17 adult patients suffering from CKD with KDOQI (Kidney Disease Outcomes Quality Initiative) stages 3–5 without hemodialysis, 14 adult CKD patients on maintenance hemodialysis and 15 renal transplant recipients and matched controls (Table 1). The study was approved by the Ethics Committee, Medical University of Vienna (#511/2007). Written informed consent was obtained from participants or parents. Patients with diabetes mellitus, nephrotic syndrome or inflammatory diseases, malignancy and infections and patients on corticosteroids or lipid lowering or mTOR inhibitors were excluded. Blood was collected after an overnight fast.

2.2. Lipid and apolipoprotein analysis and lipoprotein isolation

Serum free and total cholesterol, triglycerides, phospholipids and free fatty acids (FFAs) were measured enzymatically. Apolipoprotein (apo) A-I, apoA-II, apoB, apoC-II, apoC-III and apoE were determined by immunoturbidimetry. Plasma LDL and HDL were isolated by sequential flotation ultracentrifugation [25].

2.3. Isolation of human monocytes and differentiation into monocyte-derived macrophages

Blood was centrifuged to remove plasma, which was replaced by RPMI medium, and was layered on Ficoll-Paque PREMIUM solution (GE Healthcare, Uppsala, Sweden) and centrifuged. Cells were collected, washed and seeded in 24-well plates (0.5×10^6 cells/well). After 2 h cells were washed, and adherent human monocytic cells were differentiated towards macrophages for one week in media containing 10% autologous serum.

2.4. Cholesterol acceptor capacity

THP-1 cells (ATTC-TIB-202, LGC Standards, Teddington, UK) were cultured and differentiated towards macrophages using PMA for three days [26]. During the last 24 h cells were trace-labeled with [^{3}H]-cholesterol (0.5 μCi/well) (Perkin Elmer, Waltham, MA, USA) and lipid-loaded using acLDL (50 μg/ml) in the presence of an LXR agonist, TO901317 (3 μM) (Sigma-Aldrich) [27]. Cells were washed and equilibrated with RPMI for 2 h, and then media were replaced with RPMI containing patient or control serum (1%) or HDL (10 μg/ml) for 6 h. Cells were lysed and lysates and media were analyzed in a β-counter (Perkin Elmer). CAC was calculated as cpm in the medium divided by total cpm (media + lysates) and expressed in percent. Efflux to plain medium was subtracted to obtain specific CAC.