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Original Article

P-cresyl sulfate accelerates left ventricular diastolic dysfunction associated with chronic kidney disease

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Abstract: Chronic kidney disease (CKD) favors the development of cardiovascular disease (CVD). Protein-bound uremic toxins retained in CKD patients are considered to be emerging risk factors for CVD. One uremic toxin, p-cresyl sulfate (PCS), has now been the subject of research interest with regard to its negative impact on cardiovascular system. However, the effect of PCS on CVD remains to be elucidated. In the present study, the association of PCS and left ventricular (LV) diastolic dysfunction in the clinical setting was investigated. 170 patients underwent hemodialysis (HD) treatment was evaluated with echocardiography. Serum biochemistries and PCS levels were also measured. To confirm the clinical findings, wild type C57/BL male mice were treated with PCS to study the possible acceleration of cardiac dysfunction. 8-wk-old mice underwent uninephrectomy (UNX) were randomly divided into PCS-treated (intake of 0.5 mg/ml PCS dissolved in double distilled water for 20 weeks) and control groups. At the end of the experiment, echocardiography and haemodynamic measurements were performed in each mouse. Of the 170 patients, a significantly greater prevalence of LV diastolic dysfunction was present in patients with higher serum PCS levels (>0.85 μg/ml) than those with lower serum PCS levels (≤0.85 μg/ml). Animal study showed a significantly greater prevalence of LV diastolic dysfunction in PCS-treated mice than control group. These findings suggest that PCS contributes to diastolic dysfunction and that targeting PCSmay be a therapeutic strategy in CKD.

Keywords: Uremic toxins, p-cresyl sulfate, diastolic dysfunction

Introduction

Cardiovascular disease (CVD) advances more rapidly in individuals with chronic kidney disease (CKD) compared with the general population [1]. Among which, a close relationship between CKD and chronic heart failure (CHF) has been demonstrated repeatedly [2-4]. Left ventricular hypertrophy (LVH) is the most commonly diagnosed cardiovascular abnormality and thought to be the main factor contributing to CHF in CKD patients [5, 6]. In patients with HF, about half percent have preserved left ventricular (LV) systolic function, referred as diastolic HF. The prognosis of patients with diastolic HF is as threatening as that of patients with systolic HF [7]. More importantly, greater CKD-associated mortality occurs in patients with diastolic HF than in those with systolic HF [8].

Traditional cardiovascular risk factors alone does not fully explain the high prevalence of CVD in the CKD population [9]. Uremic toxins are substances accumulating in CKD patients and are considered to be emerging risk factors for CVD [10, 11]. These substances are classified into 3 groups: free-water-soluble low-molecular-weight toxins, middle molecules, and protein-bound toxins [12]. Protein-bound uremic toxins are considered to exert major toxic effects because of their difficult removal by dialysis [13, 14]. In the article published in 2012, the European Uremic Toxin (EUTox) group described 23 protein-bound toxins [15], including p-cresyl sulfate (PCS). PCS, whichoriginates from bacterial protein fermentation in the large intestine mucosa [16], has now been considered as the main uremic toxin [17]. Free PCS has been demonstrated to be independently associated with increased risk of mortality in patients with CKD [18, 19]. In-depth*in vitro* studies revealed that PCS could increase the percentage of leucocytes that expressed oxida-

Table 1. Clinical and demographic characteristics of the study population

	Free PCS>0.85 μg/ml (n=85)	Free PCS≤0.85 μg/ml (n=85)	*P* value
Age, years	57.8±14.8	59.1±14.0	0.566
Male, n (%)	53(62.4)	51(60.0)	0.755
BMI (kg/m^2)	22.9±2.5	23.4±2.8	0.739
Diabetes mellitus, n (%)	9 (10.6)	8 (9.4)	0.780
Hypertension, n (%)	30 (35.3)	29 (34.1)	0.873
Laboratory data			
Albumin (g/L)	35.2±4.4	36.5±5.1	0.75
Hemoglobin (g/L)	106.56±19.43	107.95±16.43	0.618
ALT (U/L)	14.72±8.34	15.59±8.42	0.502
AST (U/L)	15.12±7.80	15.66±9.02	0.676
BUN (mmol/L)	27.50±7.18	25.68±7.49	0.112
CRE (μmol/L)	995.80±279.63	972.84±257.08	0.583
Triglycerides (mmol/L)	1.73±0.94	1.95±1.20	0.193
TC (mmol/L)	4.18±1.24	4.17±1.20	0.970
HDL-C (mmol/L)	1.05±0.37	0.94±0.31	0.037
LDL-C (mmol/L)	2.45±0.94	2.49±0.94	0.790
CRP (mg/L)	0.57±0.56	0.67±0.80	0.359
Calcium (mmol/L)	2.24±0.23	2.21±0.16	0.803
Phosphate (mmol/L)	1.19±0.09	1.16±0.10	0.795
BNP	8587.42±10408.25	6356.76±8506.64	0.141
Free PCS (μg/ml)	2.311±1.491	0.370±0.234	<0.001
Total PCS (μg/ml)	39.85±25.52	12.89±11.00	<0.001

Data are expressed as mean±SD, or for binary variables, number (frequency). PCS, p-cresyl sulfate; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; CRP, C-reactive protein; BNP, B-type natriuretic peptide.

Table 2. Echocardiographic findings

	Free PCS>0.85 μg/ml (n=85)	Free PCS≤0.85 μg/ml (n=85)	*P* value
AAO (mm)	33.91±3.05	33.69±2.90	0.729
LA (mm)	40.30±8.04	40.47±6.29	0.908
Diameter; diastolic (mm)	52.09±6.08	50.06±6.61	0.130
Diameter; systolic (mm)	34.59±5.98	32.82±5.81	0.153
IVS (mm)	10.45±1.26	10.69±1.80	0.456
LVPW (mm)	9.84±1.34	10.06±1.54	0.473
LVEF (%)	60.64±8.68	63.40±6.39	0.087
E/E'	15.38±2.18	8.15±1.98	<0.001

Data are expressed as mean±SD. PCS, p-cresyl sulfate; AAO, Aorta ascendens; LA, left atrial; IVS, interventricular septum; LVPW, left ventricular posterior wall; LVEF, left ventricular ejection fraction; E/E', E-wave/E'-wave ratio.

tive burst activity, alter endothelial function, and cause renal tubular cell damage [20-23]. PCS was also found to induce kidney fibrosis and promote insulin resistance via *in vivo* studies [24, 25]. However, the effect of PCS on HF, especially on diastolic HF, remains to be elucidated.

In the present study, we try to find the correlation between PCS andLV diastolic dysfunction both clinically and in experimental study. Results of this study are helpful for better understanding the toxicity of PCS on cardiovascular system.

Materials and methods

Patients

This study included 170 adult uremic patientswho received hemodialysis (HD) therapy three times a week for at least 6 months. Patients were excluded if there was previous history of CVD (coronary artery disease, myocardial infarction, valvular heat disease, atria fibrillation, and sever arrhythmia), malignant tumor, and systemic disease such as systemic lupus erythematosus. Also excluded were patients with LV systolic dysfunction, defined as LV ejection fraction ≤50%. Written informed consent was obtained from each enrolled patient. This study was approved by the institutional ethics committee at Rui Jin Hospital.

Data collection

Blood samples from HD patients were collected after overnight fasting before the mid-week HD treatment. Biochemical data, including albumin, hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (CRE), Triglycerides, Total Cholesterol, high-density lipoprotein-cholesterol (HDL-

Table 3. Correlations between serum free and total PCS and selected clinical and biochemical characteristics

	Free PCS		Total PCS	
	R	P	R	P
Age	-0.061	0.434	-0.079	0.317
Sex	0.032	0.680	-0.009	0.905
BMI	0.161	0.510	0.005	0.985
Diabetes mellitus	-0.060	0.439	-0.059	0.445
Hypertension	0.012	0.878	0.026	0.739
Laboratory data				
Albumin	-0.049	0.528	-0.131	0.092
Hemoglobin	0.008	0.915	0.106	0.170
ALT	-0.025	0.745	0.026	0.742
AST	-0.065	0.404	0.001	0.997
BUN	-0.001	0.985	0.036	0.648
CRE	0.048	0.531	-0.072	0.355
Triglycerides	-0.091	0.242	-0.048	0.543
TC	0.029	0.707	0.043	0.579
HDL-C	0.096	0.220	0.119	0.128
LDL-C	0.010	0.895	0.026	0.744
CRP	-0.015	0.858	-0.090	0.274
Calcium	0.027	0.701	0.042	0.564
Phosphate	0.030	0.704	0.045	0.604
BNP	0.058	0.463	0.144	0.096
AAO	0.012	0.906	-0.090	0.394
LA	-0.089	0.397	0.047	0.656
Diameter; diastolic	0.118	0.258	0.115	0.276
Diameter; systolic	0.086	0.414	0.119	0.257
IVS	-0.049	0.638	-0.014	0.895
LVPW	-0.063	0.550	-0.037	0.726
LVEF	-0.078	0.461	-0.124	0.246
E/E'	0.345	<0.001	0.101	0.074

PCS, p-cresyl sulfate; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; CRP,C-reactive protein; BNP, B-type natriuretic peptide; AAO, Aorta ascendens; LA, left atrial; IVS, interventricular septum; LVPW, left ventricular posterior wall; LVEF, left ventricular ejection fraction; E/E', E-wave/E'-wave ratio.

Table 4. Logistic regression analyses for left ventricular (LV) diastolic dysfunction

Variable	OR (95% CI)	*P* value
Free PCS	0.982-2.739	0.017
Total PCS	0.963-1.029	0.442

CI, confidence interval.

C), low-density lipoprotein-cholesterol (LDL-C), calcium, phosphate, C-reactive protein (CRP), and B-type natriuretic peptide (BNP) were measured.

After blood sampling, echocardiography was performed in each patient using a commercially available system (Vivid 7, GE Healthcare, Wisconsin, U.S.) with a 1.5- to 4.0- MHz transducer. Parameters of the cardiac cycle were recorded by M-mode echocardiography, and aorta ascendens (AAO), left atrial (LA), LVdiastolic diameter, LV systolic diameter, interventricular septum (IVS), LV posterior wall (LVPW), and LV ejection fraction (LVEF) were calculated.The mitral and pulmonary venous flow were measured using pulsed Doppler echocardiogram including peak flow velocity of early diastole (E), peak flow velocity of atrial contraction (A). The mitral annular peak early myocardial tissue velocity (E') and peak late (or atrial) myocardial tissue velocity (A') were measured using tissue-doppler imaging by placing a sample volume on the septal from the apical 4-chamber view. E/E' ratio was calculated.

PCS assay

PCS concentrations in serum samples were determined using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). In brief, an aliquot of 80 μl of the serum sample was added to 20 μl HCl (6 mol/L) to deproteinate the sample; then, PCS was extracted using 1 ml isopropyl ether. Next, 40 μl NaOH (0.05 mol/L) and the internal standard (IS), methanol, were added to the sample and the solution was evaporated under an N_2 stream until dry. The dried extracts were resolved with 100 μl 50% aqueous methanol and 10 μl supernatant was injected for LC-MS/MS analysis. To calculate the concentration of PCS, a calibration curve (peak height ratio PCS/IS versus PCS concentration) was made from standard solutions (30 mg/L).

Animal preparation

All experiments were performed in C57/BL mice, which were obtained from Shanghai Laboratory Animal Center of the Chinese Academy of Science (SLACCAS, Shanghai, China) at the age of 7 wk. The mice were housed in polycarbonate cages and kept in a temperature controlled room at 21°C to 23°C and 50%±15% relative humidity throughout the experiment. One week later, they were subjected to uninephrectomy (UNX) according to the method we previous reported [26] and then

Table 5. Blood pressure and heart rate in control-UNX and PCS-UNX mice before and after 20 weeks of PCS treatment

	Control-UNX (n=6)	PCS-UNX (n=6)	*P* value
8 weeks			
Mean systolic pressure (mmHg)	95±5	97±6	0.73
Mean diastolic pressure (mmHg)	49±4	50±3	0.82
Heart rate	703±19	709±25	0.71
27 weeks			
Mean systolic pressure (mmHg)	93±6	101±6	0.23
Mean diastolic pressure (mmHg)	55±3	66±8	0.22
Heart rate	727±22	717±38	0.74

All data were given mean ± SD. UNX, unilateral nephrectomy.

Table 6. Serum biochemistry in control-UNX and PCS-UNX mice after 20 weeks of PCS treatment

Serum biochemistry	Control-UNX (n=6)	PCS-UNX (n=6)	*P* value
Albumin (g/L)	3.62±0.48	3.64±0.60	0.95
ALT (U/L)	85.5±20.3	124.1±34.2	0.30
AST (U/L)	137.1±22.9	153.1±20.8	0.65
BUN (mmol/L)	9.5±0.7	11.5±0.8	0.007
CRE (μmol/L)	2.27±0.23	2.54±0.21	0.48
Cholesterol (mmol/L)	2.60±0.65	2.98±0.43	0.82
HDL-L (mmol/L)	2.01±0.63	1.21±0.29	0.15
LDL-L (mmol/L)	2.10±0.49	2.37±0.50	0.74
Triglycerides (mmol/L)	1.68±0.45	1.48±0.35	0.44
CK (U/L)	366.9±55.4	401.4±96.7	0.52
CK-MB (U/L)	460.7±88.6	550.3±96.1	0.39
LDH (U/L)	392.1±55.8	450.2±71.2	0.31

Male C57/BL mice were administrated with PCS or control double distilled water from the age of 8 weeks through 28 weeks. At sacrifice, serum was prepared and evaluated for levels of biochemistry. All data were given mean ± SD. UNX, unilateral nephrectomy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRE, creatinine; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; CK, creatine kinase; CK-MB, creatine kinase isoenzyme MB; LDH, lactate dehydrogenase.

divided into PCS-treated (PCS-UNX) and control (control-UNX) groups (n = 6 in each group). The PCS-treated mice were fed with 0.5 mg/ml PCS dissolved in double distilled water for 20 weeks. In the contrast, the control mice received daily double distilled water. Blood pressure (BP), heart rate, and serum biochemistry at the end the experiment were measured to calculate the characters in PCS-treated and control groups. All the procedures described were approved by the Animal Use and Care Committee of Shanghai Jiao Tong University School of Medicine (approval number: SYKX-2008-0050).

BP measurement

Mean arterial blood pressure (BP) and heart rate (HR) were measured in awake, resting mice with a tail-cuff system (BP 2000, Visitech Systems, Apex, NC, USA) that uses a photoelectric sensor to detect the tail blood flow [27]. Mice were first familiarized to the procedure with repeated BP measurements for four consecutive days as described previously [28]. Reported BP was the mean of 10 measurements per mouse on the fifth day after surgery. BP measurement accuracy was assured via regular calibration of the pressure transducer. The average intra-individual variability in BP measurements was 6.1%, and this is within normal limits of variation.

Animal serumbiochemistry

Blood samples were taken from each mouse group after anesthesia via retro-orbital venous plexus puncture and blood was collected in heparinized microtubes. Serum was then separated by centrifugation at 1,000 × g for 10 min at 4°C. Serum biochemistries (albumin, ALT, AST, urea, creatinine, cholesterol, HDL-C, LDL-C, triglycerides, creatine kinase [CK], creatine kinase isoenzyme MB-[CK-MB], and lactate dehydrogenase [LDH] were measured with a Chemix-180 autoanalyzer (Sysmex, Koube, Japan).

Animal echocardiography

To determine whether PCS treatment had any effect on mice's cardiac function, we performed M-mode echocardiography with the small animal echocardiography analysis system of Vevo 770 (Visualsonics Inc., Toronto, Canada). Briefly, mice were lightly anesthetized by inhalation of 1.5% isoflurane, and the upper sternal and subxiphoid areas were shaved and then moistened for better acoustic coupling. Electrode was attached to mice limbs and parameters of the cardiac cycle were recorded by M-mode echocardiography with a 30 MHz transducer. LV systolic and diastolic diameters, LV systolic

Table 7. Parameters of cardiac function in control-UNX and PCS-UNX groups

	Control-UNX (n=6)	PCS-UNX (n=6)	*P* value
Diameter; systolic (mm)	1.62±0.73	1.99±0.57	0.310
Diameter; diastolic (mm)	3.31±0.48	3.42±0.92	0.635
Volume; systolic (uL)	10.21±10.76	14.46±8.74	0.432
Volume; diastolic (uL)	45.72±16.47	49.08±13.06	0.679
Stroke Volume (uL)	35.51±7.24	34.61±7.54	0.829
LV Mass	81.44±14.05	83.36±18.03	0.836
Ejection Fraction (%)	81.93±14.50	72.44±12.79	0.221
Fraction Shortening (%)	52.60±14.31	42.56±12.70	0.192
Cardiac Output (mL/min)	15.02±4.36	14.16±3.39	0.685
Heart Rate (BMP)	416.85±51.60	408.66±37.46	0.736
IVS; diastolic (mm)	0.9077±0.1971	0.9003±0.1303	0.934
IVS; systolic (mm)	1.4838±0.2651	1.4078±0.2195	0.570
LVPW; diastolic (mm)	0.9108±0.1754	0.8714±0.1322	0.640
LVPW; systolic (mm)	1.4976±0.2526	1.3542±0.2350	0.298
E/E'	42.461±2.681	81.260±4.2731	0.003

All data were given mean ± SD. IVS, interventricular septum; LVPW, left ventricular posterior wall; E/E', E-wave/E'-wave ratio.

Table 8. Left ventricular haemodynamic function

	Control-UNX (n=6)	PCS-UNX (n=6)
HR (bpm)	297±41	315±46
ESP (mmHg)	115.3±15.2	110.8±13.5
EDP (mmHg)	12.2±3.7	18.5±4.1[a]
ESPVR slope (mmHg/μL)	8.4±1.3	8.8±1.1
Diastolic stiffness	0.016±0.003	0.137±0.031[a]

All data were given mean ± SD. [a]*P*< 0.05 for difference from controls.HR, heart rate; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESPVR, end-systolic pressurevolume relationship.

and diastolic volumes, stroke volume, LF mass, ejection fraction, fraction shortening, cardiac output, IVS diameters, and LVPW diameters were calculated. Furthermore, the peak E velocity was determined using blood flow Doppler and the mitral annular diastolic velocity (E') was measured using Doppler tissue imaging, E/E' ratio was then calculated.

Haemodynamic measurements

The animals were anaesthetized and a 1.4 F micro-conductance pressure catheter (ARIA SPR-719; Millar-Instruments, Houston, TX, USA) was positioned in the LV through right external carotid artery. Under stable hemodynamic conditions, the baseline indices were initially measured. A silk thread was then placed under the inferior vena cava just above the diaphragm to change the LV preload and the pressure-volume loop was drawn. Systolic function was quantified by LV end-systolic pressure (ESP) and the slope of the ESP-volume relationship (ESPVR). Diastolic function was measured by LV end-diastolic pressure (LVEDP) and end diastolic pressure-volume relationship (EDPVR), which is an indicator of LV stiffness, as determined from an exponential fit to the end-diastolic pressure-volume points.

Measurement of inflammatory cytokines

Serum samples were collected from each mice, and mice interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF-a), and monocyte chemotactic protein (MCP)-1 ELISA kits (R&D, Minnesota, USA) were used to measure inflammatory cytokine levels according to the instructions of the manufacturer.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD). Categorical variables are described by counts and percentages. The total group of patients was dichotomized based on the serum level of PCS (>0.85 μg/ml or ≤0.85 μg/ml; median as cutoff) for descriptive and analytical purpose. Student's t-test and chi-square test were used to assess statistically significant differences among different groups in clinical and animal studies. To examine the correlations between PCS and other variables, Spearman and Pearson test were used. Logistic regression was used for multivariate analysis of independent variables. A value of *P*< 0.05 was interpreted as statistically significant. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical findings

Table 1 depicts the demographic, clinical and biochemical characteristics of 170 analyzed

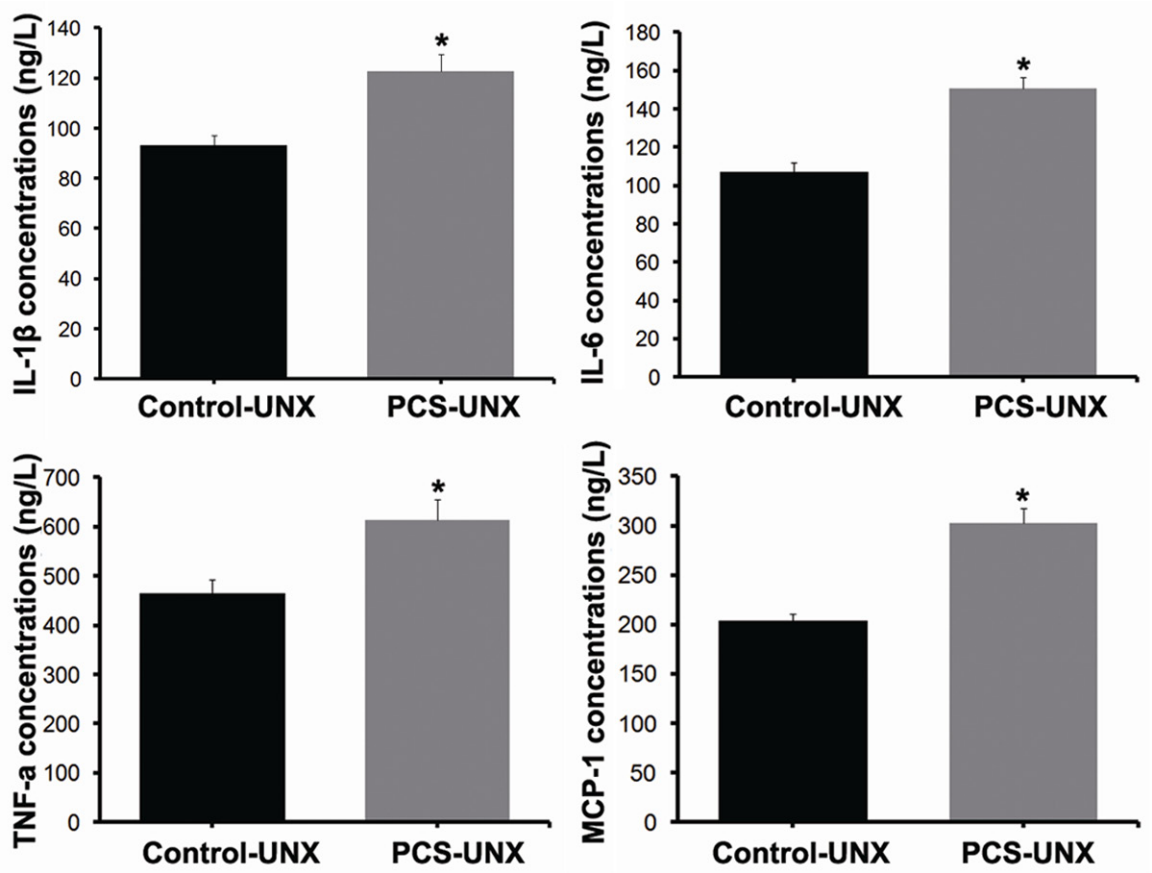

Figure 1. PCS increased inflammatory factors expression in experimental mice. Data representing serum inflammatory factors levels, as measured by ELISA, showing significantly increased IL-1β, IL-6, MCP-1, and TNF-ain PCS-treated mice compared with controls. Data are expressed as mean + SD. $^{*}P< 0.05$ vs. control.

patients. The prevalence of LV diastolic dysfunction was significantly lower in the lower PCS (≤0.85 mg/L) group than in the higher PCS group (>0.85 mg/L), as a significantly increased E/E' was present in the higher PCS group. Other echocardiography parameters such as AAO, LA, LV systolic and diastolic diameters, IVS, LVPW, and LVEF were not significantly different between two groups (**Table 2**). The univariate correlations between serum total and free PCS levels and the clinical and biochemical characteristics of the study population are shown in **Table 3**. A positive correlation was observed between the free PCS serum levels and E/E', and total PCS levels were positively and significantly correlated with free PCS levels. Further multivariate linear regression analyses indicated that E/E' was independently associated with free PCS levels ($P = 0.017$), whereas no independent variable associated with total PCS levels. The results of univariate and multivariate logistic regression analyses to predict the presence of LV diastolic dysfunction are list in **Table 4**.

Effect of PCS on indices of BP andserum biochemistry

After 20 weeks of PCS treatment, the mean BP in experimental mice did not differ in PCS-UNX and control-UNX groups (**Table 5**). Serum biochemistry including albumin, ALT, AST, total cholesterol, HDL-C, LDL-C, triglycerides, CK, CK-MB, and LDH did not differ in two groups (**Table 6**).

PCS promote left ventricular diastolic dysfunction

Echocardiographic analysis showed E/E' ratio was significantly increased in PCS-treated mice compared with controls (0.49±0.27 for PCS and 1.22±0.47 for controls, $P< 0.05$; n = 6 for both groups), while there was no difference of LVEF in different groups, indicating that PCS

could promote left ventricular diastolic dysfunction (**Table 7**). **Table 8** shows the results of the hemodynamic study by cardiac catheterization 20 weeks after PCS treatment. Load-independent parameters measured by pressure-volume loop analysis revealed that diastolic function was worse in PCS-treated mice, with an increase in LVEDP and diastolic stiffness, as compared to control. None of the systolic function related parameters, LVESP and ESPVR slope, were significantly different between PCS and control groups.

Inflammatory factor levels

Relative to controls, PCS treatment significantly increased IL-1β, IL-6, MCP-1, and TNF-a production in the experimental mice. These results indicated that PCS may promote left ventricular diastolic dysfunction via its pro-inflammatory activity (**Figure 1**).

Discussion

Patients with CKD are at high risk of CVD [1, 29]. The main events observed in CKD patients include HF, atherosclerosis, arterial stiffness, vascular calcifications, et al [11, 30]. Though acceleration of coronary artery disease (CAD) and LVH are major cardiac problems observed in CKD patients and thought to be contributory to CKD-related HF [31], the exact causes are still poorly defined. For a long time, it was unclear whether or not this observation is fully explained by the high prevalence of classical cardiovascular risk factors in uremia (e. g. diabetes, hypertension, and the presence of renal dysfunction per se lead to activation of the renin-angiotensin system, and dyslipidemia) [32-34].

Recently, more and more studies have showed the involvement of uremic toxins, especially protein-bound toxins, in uremic HF, partly due to the difficulty of removing these toxins using traditional dialysis therapy [35, 36]. In fact, one important uremic toxin, indoxyl sulfate (IS), has been demonstrated to have pro-fibrotic and pro-hypertrophic effects on cardiac myocyte *in vitro* [37]. The clinical relation of serum IS levels and left ventricular diastolic dysfunction was then demonstrated by Sato et al [38]. Using a subtotal-nephrectomized (5/6-STNx) rat model, IS treatment had development of diastolic dysfunction determined by Doppler echocardiography [39]. In the present study, we explored the causal relationship between diastolic dysfunction and PCS accumulation clinically and in animal models, which is helpful for better understanding the toxicity of uremic toxins on HF.

It is known that the prognosis of patients with diastolic HF is as threatening as that of patients with systolic HF [7]. Furthermore, greater CKD-associated mortality occurs in patients with diastolic HF than in those with systolic HF [8]. Pro-inflammatory cytokine activation is a critical mechanism in the progression of both HF [40] and CKD [41]. Markers of inflammation are predictive of all-cause mortality and cardiac death in patients with CKD [42]. Pro-inflammatory cytokines such as IL-1β, TNF-a, and IL-6 stimulate cardiac remodeling and heart failure through activatingmitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-kB) pathways [43-45]. In the animal study, treatment of mice with PCS increased the expression of IL-1β, IL-6, MCP-1, and TNF-a, which may partly explain the underlying mechanism of the cardiac toxicity of PCS.

Conclusion

Our data show that PCS is associated with the progression of diastolic dysfunction. This might beattributed to its pro-inflammatory activity.

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Disclosure of conflict of interest

None.

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