

Echocardiographic effects of eplerenone and aldosterone in hypertensive rats

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1. ABSTRACT

The effects of aldosterone receptor blockade on echocardiography in spontaneously hypertensive rats (SHR) are not fully characterized. In this study, multiple echocardiographic parameters were compared for 42 weeks between SHR versus Wistar-Kyoto rats (WKY) serving as normotensive controls. In addition, echocardiographic parameters were compared for 28 weeks between the SHR versus SHR treated with eplerenone 100 mg/kg/day or spironolactone 50 mg/kg/day. Compared to normotensive WKY rats, SHRs had significantly increased systolic blood pressure, increased cardiac mass, increased isovolumic relaxation time (IVRT), decreased E/A ratio, increased mitral closure opening time interval (MCO) and increased Tei index. Both eplerenone and spironolactone significantly decreased systolic blood pressure compared to the SHR controls. The spironolactone treatment group demonstrated significant increases in heart rate and cardiac output and a decrease in cardiac index compared to SHR controls. Any aldosterone blockade in SHR protected against the increased cardiac mass. Similar to clinical echocardiographic observations, hypertension in rats results in left ventricular hypertrophy (LVH) and diastolic dysfunction and aldosterone receptor blockade reduces LVH in SHR.

2. INTRODUCTION

Clinically, congestive heart failure (CHF) is associated with systolic or diastolic dysfunction or a combination of both. Management options are often based on an understanding of pathophysiologic mechanisms, which lead to the development of a given condition. The efficacy of the aldosterone receptor antagonist, spironolactone, to abort or delay the progression of systolic heart failure was shown in clinical trials (1, 2). In the Randomized Aldactone Evaluation Study (2), a standard treatment regimen of an angiotensin-converting-enzyme inhibitor (ACEI) and a diuretic, with or without digoxin, plus spironolactone (Aldactone) was compared to a similar regimen plus placebo in patients with New York Heart Association (NYHA) Class III-IV CHF. Data from this study demonstrated a 30% decrease in overall mortality and hospitalization for cardiac events in patients treated with spironolactone. However, spironolactone has important adverse effects related to interactions with sex-hormone receptors. A more selective aldosterone receptor antagonist, eplerenone, has shown similar benefits to spironolactone after acute myocardial infarction (1). However, no major human or animal studies have evaluated its usage in diastolic dysfunction. Therefore, to better explore treatment options for diastolic dysfunction, it

is important to understand the mechanisms behind its evolution and progression. In contrast to the treatment of CHF caused by systolic dysfunction, few clinical trials are available to guide the treatment of patients with diastolic dysfunction. In addition, no human or animal studies have directly compared the echocardiographic effects of spironolactone and eplerenone.

The natural history of diastolic heart failure is not well characterized. Hypertrophy of the myocardium is an early milestone during the clinical course and an important risk factor for subsequent cardiac morbidity and mortality. Left ventricular hypertrophy (LVH) can occur due to genetic abnormalities or in response to increased wall stress resulting from physiologic or pathologic states. The most characteristic cellular feature of this process is the enlargement of existing myocytes caused by an accumulation of sarcomeric proteins and reorganization of myofibrillar structures (3). Myocardial hypertrophy leads to slowed or incomplete ventricular relaxation and diastolic heart failure results from impaired ability of the ventricles to accept blood. Abnormal alterations in the collagen matrix impair myocyte re-lengthening, leading to relaxation abnormalities, progressive diastolic dysfunction and heart failure (4). The major clinical consequence of diastolic failure is an elevation of the ventricular filling pressure leading to a high pulmonary venous pressure, causing pulmonary congestion.

3. MATERIALS AND METHODS

3.1. Experimental protocol for echocardiography

The experimental procedures were performed in accordance with guidelines of the National Institutes of Health and American Association for the Accreditation of Laboratory Animal Care (AAALAC), and approved by the Scott and White Memorial Hospital/Texas A&M Health Science Center Institutional Animal Care and Use Committee, Temple, TX.

Anesthesia was induced with 4% isoflurane combined with 3 L/min oxygen. Then, a mixture of 0.025 mL of xylazine (10 mg/mL) and 0.025 mL of ketamine (100 mg/mL) was administered at a minimal dose of 0.05 mL intramuscularly. The animals were placed on a warming table to maintain normothermia. Echocardiography was then performed using Agilent Sonos 5500, Hewlett Packard, Palo Alto, CA with a 12-MHz probe. Anesthetized rats were allowed to breathe spontaneously with nose cone supplemental oxygen during echocardiography. Using American Society of Echocardiography guidelines, electrocardiographic, and transthoracic echocardiographic parameters or calculations were recorded (5). All measurements were made online and optimal digital images were selected from more than 10 cardiac cycles. Left ventricular (LV) end-systolic and end-diastolic areas were traced in a single-plane apical 4-chamber view and online Simpson's rule ejection fraction was calculated using the modified single-plane method. Systolic blood pressure was recorded by tail cuff manometry just prior to each echocardiogram. Heart rate was recorded at the beginning of echocardiography from

the ECG monitor. Standard formulas were used for echocardiographic calculations (6).

3.2. Experimental protocol for treatments

For the study, sixty adult male rats were used. Forty-five 3-week-old Spontaneously-Hypertensive rats (SHR) were randomized into treatment and control groups. The treatment group was further randomized into an eplerenone group and a spironolactone group. Fifteen age-matched Wistar-Kyoto rats (WKY) served as normotensive controls. Rats assigned to eplerenone group (SH_EPL) were treated with 100 mg of eplerenone/kg/day. The spironolactone group (SH_ALD) was treated with 50 mg of spironolactone/kg/day. The eplerenone and spironolactone was mixed into dry rat chow in a similar fashion. Prior studies have shown that rats of this size consume ~40 grams of chow per day. The treatment period began on day 0 and continued to the end of the study. At day 0 and at 4-week intervals, transthoracic echocardiographic examination was performed. After administration of anesthesia and just before echocardiography, blood pressure readings were obtained using a tail cuff method (Advanced Auto-Inflate Blood Pressure Monitor, Harvard Apparatus).

3.3. Statistical Analysis

Linear Mixed effects models were utilized to test treatment effect and time effect with and without an interaction between treatment and time effects. When an interaction between treatment and time effects was non-significant, it was excluded from the model. Different covariance structures of error were used and the one with lowest AIC (Akaike Information Criterion) value was selected. Only significant results without interaction are reported. Contrast analyses were utilized to compare treated (SH_ALD and SH_EPL) to healthy control and treated (SH_ALD and SH_EPL) with SH Placebo. A p-value of less than 0.05 indicated a significance of overall treatment effect and 0.025 (Bonferroni-adjusted) was used a significance of contrast analyses. SAS 9.2 (SAS Institute Inc., Cary, NC) was used for statistical analysis.

When boxplots display the data, the box represents the middle 50% of the data. The line through the box represents the median. The lines (whiskers) extending from the box represent the upper and lower 25% of the data. The dot in each box represents the mean. The line on each plot connects the means of the data.

4. RESULTS

4.1. Echocardiographic effects of hypertension

By 42 weeks, 20 rats (10 in WKY control and 10 in SH Placebo) had completed the repeated anesthesia-echocardiographic observation and they are the sample for this report. Forty three outcomes, including Systolic BP and heart rates, were measured at 7 time points 0, 7, 14, 21, 28, 35, and 42 weeks). Figure 1 displays similar weight gain of the WKY versus SH placebo control strains. The overall mean weight of 363 ± 5.8 gm for WKY strain was significantly higher (p-value = 0.002) than 339 ± 5.1 gm for SHR strain. Thus, observations potentially affected by rat

Table 1. Significant variables for repeated measures analysis of WKY vs SHR (without interaction)

Variable	SHR LS Mean \pm SE	WKY LS Mean \pm SE	P value SH Placebo vs. WKY
SYS BP	140.6 \pm 2.568	103.8 \pm 2.734	<.0001
Cardiac Mass/body wt (mg/g)	213.9 \pm 5.783	194.31 \pm 5.783	0.0297
Mitral inflow Doppler E/A ratio	2.303 \pm 0.060	2.366 \pm 0.061	0.0041
IVRT (msec)	26.97 \pm 0.77	23.14 \pm 0.82	<.003
MCO	136.20 \pm 1.417	120.2 \pm 1.474	<.0001
Tei Index	0.406 \pm 0.028	0.301 \pm 0.029	0.0169

Abbreviations: WKY= Wistar-Kyoto rats, SHR= Spontaneously-hypertensive rats placebo group

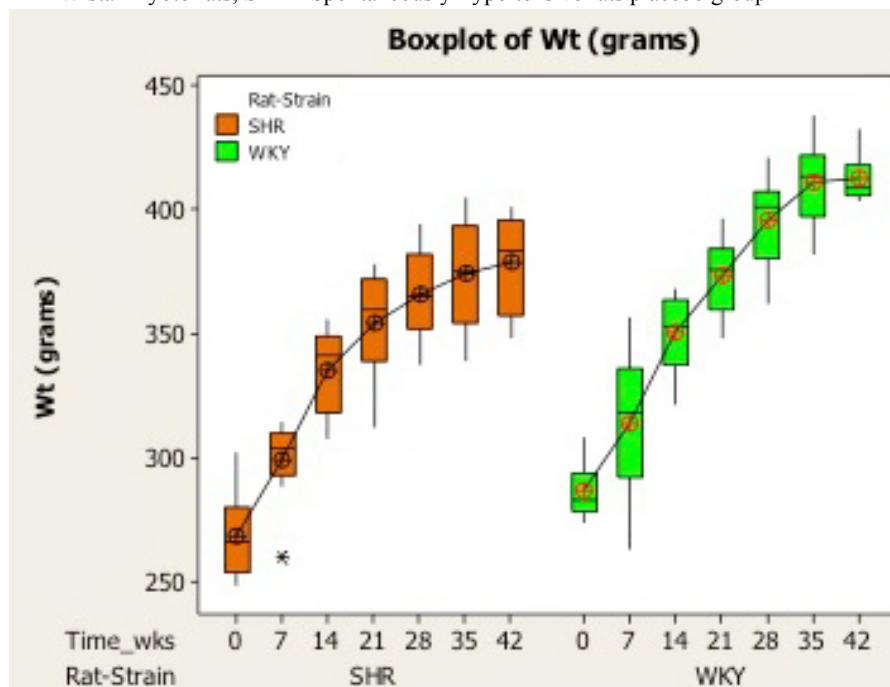


Figure 1. boxplot. Each box represents the middle 50% of the data. The line through the box represents the median. The lines (whiskers) extending from the box represent the upper and lower 25% of the data. The line on each plot connects the means of the sample. Rat's weight increased with time until 28 weeks for both Wistar-Kyoto rats (WKY) and spontaneously hypertensive placebo (SHR) and then changed little after 28 weeks.

size are normalized by dividing rat weight. Figure 2 presents the systolic blood pressure of the WKY versus SHR strains. SHR least-squares mean systolic blood pressure of 140.6 \pm 2.6mm Hg was significantly higher than 103.8 \pm 2.7 mm Hg for WKY strain.

4.1.1. Structural effects

SHR cardiac mass of 213.9 \pm 5.8 mg/gram was significantly higher than WKY cardiac mass 194.3 \pm 5.8 mg/gram (p <0.05).

4.1.2. Effects on systolic function

Figure 3 compares the cardiac index between SHR and WKY strains over time. Cardiac index did not differ between strains at any single observation time. From 0 weeks to 42 weeks, cardiac index decreased significantly for both strains being 22 \pm 2.8 to 14 \pm 1.9 ml/min/100 gm for WKY and 22 \pm 2.7 to 11 \pm 1.1 ml/min/100 gm for SHR (p <0.001).

4.1.3. Effects on diastolic function

Table 1 indicates that SHR had a significant decrease in mitral inflow E/A ratio and an increase

isovolumic relaxation time (IVRT). In addition, the SHR demonstrated an increase in the MCO and Tei index, compared to WKY. The aortic ejection time did not differ between SHR and WKY, indicating that the increased Tei index observed in SHR resulted from increased MCO.

4.2. Echocardiographic effects of spironolactone vs eplerenone

Comparison of the SHR controls (SH Placebo) to the spironolactone (SH_Ald) and the eplerenone treatment (SH_Epl) groups demonstrates the effects of aldosterone receptor blockade on spontaneous hypertension. All 3 groups had rapid weight gain until a plateau after the 20th week. No significant difference of weights between the treatment groups was observed (p =0.235). As seen in table 2, the spironolactone and eplerenone treated groups' systolic blood pressure was significantly lower than the placebo group. The reduction in systolic blood pressure was similar for spironolactone and eplerenone treatment. The LV apex in the rat heart tends to be midline cranial caudal axis, thereby limiting a parallel Doppler angle of insonation with the LV outflow tract; whereas, the parasternal short-axis Doppler angle of insonation usually

Table 2. Repeated measures analysis results with 28 weeks data set for significant variables (Comparison of *SH ALD vs. ***Placebo; **SH EPL vs. ***Placebo). (Without interaction)

Variable	SH ALD vs. Placebo Mean Difference (SE)		p-value	SH EPL vs. Placebo Mean Difference (SE)		p-value
SYS_BP	-8.8051	3.1576	0.0082	-11.8831	3.1834	0.0006
HR (bpm)	15.4745	5.8249	0.0115	7.4013	5.8674	0.2148
PA CO (ml/min)	9.9326	3.1911	0.0035	-1.4975	3.2321	0.6458
CI (ml/min/100gm)	2.2695	0.8637	0.0123	0.7595	0.8739	0.3902
Cardiac Mass (mg)	-71.3886	28.7471	0.0175	-20.1903	28.9314	0.4895

*SHR spironolactone treated, **SHR eplerenone treated, *** = SHR placebo group, SYS_BP = systolic blood pressure, HR= heart rate, PA-CO=cardiac output estimated from pulmonary artery VTI, CI=cardiac index

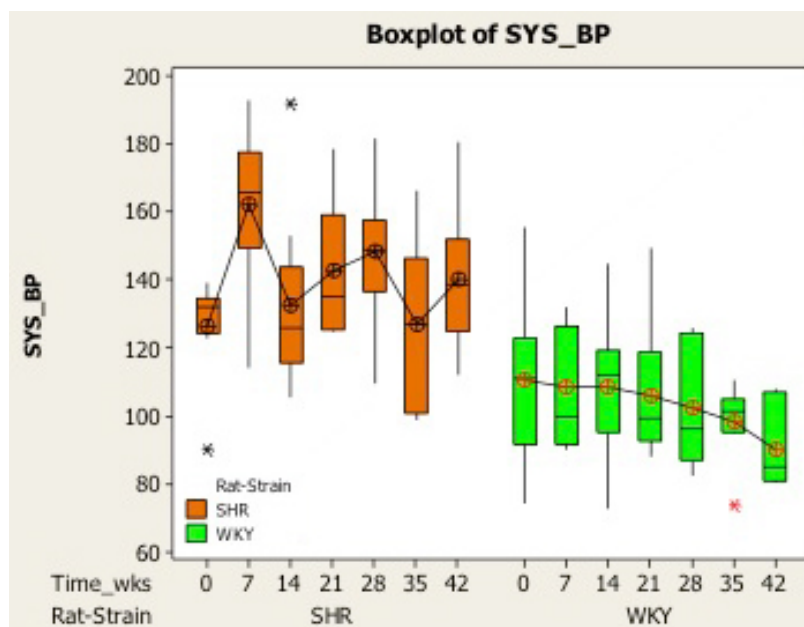


Figure 2. boxplot Systolic blood pressure (SYS_BP) was consistently higher for spontaneously hypertensive placebo (SHR) rats than Wistar-Kyoto rats (WKY) (See Figure 1 for boxplot definitions).

obtains the desired cosine of 0 degrees. For this reason, the relative changes in cardiac output were calculated by substituting the RVOT VTI for LVOT VTI in the standard cardiac output formula. This calculation is label PA CO in table 2. The spironolactone treatment had a significant increase in heart rate, increase in cardiac output and decrease in cardiac mass compared to SHR controls; whereas, the eplerenone treatment group did not.

5. DISCUSSION

Heart failure with preserved ejection fraction in the community is more prevalent in women (7). If female gender has a different effect on hypertensive echocardiographic changes, these effects may not have been detected by a study that involved only male rats. The increase in cardiac mass (LVH) was expected in spontaneously hypertensive rats compared to controls. Recent epidemiological reports indicate that heart failure is the primary event that occurs with LVH (8). In the Cardiovascular Health Study, LVH was a risk factor for the development of decreased left ventricular ejection fraction within 5 years (9). In the LIFE study, reversal of LVH with angiotensin receptor blockade improved clinical outcomes

(10).

Using WKY and SHR rats instrumented with chronic electromagnetic flow probes on the ascending aorta and arterial pressure catheters, Smith and Hutchins (11) reported hemodynamics up to 120 days (~17 weeks). The rat mean weight in their study was in the range of 311-314 gm, which is comparable to our current study 21-week range of 353-373 gm. At 17 weeks, they observed a 45% higher mean arterial pressure in SHR vs. WKY rats, which is comparable to the 35% increase in systolic blood pressure observed in our study. As shown in figure 3, the current study confirmed Smith and Hutchins (11) observation of decreasing cardiac index with time for both rat strains. Their observation of a transiently higher cardiac index in SHRs from 31 to 42 days (4.4 to 5.8 wks.) was probably not observed in our current study because the earliest echocardiograms were performed later (7 weeks after baseline measurements). The current study also differed by using an echocardiographic estimate of cardiac output in anesthetized rats instead of implanted flow probes in unanesthetized rats.

Decreased E/A ratio and increased isovolumic relaxation time (IVRT) are some of the echocardiographic

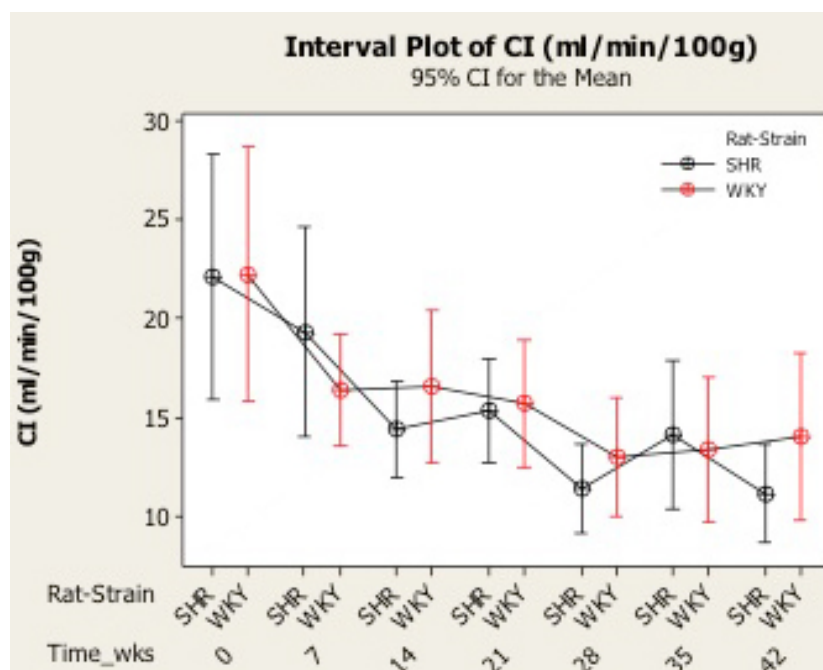


Figure 3. *interval plot* Cardiac index comparison between SHR and WKY strains over time.

criteria for Grade I diastolic dysfunction (12). The calculation of index of myocardial performance (Tei Index) used aortic ejection time (ET) and mitral valve closure to opening time (MCO) (12). MCO is the sum of ET, isovolumic contraction time (IVCT) and (IVRT). Systolic dysfunction results in prolongation of IVCT and shortening of ET. Both systolic and diastolic dysfunction results in abnormality of myocardial relaxation that prolongs the IVRT. As listed in table 1, compared to normotensive WKY rats, SHR rats had a significant increase in MCO resulting in an associated increase in the Tei index. Since ET was not significantly different between the two strains, then the increase in MCO was related to an increase in IVCT or IVRT are both. In the context of the finding of other abnormal parameters of diastolic dysfunction, it is likely that the abnormally increased Tei index relates to diastolic dysfunction in the SHR strain. The Tei index is independent of heart rate and blood pressure and does not rely on geometric assumptions (13). It is reproducible and has prognostic value in several clinical conditions. For example, The Tei index predicted the development of heart failure (HF) in a cohort of elderly men without baseline LV systolic dysfunction (14).

Compared to placebo treated SHR rats, either spironolactone or eplerenone treated rats had a similar 7% - 9% decrease in systolic blood pressure. Since a dose response study was not performed for either drug, spironolactone's sole effects on increasing cardiac index and decreasing cardiac mass could be dose related. Also, the increased heart rate and cardiac index could possibly be related to nonspecific interactions of spironolactone with progesterone receptors and androgen receptors (15).

In SHR, aldosterone blockade parallels that

reported in clinical studies. In the 4E Left Ventricular Hypertrophy Study (16), aldosterone receptor blockade clinically reduced systolic blood pressure 15% compared to 9% in SHR. Based on clinical magnetic resonance imaging in the 4E Left Ventricular Hypertrophy Study (16), eplerenone clinically decreased cardiac mass comparable to this study observing decreased cardiac mass in the spironolactone treated SHR. In another study (17), the addition of spironolactone to antihypertensive therapy, clinically reduced echocardiographic cardiac mass 4% compared to 9% in SHR.

In conclusion, spontaneous hypertension in rats results in increased cardiac mass, diastolic dysfunction and increased Tei index. Aldosterone receptor blockade for 28 weeks in SHR reduced systolic blood pressure and cardiac mass on a scale comparable to clinical studies. To our knowledge, this is the first study to demonstrate differential effects between spironolactone and eplerenone treatments under hypertensive conditions, in which spironolactone improved cardiac index and reduced cardiac mass.

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