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Damage of the endothelial glycocalyx in chronic kidney disease

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ABSTRACT

Background and objectives: The endothelial glycocalyx (eGC), a mesh of anionic biopolymers covering the luminal surface of endothelial cells, is considered as an intravascular compartment that protects the vessel wall against pathogenic insults in cardiovascular disease. We hypothesized that chronic kidney disease (CKD) is associated with reduced eGC integrity and subsequent endothelial dysfunction.
Methods & results: Shedding of two major components of the eGC, namely syndecan-1 (Syn-1) and hyaluronan (HA), was measured by ELISA in 95 patients with CKD (stages 3–5) and 31 apparently healthy controls. Plasma levels of Syn-1 and HA increased steadily across CKD stages (5- and 5.5-fold, respectively $P < 0.001$) and were independently associated with impaired renal function after multivariate adjustment. Furthermore, Syn-1 and HA correlated tightly with plasma markers of endothelial dysfunction such as soluble fms-like tyrosine kinase-1 (sFlt-1), soluble vascular adhesion molecule-1 (sVCAM-1), von-Willebrand-Factor (vWF) and angiopoietin-2 ($P < 0.001$). Experimentally, excessive shedding of the eGC, evidenced by 11-fold increased Syn-1 plasma levels, was also observed in an established rat model of CKD, the 5/6-nephrectomized rats. Consistently, an atomic force microscopy-based approach evidenced a significant decrease in eGC thickness (360 ± 79 vs. 157 ± 29 nm, $P = 0.001$) and stiffness (0.33 ± 0.02 vs. 0.22 ± 0.01 pN/nm, $P < 0.001$) of aorta endothelial cell explants isolated from CKD rats.
Conclusion: Our findings provide evidence for damage of the atheroprotective eGC as a consequence of CKD and potentially open a new avenue to pathophysiology and treatment of cardiovascular disease in renal patients.

1. Introduction

Patients with chronic kidney disease (CKD) exhibit endothelial dysfunction and accelerated vascular disease leading to increased morbidity and mortality due to cardiovascular events [1–3]. The underlying mechanisms, however, are still not fully understood. Endothelial dysfunction has long been ascribed to a malfunction of the endothelial cell itself. Recent studies, though, provided compelling evidence that the endothelium is protected against pathogenic insults by a highly hydrated negatively charged "fire-wall" called the glycocalyx. The endothelial glycocalyx (eGC), a carbohydrate-rich mesh of large anionic polymers, lines the luminal side of the endothelium along the entire vascular tree [4]. It is composed of core proteoglycans, especially those of the syndecan family, to which both highly sulfated glycosaminoglycans and hyaluronan are attached [5,6]. Measuring about 0.5–2 μm in thickness, the eGC forms an integral part of the vascular barrier alongside the endothelial cell itself [7].

Having been neglected over decades, important physiological properties were attributed to the eGC during the last years. Given its strategic location as the interface between blood and endothelium, the intact glycocalyx prevents transvascular protein leakage and reduces leukocyte–endothelial interactions [8–10]. Beyond that, the glycocalyx contributes to the regulation of redox state and is crucially involved in the mediation of shear-induced nitric oxide release as well as physiologic anticoagulation [4,6,11]. Its structure is fairly stable but also in healthy endothelium subject to a permanent dynamic equilibrium between biosynthesis of new components and shear dependent removal, the so-called shedding, of existing constituents [12].

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Damage to eGC, however, can occur under exposure to inflammatory or atherogenic noxae, such as endotoxin [13] or TNF-alpha [14,15], oxidized LDL [11,16], excess atrial natriuretic peptide [17,18], hyperglycaemia [19], and hypervolemia [20]. All of these eGC-deteriorating stimuli occur abundantly in CKD. Given the fact that the mechanisms of accelerated atherosclerosis and cardiovascular disease in CKD patients are still not fully understood, we aimed to investigate whether eGC damage might be a mediating factor in this process. Thus, we sought to assess the impact of chronic renal impairment on 1) circulating markers of eGC damage and endothelial dysfunction in CKD patients, as well as on 2) eGC structure (i.e. thickness and stiffness) itself of aorta endothelial cell explants isolated from CKD rats.

2. Materials & methods

2.1. Patients

The medical ethics committee at University Hospital Münster (Münster, Germany) approved the study and written informed consent was obtained from all subjects. In total, 95 stable Caucasian patients with CKD stages 3–5 (eGFR $\leq$ 60 ml/min/1.73 m^2) were included in this study. Patients with presumable eGC alterations due to acute or chronic infections, malignancy, history of organ transplantation, or pregnancy were excluded. CKD patients were divided according to the National Kidney Foundation's classification of CKD. Subjects with eGFR $\geq$60 ml/min/1.73 m^2 and without evidence of proteinuria served as controls. The control group (n = 31) consisted of healthy volunteers and patients scheduled for elective surgery at University Hospital Münster. All subjects continued their regular medication, including antihypertensive agents and statins. Demographic, clinical, and biochemical characteristics of patients and controls are summarized in Table 1.

2.2. Data collection and blood sampling

Demographic data and details of medical history were collected at enrollment. Hypertension was defined as systolic blood pressure $\geq$140 mmHg and diastolic blood pressure $\geq$90 mmHg or current antihypertensive therapy. The clinical definition of diabetes included fasting glucose $\geq$126 mg/dl, intake of oral hypoglycaemic agents, or insulin use. Cardiovascular risk was estimated using the Framingham Risk Score. Estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation, as previously described [21].

Blood samples from patients and control subjects were collected into ethylenediaminetetraacetic acid (EDTA) tubes, centrifuged, divided into aliquots and stored at −80 °C. Serum creatinine, HbA1c, cholesterol and urinary protein were measured using standard laboratory techniques (Center for Laboratory Medicine, University Hospital Münster). In dialysis patients, blood samples were drawn pre-dialysis.

Table 1
Baseline characteristics of all participants.[a]

	Estimated GFR (ml/min/1.73 m^2)[e]				*P* Value
	≥60 (n = 31)	30 to 59 (n = 26)	15 to 29 (n = 35)	<15 (n = 34)	
Age (yr)	45 ± 21	68 ± 16	67 ± 16	62 ± 13	<0.001[b]
Male gender [% (n)]	65 (20)	46 (12)	46 (16)	65 (22)	0.215[d]
Creatinine (mg/dl)	0.9 (0.7–1.0)	1.3 (1.1–1.5)	2.0 (1.8–2.3)	4.5 (3.3–5.5)	<0.001[c]
eGFR (ml/min/1.73 m^2)[e]	95 (79–110)	40 (36–48)	22 (18–25)	6 (5–10)	<0.001[c]
Proteinuria (g/24 h)	0.0 (0.0 - 0.0)	0.0 (0.0–0.7)	0.3 (0.2–1.5)	0.7 (0.2–1.3)	<0.001[c]
Cardiovascular risk factors					
Arterial hypertension [% (n)]	29 (9)	77 (20)	83 (29)	71 (24)	0.033[d]
SBP (mmHg)	130 (125–135)	135 (129–150)	130 (120–140)	130 (125–140)	0.457[c]
DBP (mmHg)	80 (80–80)	80 (75–80)	80 (70–80)	80 (78–80)	0.306[c]
BMI (kg/m^2)	23.7 (22.1–28.0)	25.5 (23.1–29.7)	27.1 (23.4–30.9)	25.4 (22.5–27.5)	0.416[c]
Cholesterol (mg/dl)	165 (134–211)	184 (134–210)	168 (142–198)	156 (130–199)	0.816[c]
HDL-cholesterol (mg/dl)	49 (45–56)	48 (39–56)	43 (36–50)	43 (39–48)	0.031[c]
Calculated LDL-cholesterol (mg/dl)	129 (109–166)	114 (97–156)	100 (68–127)	97 (67–116)	0.004[c]
Diabetes [% (n)]	3 (1)	23 (6)	40 (14)	32 (11)	0.044[d]
HbA1c (%)	4.7 (4.5–5.0)	5.3 (5.0–5.7)	5.2 (4.8–6.5)	5.2 (5.0–5.7)	0.004[c]
Framingham risk score (%)	8 (1–20)	12 (4–17)	16 (5–22)	11 (6–16)	0.113[d]
Smokers [% (n)]	19 (6)	19 (5)	23 (8)	29 (10)	0.726[d]
Current medication					
ACE inhibitors [% (n)]	19 (6)	65 (17)	54 (19)	32 (11)	0.034[d]
AT1 blockers [% (n)]	13 (4)	27 (7)	17 (6)	26 (9)	0.704[d]
Erythropoietin [% (n)]	0 (0)	4 (1)	11 (4)	35 (12)	<0.001[d]
Statins [% (n)]	6 (2)	50 (13)	46 (16)	29 (10)	0.026[d]
Markers of endothelial dysfunction					
Ang-2 (ng/ml)	0.7 (0.6–1.2)	1.2 (0.9–1.8)	1.6 (1.1–3.4)	3.2 (1.7–5.7)	<0.001[c]
sFlt-1 (pg/ml)	56 (44–79)	77 (61–107)	113 (89–158)	101 (83–149)	<0.001[c]
sVCAM-1 (pg/ml)	740 (544–874)	752 (660–1128)	817 (597–1458)	1679 (1028–2233)	<0.001[c]
vWF (U/ml)	0.2 (0.1–0.5)	0.3 (0.2–0.6)	0.5 (0.2–0.9)	0.7 (0.3–1.3)	<0.001[c]
Circulating glycocalyx components					
Hyaluronan (ng/ml)	81 (48–171)	133 (62–224)	147 (86–230)	441 (163–1213)	<0.001[c]
Syndecan-1 (ng/ml)	53 (41–92)	90 (61–344)	212 (100–355)	270 (92–515)	<0.001[c]

ACE, angiotensin-converting enzyme; AT1, Angiotensin-II receptor; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycohemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

[a] In case of a normal distribution, variables are presented as mean ± SD. In case of a skewed distribution, variables are presented as median (interquartile range).

[b] Univariate ANOVA, post hoc test (Scheffé).

[c] Kruskal–Wallis one-way ANOVA.

[d] χ^2 test.

[e] GFR values were calculated using CKD-EPI formula.