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Partial nephrectomy as a model for uremic cardiomyopathy in the mouse

David J. Kennedy¹, Jihad Elkareh¹, Amjad Shidyak¹, Anna P. Shapiro¹, Sleiman Smaili¹, Krishna Mutgi¹, Shalini Gupta¹, Jiang Tian¹, Eric Morgan¹, Samer Khouri¹, Christopher J. Cooper¹, Sankaridrug M. Periyasamy¹, Zijian Xie¹, Deepak Malhotra¹, Olga V. Fedorova², Alexei Y. Bagrov², and Joseph I. Shapiro¹

¹Department of Medicine and Pharmacology, University of Toledo College of Medicine, Toledo, Ohio ²Department of Laboratory of Cardiovascular Science, National Institute on Aging, Baltimore, Maryland

Abstract

Because of the plethora of genetic manipulations available in the mouse, we performed a partial nephrectomy in the mouse and examined whether the phenotypical features of uremic cardiomyopathy described in humans and rats were also present in the murine model. A 5/6 nephrectomy was performed using a combination of electrocautery to decrease renal mass on the left kidney and right surgical nephrectomy. This procedure produced substantial and persistent hypertension as well as increases in circulating concentrations of marinobufagenin. Invasive physiological measurements of cardiac function demonstrated that the 5/6 nephrectomy resulted in impairment of both active and passive left ventricular relaxation at 4 wk whereas tissue Doppler imaging detected changes in diastolic function after 6 wk. Morphologically, hearts demonstrated enlargement and progressive fibrosis, and biochemical measurements demonstrated downregulation of the sarcoplasmic reticulum calcium ATPase as well as increases in collagen-1, fibronectin, and vimentin expression. Our results suggest that partial nephrectomy in the mouse establishes a model of uremic cardiomyopathy which shares phenotypical features with the rat model as well as patients with chronic renal failure.

Keywords

renal failure; TGF- β ; cardiotonic steroids; reactive oxygen species; fibrosis

Cardiac disease is directly responsible for the extremely high morbidity and mortality seen in patients with end-stage renal disease (ESRD) (12). Clinically, this cardiac disease of renal failure, also called uremic cardiomyopathy, is characterized by left ventricular hypertrophy and diastolic dysfunction. On this background, we have previously demonstrated that the cardiotonic steroid marinobufagenin (MBG), signaling through the Na-K-ATPase, is responsible for many of the features of experimental uremic cardiomyopathy induced by partial nephrectomy in the Sprague-Dawley rat (6). Specifically, we have noted that partial nephrectomy in the rat is accompanied by substantial elevations in blood pressure, cardiac hypertrophy, impaired left ventricular relaxation, downregulation of the sarcoplasmic

reticulum ATPase (SERCA) and cardiac fibrosis. Except for the blood pressure elevation, immunization against MBG prevents all of these abnormalities (2,6).

Although the rat is an extremely useful model to study the cardiomyopathy of renal failure (5,6), there are many genetic manipulations which are currently available in the mouse as well as greater ease of making additional genetic manipulations in a murine system. Therefore, we performed the following studies to test the feasibility of studying experimental uremic cardiomyopathy in the mouse.

METHODS

All animal experimentation described in the manuscript was conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* using protocols approved by the University of Toledo Institutional Animal Care and Use Committee. We performed a partial nephrectomy (PNx) in male CD1 mice, weighing between 25 and 27 g. PNx was performed by selective cauterization (Bovie high-temperature fine tip cautery, Aaron Medical, St. Petersburg, FL) of the entire upper and lower poles of the left kidney, leaving a 2-mm intact segment around the hilum (Fig. 1) as described by Gagnon and Duguid (3). This was followed by removal of the right kidney at the same time. Following PNx or sham surgery, conscious blood pressure was monitored weekly using the tail-cuff method (6). In a substudy to address the role of blood pressure, $n = 13$ mice were given antihypertensive therapy consisting of hydralazine (80 mg/l), reserpine (5 mg/l), and hydrochlorothiazide (30 mg/l) added to the drinking water (15).

Some mice were subsequently anesthetized with pentobarbital sodium (50 mg/kg ip) and studied with Doppler imaging at 4 and 6 wk following surgery or instrumented with a Millar 1.4-Fr catheter at either 4, 6, and 8 wk for measurement of ventricular hemodynamics [e.g., τ value, slope of regression line fit to end-diastolic pressure vs. end-diastolic volume generated by inferior vena cava occlusions (EDPVR)] as we have previously reported in the rat (2).

A Sonos 5500 system using a 14-MHz linear transducer (15-6L, Philips Medical Systems, Bothell, WA) was used to perform Doppler imaging studies as described by other workers (7,13,14). Diastolic function was assessed by examination of the early (Ea) and late or atrial (Aa) velocity waves on the tissue Doppler imaging (TDI) studies as well as the early (E) and atrial (A) wave on the flow Doppler studies. For the ventricular catheter studies, diastolic function was assessed by measuring the time constant for isovolumic relaxation (τ) to assess active relaxation and the EDPVR to assess passive relaxation. Higher values of τ and EDPVR imply impaired active and passive relaxation, respectively (2,8,10,18).

Blood was sampled for measurement of plasma MBG concentration ([MBG]), and the animal's heart was removed and studied for weight, histology (trichrome staining and morphometric analysis), and biochemical analysis as we have previously reported in the rat (2,6). Plasma [MBG] was determined following extraction on a C₁₈ column using an immunoassay employing DELPHIA as previously described (6).

Western blot analysis was performed on protein isolated from tissue homogenates as described previously (5).

Data are presented as means $\pm$ SE. Data obtained were first tested for normality. If the data did not pass the normality test, the Tukey test (for multiple groups) or the Mann-Whitney Rank Sum test was used to compare the data. If the data did pass the normality test, parametric comparisons were performed. If more than two groups were compared, one-way analysis of variance was performed before comparison of individual groups with the

unpaired Student's *t*-test with Bonferroni's correction for multiple comparisons (16). Statistical analysis was performed using SPSS software.

RESULTS

Induction of PNx resulted in rapid and sustained increases in blood pressure (Fig. 1A). Plasma [MBG] was noted to be increased about twofold 4, 6, and 8 wk following PNx compared with sham surgery ($P < 0.05$ at each time; see Table 2). For examination of left ventricular function, PNx resulted in increases in systolic function as assessed by ejection fraction, dp/dt , and the slope of the end-systolic pressure-volume relationship (ESPVR) following inferior vena cava constriction (Table 1). Regarding diastolic function, both active relaxation assessed by the time constant for isovolumic relaxation (τ) and passive relaxation assessed by the slope of the EDPVR were noted to be impaired by PNx (representative pressure-volume loops in Fig. 1B, data in Table 1). TDI measurements also noted trends of decreasing Ea and Aa velocities with time, but only the Aa velocity was significantly reduced at 6 wk. Marked ventricular hypertrophy was noted at 4, 6, and 8 wk following PNx as demonstrated by increases in the heart weight-to-body weight ratio (see Table 3). Interestingly, end-diastolic and end-systolic volumes were noted to be markedly reduced at 4 wk after PNx, using the Millar catheter system, whereas these measurements appeared to increase at 6 and 8 wk. We suspect that this "normalization" of these volumes may actually represent a conversion from concentric to eccentric hypertrophy and a worsening of the cardiomyopathy (9), but additional studies with longer time periods of observation will be necessary to confirm this.

Time control hearts did not demonstrate any increase in fibrosis by morphology or any cardiac hypertrophy or increase in myocyte cross-sectional area during the course of the study. Similarly, protein expression data were similar at the three time points studied. In contrast, PNx induced activation of sarcoma viral oncogene homolog (Src) and ERK at 4, 6, and 8 wk, signal transduction steps which we have previously shown to be important in signaling through the Na-K-ATPase (6). Decreases in both α_1 - and α_2 -Na-K-ATPase isoform expression were also noted as well as decreases in SERCA2a (Table 2). Trichrome staining of histological sections demonstrated increases in myocyte cross-sectional area as well as increased fibrosis (representative images in Fig. 2, quantitative data in Table 3). Increases in procollagen and vimentin expression were noted as well (Table 2).

Interestingly, the cardiac changes induced by PNx were not measurably altered by reductions in blood pressure achieved by the antihypertensive agents. The addition of these agents resulted in substantial reductions in systolic BP at 4 wk (126 ± 2 mmHg, $P < 0.01$ vs. PNx alone) but did not substantially affect the cardiac alterations induced as assessed by the heart weight-to-body weight ratio ($4.6 \pm 0.1 \times 10^{-3}$, $P =$ not significant vs. PNx alone) or the amount of fibrosis on trichrome staining ($14 \pm 3\%$, $P =$ not significant vs. PNx alone) assessed after 4 wk.

DISCUSSION

Our laboratory has been interested in the role that cardiotonic steroids play in the pathogenesis of uremic cardiomyopathy. Specifically, we have noted that a number of biochemical, physiological, and morphological changes occur with PNx in the rat and that MBG, signaling through the Na-K-ATPase, appears to be important in this process. However, our studies are still inconclusive without the capacity to examine the actual signaling process in vivo. We performed the studies in this report in the mouse to establish that the same phenotypical changes followed partial nephrectomy so that we might use a murine model for further mechanistic studies.

Our data demonstrated that the CD1 mouse responded quite similarly to PNx as we previously reported with Sprague-Dawley rats (2,5,6). Specifically, we were able to demonstrate sustained increases in conscious blood pressure and plasma [MBG], cardiac hypertrophy, evidence for impaired active and passive relaxation and progressive cardiac fibrosis following PNx in the male CD1 mouse. Although TDI and flow Doppler assessments were not as sensitive as the Millar pressure catheter to changes in left ventricular relaxation, the results of the TDI studies further demonstrated left ventricular diastolic dysfunction consistent with uremic cardiomyopathy. This apparent insensitivity of the echocardiographic measurements was likely a consequence of suboptimal performance rather than an inherent limitation of this technique. In general, the physiological, morphological, and biochemical alterations with PNx were similar to that which we have reported in the male Sprague-Dawley rat, where suprarenal aortic constriction was used as a control for hypertensive changes (5). Interestingly, the CD1 mouse also develops severe hypertension with suprarenal aortic constriction but very little cardiac hypertrophy or fibrosis over a similar time course to what we employed in our current study (4,17). Moreover, in the current study, “triple” antihypertensive therapy (15) substantially lowered blood pressure toward normal, but it did not attenuate the cardiac hypertrophy or fibrosis induced by PNx. Thus the cardiac changes seen in this model appear to be more dependent on the uremic milieu rather than elevations in blood pressure alone. This report demonstrates the feasibility of pursuing detailed studies of the molecular mechanisms utilizing knockout and “knockin” models that have already been established in the mouse. For example, the role of caveolin-1 can be studied using the caveolin-1 knockout mouse developed by Razani and colleagues (11). Alternatively, the role of the Na-K-ATPase as a receptor in this process can be studied using α_1 -Na-K-ATPase knockout (heterozygote), α_2 -resistant, and α_1 -sensitized mice created by Lingrell and coworkers (1,19). While these future studies are both interesting and potentially important, we believe that this brief technical report demonstrates the feasibility of using the mouse model for these studies as well as other investigations into the pathogenesis of uremic cardiomyopathy.

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Fig. 1.

Partial nephrectomy (PnX) produces hemodynamic changes consistent with fibrosis. *A*: blood pressure responses to PNx compared with control over the period of study. Values are means $\pm$ SE. $**P < 0.01$ vs. control. *B*: representative pressure-volume loops in control and PNx animals. *C*: representative Doppler imaging tracings [tissue Doppler imaging (TDI; *top*) and flow Doppler (mitral; *bottom*)] in control and PNx animals. Early (Ea), atrial (Aa), systolic (S), early (E), and atrial (A) waves annotated on control tracings.

Fig. 2.

Representative Masson's trichrome sections of left ventricular cardiac tissue in control animals as well as 4, 6, and 8 wk after partial nephrectomy (PNx).

Table 1

Effects of PNx on various functional parameters

Measurement	Sham	PNx		
		4 wk	6 wk	8 wk
Doppler imaging	<i>n</i> = 13	<i>n</i> = 7	<i>n</i> = 7	ND
Ea wave (TDI endocardium), m/s	0.042±0.002	0.041±0.004	0.039±0.003	
Aa wave (TDI endocardium), m/s	0.039±0.003	0.032±0.006	0.030±0.001 *	
S wave (TDI endocardium), m/s	0.038±0.002	0.040±0.002	0.045±0.004	
E wave (Mitral inflow), m/s	1.11±0.07	1.08±0.06	1.06±0.09	
A wave (Mitral inflow), m/s	0.69±0.05	0.64±0.02	0.61±0.07	
Left ventricular catheter	<i>n</i> = 28	<i>n</i> = 12	<i>n</i> = 16	<i>n</i> = 14
End-systolic volume, μ l	6.7±0.3	3.7±0.2 [†]	4.1±0.3 [†]	4.2±0.3 [†]
End-diastolic volume, μ l	18.5±0.3	12.9±1.5 [†]	16.2±1.2 *	17.6±0.8
Ejection fraction, %	73±1	80±1 [†]	82±1 [†]	82±2 [†]
τ , ms	7.5±0.3	10.5±0.4 [†]	11.0±0.8 [†]	10.5±0.4 [†]
EDPVR, (mmHg/ μ l) $\times 10^4$	310±38	563±75 *	765±64 [†]	677±76 [†]

Values are means $\pm$ SE. TDI, tissue doppler imaging; PNx, partial nephrectomy; ND, not done; τ , time constant for isovolumic relaxation; EDPVR, end-diastolic pressure vs. end-diastolic volume generated by inferior vena cava occlusions. Studies were performed 4 and 6 wk, and 4, 6, and 8 wk after sham or PNx surgery for the echocardiography and ventricular catheterization, respectively. Sham surgery mice were found to have virtually identical measurements at the different time points for both Doppler imaging [4 (*n* = 6) and 6 wk (*n* = 7)] and ventricular catheterization studies [4 (*n* = 12), 6 (*n* = 8), and 8 wk (*n* = 8)]. To simplify the table, these sham surgery data have been combined.

* $P < 0.05$ vs. sham.

[†] $P < 0.01$ vs. sham.

Table 2

Effect of PNx on various biochemical measurements

Measurement	Sham (<i>n</i> = 28)	PNx		
		4 wk (<i>n</i> = 12)	6 wk (<i>n</i> = 8)	8 wk (<i>n</i> = 8)
Plasma [MBG], pmol/l	143±22	304±55 [*]	331±79 [*]	343±69 [*]
Plasma malondialdehyde μM	2.6±0.1	2.8±0.1	2.8±0.2	3.2±0.1 [*]
Western blot analyses	<i>n</i> = 18	<i>n</i> = 6	<i>n</i> = 6	<i>n</i> = 6
pSrc/Src, fraction of control	1.0±0.04	1.35±0.08 [*]	1.22±0.10	1.29±0.07 [*]
pERK/ERK, fraction of control	1.0±0.02	2.18±0.04 [†]	2.74±0.08 [†]	1.90±0.04 [†]
α ₁ -Na-K-ATPase, fraction of control	1.0±0.04	0.72±0.05 [*]	0.71±0.05 [*]	0.60±0.06 [†]
α ₂ -Na-K-ATPase, fraction of control	1.0±0.07	0.73±0.04 [*]	0.74±0.05 [*]	0.46±0.04 [†]
SERCA2a, fraction of control	1.0±0.06	0.74±0.04 [*]	0.64±0.04 [†]	0.52±0.04 [†]

Values are means ± SE. MBG, marinobufagonine concentration; SERCA, sarcoplasmic reticulum ATPase. Studies were performed 4 and 6 wk, and 4, 6, and 8 wk after sham or PNx surgery. Sham surgery mice were found to have virtually identical measurements at the different time points, and to simplify the table, the sham surgery data have been combined.

^{*} *P* < 0.05 vs. sham.

[†] *P* < 0.01 vs. sham.

Table 3

Effect of PNx on various morphological measurements

Measurement	Sham (<i>n</i> = 28)	PNx		
		4 wk (<i>n</i> = 12)	6 wk (<i>n</i> = 8)	8 wk (<i>n</i> = 8)
Heart weight/body weight $\times 10^3$	3.9 $\pm$ 0.1	4.7 $\pm$ 0.2 [†]	4.7 $\pm$ 0.1 [†]	4.8 $\pm$ 0.2 [†]
Myocyte cross-sectional area, fraction of control	1.00 $\pm$ 0.10	1.24 $\pm$ 0.11	1.38 $\pm$ 0.07*	1.45 $\pm$ 0.10 [†]
Cardiac fibrosis, fraction of control area	1 $\pm$ 1	12 $\pm$ 4*	16 $\pm$ 5 [†]	49 $\pm$ 10 [†]

Values are means $\pm$ SE. Studies were performed 4 and 6 wk, and 4, 6, and 8 wk after sham or PNx surgery. Sham surgery mice were found to have virtually identical measurements at the different time points, and to simplify the table, the sham surgery data have been combined.

* $P < 0.05$ vs. sham.

[†] $P < 0.01$ vs. sham.