

Research

Development of heart failure assessed by tissue Doppler imaging in hypertensive Dahl rats

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ABSTRACT

Objective: Tissue Doppler imaging (TDI) has been recognized as a useful tool to assess regional myocardial function. The purpose of this study was to evaluate the development of heart failure in hypertensive Dahl rats using echocardiography with TDI.

Methods: Dahl salt-sensitive (DS) rats were placed on 8% NaCl diet from 7 weeks old. As an age-matched control, DS rats were consistently placed on normal diet. In these rats, echocardiography was performed successively. We evaluated interventricular septal wall thickness (IVST), left ventricle (LV) posterior wall thickness (LVPWT), LV fractional shortening (%LVFS), LV ejection fraction (LVEF) and early diastolic velocity (peak negative velocity: PNV).

Results: Following on the rise of blood pressure (12, 18 and 25 weeks of age), IVST and LVPWT were significantly greater in DS rats fed with high-salt diet compared to control group. PNV at 18 and 25 weeks of age was significantly lower in DS rats fed with high-salt diet than control group. However, there were no significant differences on LVEF and %LVFS, between DS rats fed with high-salt diet and control group.

Conclusion: LV diastolic dysfunction preceded LV systolic dysfunction in DS rats with hypertension. Consecutive measurements of the PNV should prove valuable in follow-up of progressive LV diastolic dysfunction secondary to hypertension.

INTRODUCTION

Hypertension is one of risk factors for cardiovascular and cerebrovascular diseases, myocardial infarction, stroke, renal failure,

progressive atherosclerosis, and dementia (Agmon *et al.*, 2002; Bountiukos *et al.*, 2006). Progressive development of hypertension results in left ventricular hypertrophy (LVH), then congestive

heart failure (CHF). The increasing in the load to the left ventricle (LV) by high blood pressure (BP) makes compensated LVH, which is recognized as the prehistory of CHF. The overloading to LV goes over the ability of compensation, and then becomes CHF (*Sakata et al., 2000*).

Pulsed wave Doppler imaging of mitral inflow has been widely used to evaluate diastolic LV function. However, the mitral inflow velocity profile is affected by several factors, such as left atrial pressure, volume status, and the rate of myocardial relaxation (*Hamdan et al., 2006*). To overcome the limitation of transmitral Doppler, tissue Doppler imaging (TDI) has been developed, which has been enable to directly measure of regional myocardial velocity in real time (*Derumeaux et al., 2002*).

Accordingly, we examined LV diastolic and systolic function during the course of hypertension development using echocardiography with TDI in Dahl salt-sensitive (DS) rats.

METHODS

Animals

DS rats (DIS/Eis; Eisai, Tokyo, Japan) were used as a model showing progressive pressure-overload hypertrophy from the compensated to the decompensated stage (*Appleton et al., 1988*). They were fed a low-sodium diet (containing 0.3% NaCl) until the age of 6 weeks. Five rats were then switched to a high-sodium diet (containing 8% NaCl), whereas the remaining 9 animals were maintained on the low-sodium diet (*Ichihara et al., 2006; Nishizawa et al., 2010*). Animals on this latter diet serve as models of hypertensive LVH and heart failure at 12, 18 and 25 weeks of age, respectively. Both food and tap water were provided ad libitum throughout the experiment. BP, heart rate (HR) and body weight (BW) were recorded weekly. A cuff with a pneumatic pulse sensor was

attached to the tail. Rats were allowed to habituate to this procedure for 7 days before experiments were performed. BP and HR values were recorded on a Model MK-2000 (Muromachi Kikai Co., Ltd., Tokyo, Japan) without heating and were averaged from at least three consecutive readings obtained from each rat. All experimental procedures were performed in accordance with institutional guidelines for animal research. The investigation also conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Echocardiography

At 6, 12, 18 and 25 weeks of age, echocardiography was performed using SONOS 5500 system (Philips Medical Systems, Bothell, WA) equipped with a 12-MHz linear transducer under anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Wall thickness and LV dimensions were obtained from a short-axis view at the level of the papillary muscles. Interventricular septal wall thickness (IVST), LV posterior wall thickness (LVPWT) in diastole, and LV end-systolic and end-diastolic dimension (LVDs and LVDd, respectively) were measured, and LV fractional shortening (%LVFS) and LV ejection fraction (LVEF) were calculated. The mitral flow velocities were recorded with pulsed wave Doppler from the apical four-chamber view. The sample volume of 2 mm was placed at the tip of the mitral leaflets and adjusted to the position at which velocity was maximal. The peak flow velocity at the mitral level during rapid filling (peak E) and its deceleration time (DcT) were measured. The region of interest of 1 mm in diameter was located in the posterior wall from short axis view of the papillary muscles. The early diastolic velocity (peak negative velocity:

PNV) was measured as a diastolic function as previously described (*Shimizu et al., 1998, Ichihara et al., 2006*).

Histology

LV tissue was fixed with ice-cold 4% paraformaldehyde for 16 to 24 h, embedded in paraffin wax, and processed for histology as described (*Kato et al., 2008*). In brief, transverse sections (thickness, 3 μ m) were stained both with hematoxylin-eosin for routine histological examination.

Statistical analysis

Data are presented as mean $\pm$ SEM. Differences between 2 groups were analyzed by the unpaired Student *t* test. Multiple comparisons were evaluated by one-way analysis of variance followed by Scheffe's test. A level of $p < 0.05$ was considered statistically significant.

RESULTS

In DS rats fed with high-salt diet, systolic BP was significantly higher than in that of control group at 12, 18 and 25 weeks of age (12 weeks: 160.0 $\pm$ 26.9 vs

134.5 $\pm$ 4.9 mmHg, $p < 0.05$, 18 weeks: 189.5 $\pm$ 6.4 vs 121.0 $\pm$ 11.3 mmHg, $p < 0.05$, 25 weeks: 192.1 $\pm$ 13.8 vs 136.3 $\pm$ 15.9 mmHg, $p < 0.05$, respectively). On the other hand, HR and BW did not show significant differences between DS rats fed with high-salt diet and control group (Table 1).

The IVST and LVPWT were significantly greater in DS rats fed with high-salt diet than in control group at 12, 18 and 25 weeks of age (IVST, 12 weeks: 2.07 $\pm$ 0.14 vs 1.70 $\pm$ 0.18 mm, $p < 0.05$, 18 weeks: 2.52 $\pm$ 0.25 vs 1.99 $\pm$ 0.19 mm, $p < 0.05$, 25 weeks: 2.74 $\pm$ 0.34 vs 2.02 $\pm$ 0.09 mm, $p < 0.05$, LVPWT, 12 weeks: 1.99 $\pm$ 0.15 vs 1.64 $\pm$ 0.09 mm, $p < 0.05$, 18 weeks: 2.23 $\pm$ 0.15 vs 1.87 $\pm$ 0.14 mm, $p < 0.05$, 25 weeks: 2.33 $\pm$ 0.20 vs 1.81 $\pm$ 0.18 mm, $p < 0.05$, respectively). The PNV at 18 and 25 weeks of age were significantly lower in DS rats fed with high-salt diet than in that of control group (18 weeks: -2.94 $\pm$ 0.43 vs -3.73 $\pm$ 0.23 cm/sec, $p < 0.05$, 25 weeks: -2.58 $\pm$ 0.59 vs -3.40 $\pm$ 0.61 cm/sec, $p < 0.05$, respectively). There were no significant differences on LVDd, LVDs, LVEF, %LVFS, peak E and DcT between DS rats fed with high-salt diet and control group (Fig. 1, Table 2).

Table 1. Hemodynamic measurements in 6, 12, 18 and 25 weeks of age

		6 weeks	12 weeks	18 weeks	25 weeks
BPs(mmHg)	Control group	136.0 $\pm$ 10.9	134.5 $\pm$ 4.9	121.0 $\pm$ 11.3	136.3 $\pm$ 15.9
	High-salt diet group	132.9 $\pm$ 16.6	160.0 $\pm$ 26.9*	189.5 $\pm$ 6.4*	192.1 $\pm$ 13.8*
BPd(mmHg)	Control group	71.8 $\pm$ 4.9	67.0 $\pm$ 7.8	67.0 $\pm$ 2.8	68.7 $\pm$ 17.0
	High-salt diet group	66.9 $\pm$ 14.9	89.0 $\pm$ 14.9	98.0 $\pm$ 10.6	94.7 $\pm$ 12.6
HR(bpm)	Control group	510.2 $\pm$ 28.3	507.0 $\pm$ 5.7	479.0 $\pm$ 22.6	438.0 $\pm$ 13.1
	High-salt diet group	472.7 $\pm$ 31.9	484.0 $\pm$ 29.7	468.0 $\pm$ 7.1	471.6 $\pm$ 37.7
BW(g)	Control group	206.0 $\pm$ 5.3	352.0 $\pm$ 8.7	414.4 $\pm$ 9.7	438.0 $\pm$ 12.0
	High-salt diet group	209.1 $\pm$ 8.6	357.1 $\pm$ 13.0	422.1 $\pm$ 16.9	441.2 $\pm$ 17.8

BPs, systolic blood pressure; BPd, diastolic blood pressure; HR, heart rate; BW, body weight. Data are mean $\pm$ SEM. * $p < 0.05$ vs control group.

Table 2. Serial changes in echocardiographic parameters in control and high-salt diet groups

		6 weeks	12 weeks	18 weeks	25 weeks
IVST(mm)	Control group	1.57±0.11	1.70±0.18	1.99±0.19	2.02±0.09
	High-salt diet	1.60±0.10	2.07±0.14*	2.52±0.25*	2.74±0.34*
LVPWT(mm)	Control group	1.25±0.15	1.64±0.09	1.87±0.14	1.81±0.18
	High-salt diet	1.54±0.36	1.99±0.15*	2.23±0.15*	2.33±0.20*
LVDd(mm)	Control group	6.38±0.76	7.72±0.28	7.51±0.31	7.71±0.49
	High-salt diet	6.32±0.46	7.46±0.65	7.42±0.41	7.39±0.78
LVDs(mm)	Control group	3.11±0.35	3.93±0.38	5.16±1.42	4.36±0.43
	High-salt diet	3.13±0.39	4.14±0.84	4.08±0.62	4.08±0.36
LVEF(%)	Control group	85.7±5.0	83.6±1.5	76.7±7.5	79.6±3.6
	High-salt diet	86.1±3.1	80.5±7.6	81.1±6.1	81.1±1.9
%LVFS	Control group	50.1±6.2	47.5±1.6	39.1±3.3	43.3±3.6
	High-salt diet	50.6±4.1	44.9±8.3	45.3±5.4	44.7±2.1
Peak E(cm/sec)	Control group	98.4±17.2	108.6±9.8	93.1±17.6	101.7±11.9
	High-salt diet	96.9±9.4	108.3±9.0	95.3±8.6	97.4±8.5
DcT(msec)	Control group	44.6±5.5	43.2±6.0	38.7±5.1	36.8±2.9
	High-salt diet	41.6±5.1	43.8±5.8	36.3±6.6	32.8±3.9
PNV(cm/sec)	Control group	-3.52±0.93	-4.34±0.80	-3.73±0.23	-3.40±0.61
	High-salt diet	-3.58±1.31	-3.63±0.68	-2.94±0.43*	-2.58±0.59*

IVST, interventricular septal wall thickness; LVPWT, left ventricular posterior wall thickness; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; E, peak flow velocity at the mitral level during rapid filling; DcT, deceleration time of E; PNV, peak negative velocity. Data are mean±SEM. * $p < 0.05$ vs control group.

High-salt diet group showed increase in cross-sectional area of cardiac myocytes compared to control group at 25 weeks of age (Fig. 2).

DISCUSSION

The DS rats fed with high-salt diet at 12, 18 and 25 weeks of age showed that IVST and LVPWT were significantly thicker compared with those of control group, then built centripetal LVH. The PNV of DS rats fed with high-salt diet at 18 and 25 weeks of age was significantly lower than that of control group. However, other indices of LV systolic function such as LVEF and %LVFS did not show significant differences between DS rats fed with high-salt diet and control group. Our results indicate that DS rats fed with high-salt diet get LV

diastolic dysfunction before getting LV systolic dysfunction. The reduction in PNV may provide insight into the pathogenesis of hypertension.

Pulsed wave Doppler examination of mitral inflow has been used to assess LV diastolic function. However, the parameters; E or DcT measured by this method depend on loading conditions such as preload and left atrial pressure (*Nishimura et al., 1989*). Several studies have shown that TDI is relatively preload independent and sensitive to detect abnormalities of LV diastolic function (*Bountiukos et al., 2006, Hara et al., 2013*). In our study, there were no significant differences on peak E and DcT between DS rats fed with high-salt diet and control group at each weeks of age. Meanwhile, PNV was significantly lower in DS rats fed with

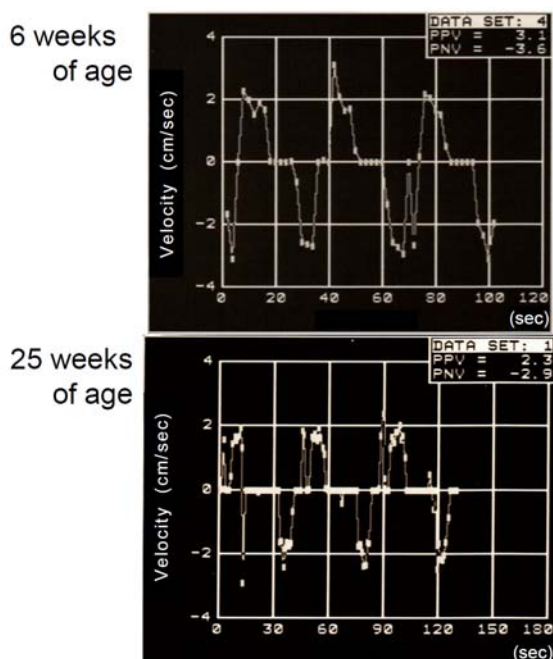
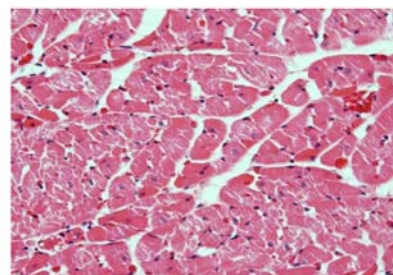


Fig. 1 Peak negative velocity of hypertensive DS rats at 6 and 25 weeks of age. The representatives of peak negative velocity at 6 weeks (upper) and 25 weeks of age (lower) are shown. PPV, peak positive velocity; PNV, peak negative velocity.

high-salt diet than control group. The PNV is thus a predictable indicator of LV relaxation compared to traditional pulsed wave Doppler.

The majority of patients with heart failure and preserved ejection have a history of hypertension. Hypertension induces a compensatory thickening of the ventricular wall in an attempt to normalize wall stress, which results in LV concentric hypertrophy, which in turn decreases LV compliance and LV diastolic filling (*Raman et al., 2010*). There is an abnormal accumulation of fibrillar collagen accompanying the hypertension-induced LV hypertrophy, which is also associated with decreased compliance and LV diastolic dysfunction (*Kakkar et al. 2010*). LV diastolic dysfunction has been recognized as a prognosis factor for heart failure as well as LV systolic dysfunction (*Acil et al., 2005; Troughton et al., 2005; Wang et al., 2005; Hamdan, 2006; Oh et al., 2006*). Serial measurements of the PNV should prove valuable in follow-up of patients with

Control group



High-salt diet group

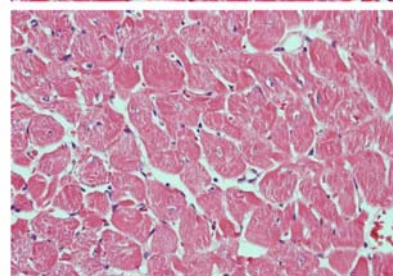


Fig.2 Morphological analysis of the left ventricle of DS rats in control and high-salt diet groups. Micrographs of myocytes in hematoxylin-eosin-stained sections of the left ventricle wall of rats in control (upper) and high-salt diet (lower) groups at 25 weeks of age are shown. Magnification, $\times 400$.

progressive LV diastolic dysfunction secondary to hypertension.

We showed that LV diastolic dysfunction preceded LV systolic dysfunction in DS rats with hypertension. The PNV provide a noninvasive index of LV diastolic function, yielding unique information about LV relaxation in patients with hypertension.

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タイトル: 高血圧ダールラットにおける組織ドプラ法を用いた心不全発症および進行過程の評価

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