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Increased concentration of circulating angiogenesis and nitric oxide inhibitors induces endothelial to mesenchymal transition and myocardial fibrosis in patients with chronic kidney disease

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ABSTRACT

Background: Sudden cardiovascular death is increased in chronic kidney disease (CKD). Experimental CKD models suggest that angiogenesis and nitric oxide (NO) inhibitors induce myocardial fibrosis and microvascular dropout thereby facilitating arrhythmogenesis. We undertook this study to characterize associations of CKD with human myocardial pathology, NO-related circulating angiogenesis inhibitors, and endothelial cell behavior.
Methods: We compared heart (n = 54) and serum (n = 162) samples from individuals with and without CKD, and assessed effects of serum on human coronary artery endothelial cells (HCAECs) in vitro. Left ventricular fibrosis and capillary density were quantified in post-mortem samples. Endothelial to mesenchymal transition (EndMT) was assessed by immunostaining of post-mortem samples and RNA expression in heart tissue obtained during cardiac surgery. Circulating asymmetric dimethylarginine (ADMA), endostatin (END), angiopoietin-2 (ANG), and thrombospondin-2 (TSP) were measured, and the effect of these factors and of subject serum on proliferation, apoptosis, and EndMT of HCAEC was analyzed.
Results: Cardiac fibrosis increased 12% and 77% in stage 3–4 CKD and ESRD and microvascular density decreased 12% and 16% vs. preserved renal function. EndMT-derived fibroblast proportion was 17% higher in stage 3–4 CKD and ESRD ($P_{trend} = 0.02$). ADMA, ANG, TSP, and END concentrations increased in CKD. Both individual factors and CKD serum increased HCAEC apoptosis ($P = 0.02$), decreased proliferation ($P = 0.03$), and induced EndMT.
Conclusions: CKD is associated with an increase in circulating angiogenesis and NO inhibitors, which impact proliferation and apoptosis of cardiac endothelial cells and promote EndMT, leading to cardiac fibrosis and capillary rarefaction. These processes may play key roles in CKD-associated CV disease.

1. Introduction

Individuals with chronic kidney disease (CKD) have high risks of developing and dying from cardiovascular (CV) disease and these risks are not fully explained by traditional risk factors [1]. Strong associations of novel factors with CV events, a failure of standard therapies to substantively impact mortality in advanced CKD, and an outsize risk of sudden death relative to myocardial infarction suggest that unique features underlie CVD in the setting of uremia [2].

Experimental models of uremia are characterized by myocardial fibrosis, loss of myocardial capillaries, and inhibition of ischemia driven neo-angiogenesis [3,4]. These changes increase capillary to myocyte distance, alter oxygen delivery, and disrupt myocardial conduction, thereby facilitating propagation of arrhythmias. Experimental studies also demonstrate altered nitric oxide (NO) bioavailability in uremia [5] which may induce secondary changes in the activity and concentration of additional angiogenesis inhibitors [6–8] thereby contributing to the observed myocardial fibrosis and capillary rarefaction. However, similar

Abbreviations: ADMA, asymmetric dimethyl arginine; ANG, angiopoietin-2; CKD, chronic kidney disease; END, endostatin; EndMT, endothelial to mesenchymal transformation; ESRD, end stage renal disease; HCAECs, human coronary artery endothelial cells; NO, nitric oxide; TSP, thrombospondin-2.

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Table 1
Baseline characteristics of the autopsy cohort.

Characteristic N (%)	Preserved function (N = 21)	Stage 3–4 CKD (N = 17)	Dialysis (N = 7)	P value*
Demographics				
Age (years), mean ± SD	58.0 ± 15.2	72.8 ± 10.5	66.6 ± 5.6	0.003
Male sex	12 (57.1)	9 (52.9)	5 (71.4)	0.70
Race				0.33
White	16 (76.2)	13 (76.5)	3 (42.9)	
Black	3 (14.3)	3 (17.7)	3 (42.9)	
Labs (mean ± SD)				
eGFR, mL/min/1.73 m^2	101.7 ± 34.2	37.6 ± 16.2	2.0 ± 0.0	<0.001
Serum creatinine, mg/dL	70.7 ± 26.5	203.3 ± 159.1	707.2 ± 0.0	<0.001
Medical history				
Diabetes	2 (9.5)	10 (58.8)	6 (85.7)	<0.001
Hypertension	16 (76.2)	14 (82.4)	7 (100.0)	0.36
Coronary disease	6 (28.6)	7 (41.2)	5 (71.4)	0.13
Myocardial infarction	4 (19.1)	7 (41.2)	4 (57.1)	0.12
Atrial fibrillation	3 (15.0)	5 (29.4)	0 (0.0)	0.21
Congestive heart failure	2 (9.5)	5 (29.4)	3 (42.9)	0.12
Obesity	5 (23.8)	5 (29.4)	2 (28.6)	0.92
Hyperlipidemia	7 (33.3)	12 (75.0)	5 (71.4)	0.03
History of cancer	1 (4.8)	3 (17.7)	0 (0.0)	0.26
Anemia	3 (15.0)	8 (53.3)	4 (57.1)	0.03
Past or present smoking	11 (61.1)	8 (50.0)	2 (28.6)	0.34
Number of diseased coronary arteries (mean ± SD)#	1.4 ± 1.5	1.7 ± 1.2	1.9 ± 1.2	0.71
Cause of death				0.51
Infection	5 (23.8)	5 (29.4)	3 (42.9)	
Cardiovascular	6 (28.6)	8 (47.1)	2 (28.6)	
Other	10 (47.6)	4 (23.5)	2 (28.6)	
Medications				
Aspirin	5 (25.0)	10 (58.8)	6 (85.7)	0.01
ACE or ARB	6 (30.0)	9 (56.3)	3 (42.9)	0.28
Statin	7 (35.0)	9 (52.9)	5 (71.4)	0.22
Beta blocker	10 (50.0)	10 (58.8)	5 (83.3)	0.35

SD = standard deviation. eGFR—estimated GFR. *ANOVA and non-parametric trend test for normally and non-normally distributed continuous variables, respectively. X^2 tests for count variables. #Percent stenosis assessed pathologically was available for 17 patients with preserved function, 16 with Stage 3–4 CKD, and 7 on dialysis. Smoking status was missing in 4 individuals (preserved GFR-3, Stage 3–4 CKD-1). All dialysis patients were on hemodialysis.

data in human disease remains sparse. We undertook this study to characterize for the first time changes in human myocardial pathology across the spectrum of CKD and assess associations with NO-related circulating angiogenesis inhibitors and their effects on human coronary artery endothelial cells (HCAECs).

2. Materials and methods

2.1. Autopsy cohort

Autopsies performed at Brigham & Women's Hospital (BWH) between 2004 and 2006 (n = 45) were included. Cases without sufficient data to estimate kidney function, history of acute kidney injury lasting >1 week, cardiac transplant, active cancer, prior thoracic irradiation, treatment with anthracyclines, congenital heart disease, idiopathic or viral cardiomyopathy, or insufficient tissue were excluded. Medical history and laboratory data were extracted from clinical records. Kidney function was estimated from outpatient serum creatinine or from the lowest stable value (replicated on ≥2 occasions) if outpatient values within 4 months were unavailable.

2.2. Serologic cohort

Individuals 18–80 years old were recruited from the coronary angiography and outpatient nephrology clinics at BWH (n = 162). Individuals with acute kidney injury, history of thoracic radiation, malignancy, receiving anti-angiogenic or immunosuppressive therapy, or requiring urgent angiography were excluded. Angiography subjects were excluded if they had a history of coronary bypass surgery. Serum and plasma were collected prior to angiography, centrifuged within 15 min at 1000 g, and stored at −80 °C. Clinical data

Table 2
Histologic findings.

Characteristic mean ± SD	Preserved function-stage 1 CKD	Stage 3–4 CKD	Dialysis	P_{trend}
Histology	(N = 21)	(N = 17)	(N = 7)	
Fibrosis (%)	10.6 ± 4.8	11.9 ± 4.5	18.2 ± 6.6	0.003
Microvessels/field	459.1 ± 110.1	402.4 ± 69.7	386.9 ± 77.5	0.04
Cardiomyocytes/field	615.8 ± 214.3	573.2 ± 99.4	550.3 ± 121.9	0.31
Microvessel density (n/μM^2)	612.2 ± 146.7	536.6 ± 92.9	515.8 ± 103.3	0.04
Myocyte size (μM^2)	623.3 ± 222.8	645.4 ± 197.4	595.4 ± 222.6	0.90
Myocyte density (n/μM^2)	821.0 ± 285.8	764.3 ± 132.5	733.8 ± 162.5	0.31
Vessels per myocyte	0.80 ± 0.19	0.73 ± 0.16	0.74 ± 0.18	0.24
Immunofluorescence	(N = 11)	(N = 8)	(N = 4)	
FSP positive cells/field	4.2 ± 1.4	4.1 ± 2.0	6.6 ± 0.7	0.04
Double FSP/CD-31 positive cells/field	0.9 ± 0.7	1.4 ± 0.7	2.3 ± 1.3	0.01
Ratio of double positive to all FSP-positive cells	0.19 ± 0.12	0.36 ± 0.11	0.36 ± 0.21	0.02

SD = standard deviation.