Eprosartan reduces cardiac hypertrophy, protects heart and kidney, and prevents early mortality in severely hypertensive stroke-prone rats

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Abstract

\textbf{Objective:} Eprosartan is a selective angiotensin II type I receptor antagonist approved for the treatment of hypertension. In the present studies, eprosartan’s ability to provide end-organ protection was evaluated in a model of cardiomyopathy and renal failure in stroke-prone rats (SP). \textbf{Methods:} SP were fed a high fat (24.5\% in food) and high salt (1\% in water) diet (SFD). Eprosartan (60 mg/kg/day) or vehicle (saline control) \textit{(n}5\textit{/group}) was administered by intraperitoneally-implanted minipumps to these SP on the SFD for 12 weeks. Normal diet fed SP and WKY rats \textit{(n}5\textit{/group}) were also included for comparison (i.e. served as normal controls). Mortality, hemodynamics, and both renal and cardiac function and histopathology were monitored in all treatment groups. \textbf{Results:} Eprosartan decreased the severely elevated arterial pressure \textit{(2}12\%\textit{; }P\textit{, }0.05\textit{)} produced by SFD but did not affect heart rate. Vehicle-treated SP-SFD control rats exhibited significant weight loss \textit{(2}13\%\textit{; }P\textit{, }0.05\textit{)} and marked mortality (50\% by week 6 and 95\% by week 9; \textit{P\text{, }0.01}). Eprosartan-treated SP-SFD rats maintained normal weight, and exhibited zero mortality at week 12 and beyond. Eprosartan prevented the increased urinary protein excretion \textit{(P\text{, }0.05)} that was observed in vehicle-treated SP-SFD rats. Echocardiographic (i.e. 2-D guided M-mode) evaluation indicated that SP-SFD vehicle control rats exhibited increased septal \textit{(2}22.2\%\textit{)} and posterior left ventricular wall \textit{(1}30.0\%\textit{)} thickness, and decreased left ventricular chamber diameter \textit{(2}15.9\%\textit{)}, chamber volume \textit{(2}32.7\%\textit{)}, stroke volume \textit{(2}48.7\%\textit{)} and ejection fraction \textit{(2}22.3\%\textit{)}, and a remarkable decrease in cardiac output \textit{(2}59.3\%\textit{)} compared to controls \textit{(all }P\text{, }0.05\textit{)}. These same parameters in eprosartan-treated SP-SFD rats were normal and differed markedly and consistently from vehicle-treated SP-SFD rats (i.e. treatment prevented pathology; all \textit{P\text{, }0.05}). Cardiac-gated MRI data confirmed the ability of eprosartan to prevent cardiac pathology/remodeling \textit{(P\text{, }0.05)}.

\textbf{Conclusion:} These data demonstrate that eprosartan, at a clinically relevant dose, provides significant end-organ protection in the severely hypertensive stroke-prone rat. It preserves cardiac and renal structural integrity, reduces cardiac hypertrophy and indices of heart failure, maintains normal function of the heart and kidneys, and eliminates premature mortality due to hypertension-induced end-organ failure.

Keywords: Angiotensin; Cardiomyopathy; Heart failure; Hypertension; Ventricular function

1. Introduction

The renin–angiotensin system plays a central role not only in the etiology of hypertension but also in the pathophysiology of several cardiovascular and renal diseases. Angiotensin II (AII), primarily via actions at the AII type 1 receptor, directly causes cellular phenotypic

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changes and cell growth, regulates the gene expression of vasoactive hormones, growth factors, extracellular matrix components and cytokines, and activates multiple intracellular signaling cascades in cardiac myocytes and fibroblasts, vascular endothelial and smooth muscle cells, and renal mesangial cells. These actions participate in the pathophysiology of cardiac hypertrophy and remodeling, heart failure, vascular thickening, arteriosclerosis, and glomerulosclerosis and renal failure [1–3]. Interfering with the synthesis of AII or blocking the type 1 receptor can reduce ventricular hypertrophy, renal function/damage and/or improve outcome in animal models of disease and in man [4–18].

Eprosartan is a potent ($K_i$ 1.4 nM) and very selective (i.e. $K_i$ at type 2 receptor , 10,000 nM) AII type 1 receptor antagonist that inhibits AII-induced vascular contraction in a competitive manner [1,2]. Its unique sympathoinhibitory activity has been demonstrated as compared to other angiotensin II receptor antagonists [19]. Therefore, eprosartan can inhibit both the direct effects of AII as well as the indirect effects that are mediated by enhanced sympathetic neurotransmission, providing a special opportunity in the treatment of cardiovascular disease [1,2,4,19]. The administration of eprosartan in experimental renal failure reduced the blood pressure response to exogenous AII challenge, normalized blood pressure, limited glomerulosclerosis and reduced proteinuria, suggesting its potential utility in the treatment of progressive renal disease [20]. In addition, administration of eprosartan to rats in a volume overload model of heart failure resulted in a progressive increase and ultimate recovery in urinary sodium excretion and blocked the development of dilated cardiomyopathy (i.e. was more efficacious than the angiotensin-converting enzyme inhibitor enalapril) [21].

No work has been conducted to determine the capacity of AII blockade in protecting the heart and kidney from damage and in preserving cardiac and renal function in a model of severe, chronic hypertension. Cardiac remodeling, including left ventricular (LV) hypertrophy, is recognized as an early prognostic marker of cardiovascular disease [22]. Independent of hemodynamic complications, uremia is associated with structural abnormalities of the heart (which can also be associated with end stage renal disease) [5]. The hypertrophic heart consists mainly of hypertrophy of cardiomyocytes, interstitial fibrosis, and vascular changes (rarefied capillaries, thickened arteriolar walls) [22,23]. We previously developed a model that exhibits such heart and kidney damage in stroke-prone rats (SP) fed a high salt and high fat diet (SFD). This model was used to evaluate the beneficial effects of carvedilol (i.e. a multiaction drug approved for treatment of heart failure) on this end-organ damage [24,25].

In order to explore eprosartan’s capacity for protection in chronic severe hypertension, we have employed this SP-SFD model of diet/severe hypertension-induced accelerated cardiovascular remodeling, renal injury and prema-

ture morbidity/death. The present work was designed to quantify systematically both the functional and structural cardiac and renal changes that occur in this SP model that are relevant to pressure overload-induced end-organ failure and to determine the efficacy of chronic eprosartan administration to maintain end-organ structural integrity and to preserve normal function under these conditions.

2. Methods

2.1. Experimental design: morbidity/mortality

Stroke-prone spontaneously hypertensive male rats (SP), progeny from the strain developed by Okamoto et al. [26–30], were obtained from the National Institutes of Health (Bethesda, MD, USA), and were bred in the Department of Laboratory Animal Science at GlaxoSmithKline (King of Prussia, PA, USA). This investigation conforms with, and animals were housed and cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by US Institutes of Health (NIH Publication No. 85-23, revised 1996). Procedures using laboratory animals were approved by the Institutional Animal Care and Use Committee of GlaxoSmithKline. Young, male SP between 10 and 13 weeks of age were adapted to individual cages and fed powdered NIH-07 diet for 2 weeks prior to treatment assignment. SP were assigned to three similar groups (n 25/group) on the basis of body weight and age. Two groups received a diet of 1% NaCl as drinking water and chow supplemented with 24.5% fat (SFD) and received two intraperitoneal implants of Alzet® osmotic pumps (Model 2ML4; Alza, Palo Alto, CA, USA). Pumps were replaced every 28 days throughout the study under aseptic surgical conditions. One of these group’s pumps contained eprosartan (delivered 60 mg/kg/day) (SP-SFD eprosartan group) and the other group’s pumps contained the vehicle, isotonic saline ((SP-SFD control group). Care was taken to reduce the variability of drug dosage administration between animals (i.e. pumps were primed and verified to be effectively operating prior to insertion into animals). The daily eprosartan dose was selected based on previous data in the rat demonstrating its renal protective effects [31]. The remaining SP group received normal water and chow (4.5% fat; NIH-07 diet; sodium5 0.33%; potassium5 0.80%) (SP-normal diet group). WKY rats (n 25) of the same age and weight were fed the normal diet and were also studied for control purposes (WKY-normal diet group). This protocol was similar to that described previously [24–26]. All other groups received 1% NaCl in the drinking water and the NIH-07 diet supplemented with 24.5% fat (salt-fat diet, SFD) throughout the 12-week study. Eprosartan was synthesized at GlaxoSmithKline. Diets were milled and formulated by Zeigler Brothers (Gardners, PA, USA) and were provided ad libitum. In our
previous studies, maintenance of this strain of rats on this high fat diet and 1% NaCl drinking water began to produce signs of neurobehavioral deficits, cardiac hypertrophy, cardiac and renal damage, and significant mortality within 6–8 weeks [24,25]. To avoid unnecessary suffering, moribund animals were euthanized (pentobarbital overdose) when daily observations indicated a failure to thrive as described previously (i.e. under these conditions, SP-SFD rats die within 2–4 days of exhibiting initial symptoms of morbidity) [24,25]. When a SP-SFD rat exhibited these symptoms, an animal from each treatment group was randomly selected and tissues were harvested from these and the moribund rat as outlined below under Heart and Kidney Histopathology.

2.2. Body weight, blood pressure and heart rate

Body weight (g), systolic blood pressure (mmHg) and heart rate in beats/min (bpm) were measured at 3-week intervals throughout the study. The systolic blood pressure and heart rate were measured using an automated tail cuff method (ITTC Life Science model 179, Woodland Hills, CA, USA).

2.3. Renal urinary protein excretion

Rats were placed in metabolism cages and 24-h urine samples were collected at weeks 5–7 as described previously [31]. Following collection, urine was stored at 220°C prior to assay. Urinary protein was determined using sulfosalicylic acid [32] and the 24-h urinary protein excretion calculated.

2.4. Echocardiographic measures of LV dimensions and cardiac performance

Transthoracic echocardiograms were obtained 6–8 weeks after initiation of treatment. Anesthesia was induced in a chamber using 5% isoflurane, and maintained with 1–2% isoflurane delivered in 100% oxygen via nose cone. The ventral thorax was shaved and images were obtained using a commercially available ultrasound unit (ATL HDI5000CV with a 5–12 MHz linear array transducer). Echocardiographic methodology was similar to that as previously described and validated [33,34]. Briefly, two-dimensional guided M-mode echocardiograms of the LV obtained at the mid papillary muscle level were recorded and subsequently analyzed. Using the leading edge method, the thickness of the septal and posterior walls, and LV chamber diameter was measured in systole and diastole. End diastolic and end systolic volumes were calculated as previously described [33,34], and were used to estimate stroke volume, percent ejection fraction and cardiac output.

2.5. Cardiac MRI determinations

Cardiac MRI determinations were also made 6–8 weeks after initiation of treatment on a small subset of animals (n 4–5 per group) in order to corroborate those measures obtained using ultrasound. An EKG signal was continually monitored to allow for cardiac (systolic and diastolic) gated data collection. All animals were continually maintained on a mixture of 1–2% isoflurane and 0.8 1/min of oxygen. MR imaging was performed using a home-built birdcage resonator on a 4.7T/40 cm Bruker ABX spectrometer equipped with a gradient coil insert. Data were collected as follows: SE: TR / TE 54600 / 13.1 ms; 256 3 4 cm; slice thickness 5 1 mm; two signal averages. Total imaging time was approximately 15 min. Following imaging, the animals were allowed to recover from anesthesia under supervision. All MR images were transferred to an SGI Unix workstation, and were evaluated/compared with the software package ANALYZE™ (CN Software, UK). Both two-dimensional (2D) and three-dimensional (3D) images were evaluated for animals in each group. Analysis of LV wall thickness was determined at several points and averaged for individual slices and then averaged for a series of three slices through the ventricle for each animal.

2.6. Heart and kidney histopathology

Tissues from a group of eight rats from each of the four experimental groups were prepared for morphological examination. When an animal in the SP-SFD vehicle control group became moribund, matched animal sacrificing was carried out for that animal and for one animal randomly selected from each of the other three groups throughout the study. Immediately upon euthanasia using an overdose of pentobarbital, whole body perfusion with 200 ml phosphate buffered saline (pH 7.2) followed by 300 ml phosphate buffered (pH 7.2) formalin was conducted. Hearts and kidneys were removed and stored in formalin. The fixed organs were trimmed of excess adipose tissue and weighed. Standard central coronal transverse sections of each kidney and three coronal sections of the ventricular portions of the hearts were then processed for quantitative and/or semiquantitative histopathological evaluations. After dehydration and processing into paraffin, sections were cut at 6 mm and stained using hematoxylin and eosin (H&E) as described in detail previously [35]. A multiparametered histopathological evaluation of processed heart and kidney tissue was then performed. Crude scores of renal damage were determined as described in detail previously [24,36,37]. Briefly, standard transverse sections were graded based on overall renal damage as follows: 0 (no damage), 6 (occasional early arterial necrosis), 1 (necrosis of a few arterioles with focal secondary tubular necrosis and regeneration), 2 (moderate arterial necrosis
with regenerative changes present in up to half of the cortical and medullary parenchyma) or 3 (extensive necrosis of arterioles with focal infarcts of glomeruli and regenerative tubular changes in over half of the parenchyma). Hypertensive cardiomyopathy was scored using a modification of procedures previously utilized in order to make values more quantitative [25]. Specifically, overall arterial hypertrophy and hyperplasia were determined as previously described [25]. All foci of degenerative myofibers, myocardial inflammation, and myofiber fibrosis on all three heart sections were counted. The values for these five parameters were totaled and multiplied by 0.1. To the resulting value were added the total number of microthrombi and myocardial infarcts found on the three heart sections to obtain the total cardiomyopathy score. An atrial/conal section of heart was not analyzed because of the paucity of tissue damage found in these areas compared to the LV in this model. As separate parameters, the number of nuclei in a cross-sectional area of the proximal right and left coronary arteries were counted to determine whether hyperplasia had developed in these segments. All histological determinations were made in a completely blind manner (i.e. sections were coded and the analysis of each section was completed without any knowledge of treatment group classification).

2.7. Determination of plasma pro-ANF

Plasma pro-ANF is a well known marker of cardiac hypertrophy and failure in man [38] and its levels in the present experimental model were determined. Blood samples were collected into heparinized tubes at time of euthanasia (i.e. when animal sacrificing was carried out) for the determination pro-atrial natriuretic factor (ANF$_{31-67}$; Prepro-ANF$_{56-92}$). Plasma was prepared and stored at 2-7°C until the time of analysis. An RIA procedure for ANF$_{31-67}$ analysis was performed in accordance with the manufacturers protocol following extraction (Phoenix Pharmaceuticals, Belmont, CA, USA). Briefly, for plasma extraction, an equal volume of 1% trifluoroacetic acid (TFA) was added to the plasma. Samples were centrifuged at 6000 g for 20 min at 4°C. The supernatant was added to C$_ {18}$ SEP-PAK columns (Waters) after first preconditioning the column with 60% acetonitrile in 1% TFA followed three times by 1% trifluoroacetic acid (3 ml, three times). Plasma solutions were loaded onto the pretreated C$_ {18}$ SEP-PAK columns and washed twice with 1% TFA. The peptide was eluted with 60% acetonitrile in 1% TFA and evaporated to dryness. The residue was dissolved in 250 μl of buffer and the RIA performed. All samples were assessed in duplicate.

2.8. Statistical analysis

All summary values are expressed as the mean±standard error of the mean. A $\chi^2$ test was used for quantal analysis of survival data. All multiple group comparisons were made by parametric or non-parametric analysis of variance for unpaired data followed by post hoc comparison using Fisher’s protected least significant difference test or the Mann–Whitney U test corrected for multiple comparisons. Correlations between endpoints were conducted using Pearson’s correlation analysis and the Searman’s rank correlation test. A probability level of $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of eprosartan on survival

As in previous studies, the introduction of the SFD to SP rats produced significant mortality within 6 weeks (Fig. 1). The mortality rate was greatest within the initial 6–9 week period when practically all SP-SFD control rats died. The overall mortality in this control group was 95% at 9 weeks. No mortality was observed over the 12 week time period (and for at least 3 additional months of observation in several animals allowed to survive for longer time) in SP-SFD eprosartan rats or in SP or WKY rats on normal chow diet.

3.2. Systolic blood pressure, heart rate and body weight

Systolic blood pressure (SBP), heart rate (HR) and body weight (BW) were recorded at 3-week intervals throughout the study. BW and age, utilized prior to the introduction of the SFD, provided a means of establishing homogeneity between the different groups. These measures were per-

![Eprosartan Decreases Mortality](image)

Fig. 1. Eprosartan eliminates the occurrence of morbidity/mortality during study period. Survival in SP maintained on SFD is presented for 12 weeks of observation. SP-SFD received intraperitoneal saline (SP-SFD control) or intraperitoneal Eprosartan (60 mg/kg/day) (SP-SFD Eprosartan). SP and WKY rats on a normal diet did not exhibit any morbidity/mortality (i.e. identical to eprosartan-treated rats; data not shown). All groups (n=25 per group) were homogeneous in terms of age and weight. (*, different from eprosartan-treated group; $P<0.001$).
Table 1

Table 1 shows the week 6 data for all groups. Body weight increased in all groups except the SP-SFD control group over the observation period. Following the SFD, this group also exhibited a significant increase in blood pressure that was reduced in the SP-SFD eprosartan group. Heart rate was not affected in any of the groups of rats.

Fig. 2. Echocardiographic images were obtained using an ATL HDI5000cv ultrasound unit with a L12-5 linear array transducer. Two-dimensional guided M-mode echocardiography (left parasternal view) was utilized. (A) Representative result of a 2D guided M-mode recording from the left ventricle of a SP on normal diet. This profile is similar to that observed in WKY rats on normal diet (not shown). (B) M-mode recording for SP-SFD control rat (i.e. treated with saline vehicle). Note thickened anterior and posterior wall and reduced contractile excursions. (C) M-mode recording for SP-SFD treated with eprosartan. Note maintenance of normal appearance similar to that in (A). (Diastole, left ventricular chamber diameter at diastole; Systole, left ventricular chamber diameter at systole; bars representing 200 ms time and 5 mm length in (C) are also presented).
3.3. Renal urinary protein excretion

Urinary protein measurements made at 5–7 weeks into the study for all groups of rats are also presented in Table 1. Vehicle SP-SFD control rats exhibited frank renal disease, as evidenced by significant proteinuria. Treatment
with eprosartan prevented this and maintained protein excretion to levels similar to those observed in SP or WKY rats on normal diet.

3.4. LV Remodeling and cardiac performance

M-mode echocardiography was used to determine LV wall thickness and chamber wall dimensions. These values were used to calculate LV end diastolic and end systolic volumes, stroke volume, cardiac output, and percent ejection fraction in the four experimental groups. Representative echocardiographic images are presented in Fig. 2. Group data collected at 5–7 weeks are presented in Figs. 3–5. In the SP-SFD control group, increased end diastolic (Diastole) LV wall thickness (i.e. hypertrophy in septal and posterior walls) was observed over eprosartan-treated SP-SFD rats (Fig. 3). Decreased end diastolic chamber diameter in SP-SFD control rats was also observed to be prevented by eprosartan treatment (Fig. 3). Also, decreased end diastolic volumes (EDV) due to SFD in SP were prevented by eprosartan, which preserved normal ventricular chamber volume (Fig. 4). SP-SFD control rats exhibited significantly decreased stroke volume, ejection fraction and cardiac output (Figs. 4 and 5). The suggested improvements in contractility produced by eprosartan were clearly substantiated by the preservation of normal stroke volume (Fig. 4) and ejection fraction (Fig. 5A) (i.e. maintained/preserved similar to that observed in the control groups) that occurred in drug-treated SP-SFD. The functional cardiac protection produced by eprosartan treatment was further demonstrated by its prevention of the remarkable decrease in cardiac output observed in SP-SFD control rats (Fig. 5B) (i.e. again a maintained/preserved function similar to that observed in the control groups preserved by eprosartan).

3.5. Cardiac MRI determinations

Cardiac-gated magnetic resonance images were collected as cardiac slices in 2D and then rendered in 3D at weeks 5–7. Figs. 6 and 7 present representative in vivo MR images of 2D and 3D views of eprosartan-treated and control hearts. The increased LV wall thickness and decreased LV chamber size observed in SP-SFD controls compared to SP-SFD eprosartan rats in end-diastole is depicted in 3D rendered images (Fig. 6). The reduced ventricular wall hypertrophy visualized during end systolic and end diastolic gating is depicted in 2D images (Fig. 7). MR images clearly corroborate the ultrasound data. This is substantiated in both 2D and 3D viewpoints and emphasizes the dramatic effects of eprosartan to reduce cardiac remodeling (i.e. SP-SFD controls vs. SP-SFD eprosartan groups). Fig. 8 provides group data on LV posterior wall thickness and chamber dimensions measured at diastole and systole using MRI. Although sample size is smaller for MRI determinations, the data are in close agreement and demonstrate the preservation of normal wall thickness produced by eprosartan treatment. These MRI data, together with the ultrasound measures, demonstrate the significant eprosartan effects using two different imaging technologies, and cross-validate both endpoints in monitoring heart morphological changes in this model.

3.6. Cardiac and renal histopathology

Organ weights are presented in Table 2. Heart and kidney weights and heart weight indices (i.e. organ weight/100 g body weight) of all three SP groups were greater than those of the WKY group. Also, the heart weight indices of the four experimental groups, to a large degree, substantiated the in vivo measurements made using ultrasound and MRI (Table 2 compared to Figs. 3 and 8). Kidney weight indices showed a similar relationship. Histopathological scoring (i.e. kidney and heart end-organ damage) of standard tissue sections is summarized as total scores in Table 2 and is presented as all heart component scores in Table 3. Marked and significantly increased total
In-vivo 3-D MRI

Fig. 6. MRI measures corroborate those obtained using ultrasound. Cardiac-gated magnetic resonance imaging was performed in a 4.7T/40 cm Bruker Biospec imaging spectrometer. Representative 3D images (rendered during diastole) depict the left ventricular wall hypertrophy of a SP-SFD control rat (hypertrophy in A) compared to a SP-SFD eprosartan fed rat (B), which is similar to an SP rat fed a normal diet (C) or to WKY rats fed a normal diet (data not shown).

heart and kidney histopathology scores indicating advanced end-organ injury (Table 2) were observed in the SP-SFD control group. These scores were reduced by eprosartan treatment (i.e. in SP-SFD eprosartan rats) to levels at or below those of SP or WKY rats on normal diet (i.e. normal histological appearance/end-organ conditions). Scores of each of the components that made up the total cardiomyopathy score as well as those for the degree of hyperplasia of the proximal right and left coronary arteries (Table 3) were similarly reduced by eprosartan. Kidney crude scores and cardiomyopathy scores for individual rats were highly correlated ($r = 0.94; P < 0.0001$).

3.7. Plasma pro-ANF$_{31-67}$

The pro-ANF$_{31-67}$ RIA had no detectable cross-reactivity with insulin, vasopressin, somatostatin, adrenocorticotropic hormone, or oxytocin (data not shown). Furthermore, it was shown to have less than 0.5% cross-reactivity with the pro-ANF 1–30 and ANF peptides. This RIA is useful in rat, human and mouse because prepro-ANF is highly conserved in these species [39]. In addition, the average sample variability when performed in duplicate was only 1.8%. Using the assay, plasma levels of ANF$_{31-67}$ were compared between the SP-SFD control ($n = 12$), SP-SFD eprosartan ($n = 8$), SP-normal diet ($n = 8$) and WKY normal diet ($n = 12$) groups. All plasma samples were collected immediately prior to euthanasia. Plasma ANF$_{31-67}$ levels in the SFD-SP control group were significantly ($P < 0.05$) elevated when compared to WKY-normal diet or SP-SFD eprosartan groups (see Table 1). Therefore, the increased ANF$_{31-67}$ levels in SP-SFD animals were reduced to more normal levels due to eprosartan treatment.

4. Discussion

SP rats were originally developed by selectively in-breeding SHR for severe hypertension, and resulted in a rat strain that developed stroke spontaneously [26–30]. Placing SP rats on a SFD provides an dynamic cardiovascular disease model [24,25]. This SP-SFD model can be described generally as the occurrence of accelerated end-organ injury (i.e. brain, renal and cardiovascular) that includes vascular injury and dysfunction (e.g. coronary artery hypertrophy and hyperplasia), renal vascular, glomerular and tubular lesions, cardiac hypertrophy, vascular lesions, myofiber degeneration, myocardial inflammation fibrosis, and coronary artery microthrombosis (sometimes with microinfarction), and stroke (both cerebral hemorrhage and ischemic infarcts). Eprosartan provided significant renal and cardiac end organ protection as kidney histopathology and urinary protein excretion were reduced, cardiac myopathy and hypertrophy were reduced and normal cardiac function (i.e. as determined by both ultrasound and MRI technology) was preserved. It also eliminated morbidity/mortality throughout the 3 months in this study, and for some rats even much longer (i.e. for up to 6 months of additional observation long after
Fig. 7. Representative 2D images demonstrating reduced ventricular wall hypertrophy visualized during end systolic and end diastolic gating. Images depict the left ventricular wall hypertrophy of a SP-SFD control rat (hypertrophy in A) compared to a SP-SFD eprosartan fed rat (B), which is similar to an SP rat fed a normal diet (C) or to WKY rats fed a normal diet (data not shown).

all vehicle-treated SP SFD rats had died). These protective effects may reflect its reduction in vascular tone as well as prevention of decompensating ventricular remodeling and renal toxicity/pathology. Several indices of LV hypertrophy were reduced by drug treatment, including the well-known plasma marker of cardiac hypertrophy and failure, pro-ANF [38]. Other SP studies employing traditional neurohumoral antagonists have shown protection [24–26,37,40], and several antihypertensive agents that have been shown to increase survival and to decrease blood pressure in SP rats (for review see [40]). However, the present protection achieved with eprosartan appears to be greater than for any other compounds evaluated to date. Recent observations indicate that severe heart failure ($50\%$ reduction in cardiac output) and stroke also accompanies the morbidity in these SP-SFD rats (data not shown). In the present study, animals were healthy during functional and imaging studies, thus representing normal or prefailure conditions/difference between the groups.

Deterioration of LV function was evident in the SP-SFD control group, and significant reductions in stroke volume and cardiac output were that were inhibited significantly by eprosartan treatment, suggesting a preservation of cardiac contractility. Eprosartan did not significantly increase end diastolic volume or reduce end systolic volume, but it did preserve normal morphology and function reflected in improved contractility compared to the obvious cardiac functional deficits observed in SP-SFD control rats. Both ultrasound (local M-mode determination) and MRI (global average determination) modality measurements closely corroborate cardiac remodeling changes/differences and were able to detect significant improvement in the heart due to eprosartan treatment. These in vivo measurements of morphology have obvious advantages over histopathological determinations of LV measurements [25] because they link more directly to function monitored during life. However, the present histopathological analyses also clearly demonstrate the cumulative, complex end-organ injury/
pathology profile for both heart and kidney in this model [24,25] and illustrate the dramatic end-organ protection afforded by eprosartan under these conditions.

Improved eprosartan heart or kidney to body weight ratios was not due to an absolute reduction in the heart or kidney weights but was due to a reduction in body weight in the SP-SFD control rats. Previous studies suggest that the body weight loss in these rats is associated with the development of end-organ injury. Improved health and improved weight gain is associated with drug protection [24, 25]. The importance of ultrasound and MRI technologies in detecting heart pathology and drug protection should be emphasized. LV remodeling is more clearly evident in vivo, related to wall thickness and chamber changes primarily in the SP-SFD controls. It should be noted that these rats are very young, and that the acceleration of heart remodeling occurs only due to SFD during this short period of observation. However, cardiomyopathy

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>Heart weight index (g/100 g BW)</th>
<th>Total cardiac scores</th>
<th>Kidney weight (g)</th>
<th>Kidney weight index (g/100 g BW)</th>
<th>Total renal scores</th>
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<tr>
<td>SP-SFD control control</td>
<td>2106 17 11,1</td>
<td>1.136 0.04</td>
<td>5.256 0.35 11,1</td>
<td>13.16 1.3 11</td>
<td>2.656 0.10</td>
<td>1.306 0.07 11,1</td>
<td>2.416 0.05 11,1</td>
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<tr>
<td>SP-SFD Eprosartan</td>
<td>3136 13*</td>
<td>1.256 0.05 1</td>
<td>4.026 0.21 1</td>
<td>1.26 0.2*</td>
<td>2.776 0.20</td>
<td>0.886 0.04 3§</td>
<td>0.226 0.07 1</td>
</tr>
<tr>
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<td>1.12 0.05 1</td>
<td>3.656 0.06 1</td>
<td>3.56 0.7*</td>
<td>2.466 0.14</td>
<td>0.716 0.02*</td>
<td>0.756 0.03 1</td>
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<tr>
<td>WKY-normal diet</td>
<td>3576 21*</td>
<td>0.986 0.07 1</td>
<td>2.786 0.13 1</td>
<td>0.56 0.3*</td>
<td>2.136 0.08 1</td>
<td>0.616 0.04*</td>
<td>0.196 0.08 1</td>
</tr>
</tbody>
</table>

* $P$, 0.05 different from SP-SFD control.
$P$, 0.05 different from SP-Normal Diet.
$P$, 0.01 different from all other groups; n=5 8 in each group. Data collected from matched sacrifice rats from other groups as SP-SFD Control rats became moribund (i.e. from weeks 4–9; n=5 8 per group; see Fig. 2). Total cardiac scores are derived as described in text from the individual indices listed in Table 3.

$P$, 0.05 different from WKY-Normal Diet.
and renal failure can be expected to occur in the high risk SP rats on a normal diet as reflected by their short life span under these conditions (e.g. in our laboratories they live on the average of only 1 year) compared to SHR (e.g. ~2 years) and WKY rats (e.g. ~3 years). Indeed, SP rats on a normal diet do not quite express increased ANF, which also can be expected to increase at a later age in this strain.

The cardiomyopathic changes observed in the present model of severe hypertension include myocardial/myofibroblast hypertrophy as well as interstitial and perivascular fibrosis [25]. These changes suggest that both cardiomyocytes and cardiac inflammatory cells with fibroblastic proliferation participate in ventricular remodeling and the attendant diastolic and systolic dysfunction. Thus, an activated RAS system (ligands and receptors) in early heart failure, perhaps mediated by stretch and hypoxia, may act in an endocrine, paracrine or autocrine fashion to initiate cardiomyocyte hypertrophy as well as activating cardiac fibroblast proliferation and collagen matrix deposition. There is ample evidence demonstrating renal AII receptor-mediated hypertrophic/remodeling effects of AII [41]. For example, AII induces proliferation of cardiac fibroblasts which synthesize collagens, and AII also provides an indirect contribution of stress to the heart by increasing ventricular wall tension through an increase in vascular resistance mediated by stimulation of all vascular smooth muscle receptors [6–9,22]. The ability of AII to exert a direct effect on cardiac hypertrophy independent of its effect on blood pressure or the circulating renin–angiotensin system may be key in the present model of hypertensive hypertrophic cardiomyopathy. Although the small decrease in blood pressure may contribute to eprosartan protective effects, significant protection in this model has been observed by drugs that do not affect blood pressure or the renin–angiotensin system [24, 25]. However, substantial evidence now exists for a functional intracardiac renin–angiotensin system (i.e. a renin–angiotensin system and AII receptors in cardiac tissue suggests the existence of an autocrine/paracrine system that has effects independent of AII derived from the circulatory system) [16]. Recent data suggest that a synergism between mechanical load and AII type 1 receptor activation results in cardiac hypertrophy [4,11,16–18]. Therefore, the available data indicate that both the circulating and local renin–angiotensin (aldosterone) systems promote the development of myocardial hypertrophy in hypertensive heart disease culminating in chronic heart failure. Certainly the local renin–angiotensin system needs to be evaluated in more detail in the present experimental model. Previously, we have shown the importance of the circulating plasma renin activity and aldosterone in the stroke-prone strain [37], and in the present model of end-organ injury [24].

Selective blockade of the AII type 1 receptor represents therapeutic advantages for interrupting the renin–angiotensin system over angiotensin converting enzyme (ACE) inhibitors (i.e. providing for the potential beneficial effects of angiotensin type 2 receptor stimulation without altering bradykinin metabolism) [6,7]. Growing evidence indicates that AT type 2 receptor stimulation has beneficial effects over and above those of ACE inhibitors on the vasculature (e.g. results in reduced blood pressure, inhibition of cell proliferation and hypertrophy, reduced signaling associated with vascular injury, and reduced effects on bradykinin levels) [1,2,7].

Lastly, the dose level/regimen selected for the present study was based on previous data derived from renal protective effects in the rat [20], and is relevant to doses/blood levels employed in the clinic [42–45]. In man, eprosartan is well tolerated and may be more effective than enalapril in reducing systolic blood pressure [42,44]. Also, additional treatment with the AT type 1 receptor antagonist eprosartan, given to severe heart failure patients who received digitalis, diuretics and ACE inhibitors, resulted in a beneficial effect by increasing cardiac output. This effect may be due to eprosartan’s additional property of blocking the autocrine interaction of locally-generated AII at vascul-
lar and myocardial type 1 receptors as well as the influence on prejunctional AT1 type 1 receptors located on sympathetic nerve terminals [43].

In summary, AT1 type 1 receptor blockade using eprosartan provides chronic end organ protection in a genetic and diet dependent model of severe hypertension. Chronic treatment with eprosartan at a clinically relevant dose attenuates ventricular hypertrophy maintains cardiac and renal structural integrity, improves cardiac and renal function and prolongs survival in a spontaneously developing model of severe hypertension-induced cardiac hypertrophy and heart and kidney failure. The results provide additional data that support the AT type 1 receptor as an important pathomediator of heart and renal failure. AT type 1 receptor antagonism using eprosartan should provide an important therapeutic approach to the treatment of these frequently occurring and important diseases.

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