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Importance of blood pressure variability in organ protection in spontaneously hypertensive rats treated with combination of nitrendipine and atenolol[1]

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KEY WORDS atenolol; nitrendipine; drug combinations; baroreflex; blood pressure; blood pressure variability; organ protection; hypertension

ABSTRACT

AIM: To study the importance of reduction of blood pressure variability (BPV) in the organ protection of long-term treatment with combination of nitrendipine and atenolol, which was abbreviated as Nile, in spontaneously hypertensive rats (SHR). **METHODS**: Combination of nitrendipine (10 mg· kg^{-1}· d^{-1}) and atenolol (20 mg· kg^{-1}· d^{-1}) was given in SHR chow for 12 weeks. Blood pressure (BP) was then recorded during 24 h in conscious state. After the determination of baroreflex sensitivity (BRS), rats were killed for organ-damage evaluation. **RESULTS**: Long-term treatment with Nile significantly decreased BP and BPV, ameliorated impaired BRS, and obviously diminished end-organ damage in SHR. The indices of left ventricular and aortic hypertrophy, and glomerulosclerosis score were all positively related to BP and BPV, and negatively related to BRS in untreated and Nile-treated SHR. Multiple-regression analysis showed that decrease in left ventricular and aortic hypertrophy was mainly related to the decrease in systolic BPV, and amelioration in renal lesion was mainly determined by increase in BRS. **CONCLUSION**: Long-term treatment with Nile possessed obvious organ protection in SHR. Besides the BP reduction, the decrease in BPV and the restoration of BRS may importantly contribute to this organ protection.

INTRODUCTION

It is well documented that blood pressure variability (BPV) is increased in both hypertensive humans and hypertensive animals[1-3]. Furthermore, BPV was found as an important factor determining the end organ damage (EOD) in hypertension[4-6]. In other words, the instability of BP could produce organ damage. Based on this consideration, it has been proposed that an antihypertensive drug with a BP-stabilizing effect would offer additional benefit in the treatment of hypertension.

A combination of two antihypertensive drugs with relative light dose of each is often clinically used. We have studied the synergism of nitrendipine and atenolol on blood pressure (BP) reduction in spontaneously hypertensive rats (SHR), renovascular hypertensive rats, and DOCA-salt hypertensive rats[7]. The combination of nitrendipine and atenolol (1:2) was abbreviated as Nile and it is actually in clinical trial (Phase II). It was found that there were 2 obvious advantages in the antihypertensive effect of Nile: (1) an obvious synergism on BP reduction. This synergism may reduce the doses

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of each drug required in the treatment of hypertension; (2) a rapid and persistent antihypertensive effect with a duration up to 24 h. This feature decreases the times of drug administration and in turn decreases the BP fluctuation produced by multiple drug administrations[7]. However, we do not yet know whether Nile decreases BPV and has any effect on organ protection. The present work was designed to investigate the effect of Nile on BPV and organ protection in SHR.

METERIALS AND METHODS

Animals and reagents Male SHR with an age of 12 weeks were provided by the Animal Center of Second Military Medical University. The rats were housed with controlled temperature (23-25 °C) and lighting (8:00-20:00 light, 20:00-8:00 dark) and with free access to rat chow and tap water. Nitrendipine was purchased from Nanjing Pharmaceutical Co Ltd, China, and atenolol was purchased from Shanghai Second Pharmaceutical Co Ltd, China.

Drug administration Nitrendipine and atenolol were mixed in the rat chow. The consumption of rat chow containing drugs was determined previously. The content of drugs in the rat chow was calculated according to the chow consumption and the ingested dose of nitrendipine and atenolol were about 10 and 20 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. The control group received the same diet without the drugs. After 12 weeks of drug administration, BP was recorded during 24 h, and then BPV was calculated and baroreflex sensitivity (BRS) was determined in conscious rats. And histopathological examinations were performed after BP, BPV, and BRS studies.

BP and BPV measurement Systolic BP (SBP), diastolic BP (DBP), and heart period (HP) were continuously recorded using the described technique[8,9]. Briefly, rats were anesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). A floating polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for BP measurement, and another catheter was placed into the left femoral vein for iv injection. The catheters were exteriorized through the interscapular skin. After a 2-d recovery period, the animals were placed for BP recording in individual cylindrical cages containing food and water. The aortic catheter was connected to a BP transducer via a rotating swivel that allowed the animals to move freely in the cage. After about 14-h habituation, the BP signal was digitized by a microcomputer. SBP, DBP, and HP values from every heartbeat were determined on line. The mean values of these parameters during a period of 24 h were calculated and served as SBP, DBP and HP. The standard deviation over the mean was calculated and defined as the quantitative parameter of BPV, ie systolic blood pressure variability (SBPV), diastolic blood pressure variability (DBPV), and heart period variability (HPV).

Baroreflex sensitivity (BRS) measurement To determine the function of arterial baroreflex in conscious rats, the methods widely used are derived from that of Smyth firstly applied for human[10]. The principle of this method is to measure the prolongation of HP in response to an elevation of BP. With some modifications, this method was used in conscious rats[11,12]. A bolus injection of phenylephrine was used to induce an elevation of BP. The dose of phenylephrine was adjusted to raise SBP between 20-40 mmHg. HP was plotted against with SBP for linear regression analysis; the slope of SBP-HP was expressed as BRS (ms/mmHg).

Morphological examination The rat was weighed and killed by decapitation. The thoracic and peritoneal cavities were immediately opened. The right kidney, aorta and heart were excised and rinsed in cold physiological saline. The right kidney was blotted, and weighed. The left ventricle was isolated, blotted, and weighed. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 30-mm-long segment of thoracic aorta was harvested, blotted, and weighed. Ratios of left ventricular weight to body weight (LVW/BW), right ventricular weight to body weight (RVW/BW), ventricular weight to body weight (VW/BW), left ventricular weight to right ventricular weight (LVW/RVW), and aortic weight to the length of aorta (AW/length) were calculated[13,14]. Histopathological observation was also carried out with our conventional method[15]. Briefly, immediately after gross detection, all samples of left ventricles in 2- to 3-mm-thick slices, aortae and kidney were immersed in formalin solution for more than 1 week, dehydrated in ethanol, cleared in dimethylbenzene and embedded in paraffin. Then the 5-μm-thick sections were prepared and stained with hematoxylin and eosin for light microscopic evaluation.

Glomerulosclerosis score (GSS) For the semiquantitative evaluation of glomerulular damage, the GSS was defined as previously described[16]. On the light microscopic specimens, approximately 50 glom-

eruli from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded according to the degree of sclerosis: 0, if no mesangial expansion; 1, if mild mesangial expansion (less than 30 % of a glomerular area); 2, if moderate mesangial expansion (30 %-60 % of a glomerular area); 3, if marked mesangial expansion (more than 60 % of a glomerular area); and 4, if the sclerosis was global. This was performed by one observer in a blind fashion using coded slides. A weighed composite sclerosis score was then calculated for each kidney according to the following formula: glomerulosclerosis score=[1×(number of grade 1 glomeruli)+2×(number of grade 2 glomeruli) +3×(number of grade 3 glomeruli)+4×(number of grade 4 glomeruli)]×100/(number of glomeruli observed).

Statistical analysis Data were expressed as mean±SD. Comparisons between 2 groups were made by unpaired *t*-test. The relationships between hemodynamic parameters and organ damage parameters were analyzed by classic univariate correlation analysis. Stepwise multiple-regression analysis was performed to study the independent effect of hemodynamic parameters on organ damage. F to enter and F to remove were set to $P<0.05$ and $P>0.10$, respectively. $P<0.05$ was considered statistically significant.

RESULTS

Effects of long-term treatment with Nile on BP, BPV, and BRS in SHR In conscious SHR, Nile significantly decreased 24-h SBP, SBPV, DBP, and DBPV with a significant increase in HP and HPV. In terms of ABR function, it was found that Nile markedly enhanced BRS in treated rats (Tab 1).

Effects of long-term treatment with Nile on organ damages in SHR In Nile-treated SHR, LVW/BW, VW/BW, LVW/RVW, AW/BW, and GSS were significantly decreased when compared with untreated rats (Tab 2). Under microscope, left ventricular, aortic and renal tissues from SHR demonstrated obvious pathological changes, including cardiomyocyte hypertrophy, myocardial interstitial and perivascular fibrosis, wall thickening of intramyocardial arterioles and aortae, destruction of vascular internal elastin membrane, glomerular atrophy and fibrosis, and renal vascular sclerosis. These characteristic changes were markedly attenuated by long-term treatment with Nile (Fig 1).

Relationships between BP, BPV, BRS and organ damages in SHR It was found that LVW/BW, an index for left ventricular hypertrophy, AW/length, an index for aortic hypertrophy, and GSS, an index for renal damage were all positively related to BP and BPV, and negatively related to BRS (Tab 3). The relative dependencies of organ damage on hemodynamic parameters were assessed by stepwise multiple-regression analysis. Both LVW/BW and AW/length were independently associated with higher SBPV ($\beta=0.850$, $P<0.01$; and $\beta=0.801$, $P<0.01$, respectively). While GSS was independently associated with lower BRS ($\beta=-0.826$, $P<0.01$).

Tab 1. Effects of long-term treatment with combination of nitrendipine and atenolol on hemodynamics in spontaneously hypertensive rats (SHR). *n*=7. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* SHR.

	SHR	Nit+Ate
SBP/mmHg	190±11	149±15^c
SBPV/mmHg	15.4±2.0	11.0±1.8^c
DBP/mmHg	121±13	98±9^c
DBPV/mmHg	11.7±2.2	9.0±1.6^b
HP /ms	160±11	187±21^c
HPV/ms	26±4	31±4^b
BRS/ms·mmHg^{-1}	0.30±0.17	1.0±0.3^c

SHR, spontaneously hypertensive rats; Nit+Ate, combination of nitrendipine and atenolol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HP, heart period; SBPV, systolic blood pressure variability; DBPV, diastolic blood pressure variability; HPV, heart period variability; BRS, baroreflex sensitivity.

Tab 2. Effects of combination of nitrendipine and atenolol on pathological changes in ventricles, kidneys and aortae in spontaneously hypertensive rats. *n*=7. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* SHR.

	SHR	Nit+Ate
LVW/BW(mg/g)	3.6±0.3	2.9±0.4^c
RVW/BW(mg/g)	0.73±0.07	0.78±0.13
VW/BW(mg/g)	4.4±0.4	3.7±0.5^b
LVW/RVW	5.0±0.5	3.8±0.5^c
AW/length (mg/mm)	1.48±0.11	1.09±0.10^c
GSS	61±9	43±6^c

SHR, spontaneously hypertensive rats; Nit+Ate, combination of nitrendipine and atenolol; LVW, left ventricular weight; BW, body weight; RVW, right ventricular weight; VW, ventricular weight; AW, aortic weight; GSS, glomerulosclerosis score.

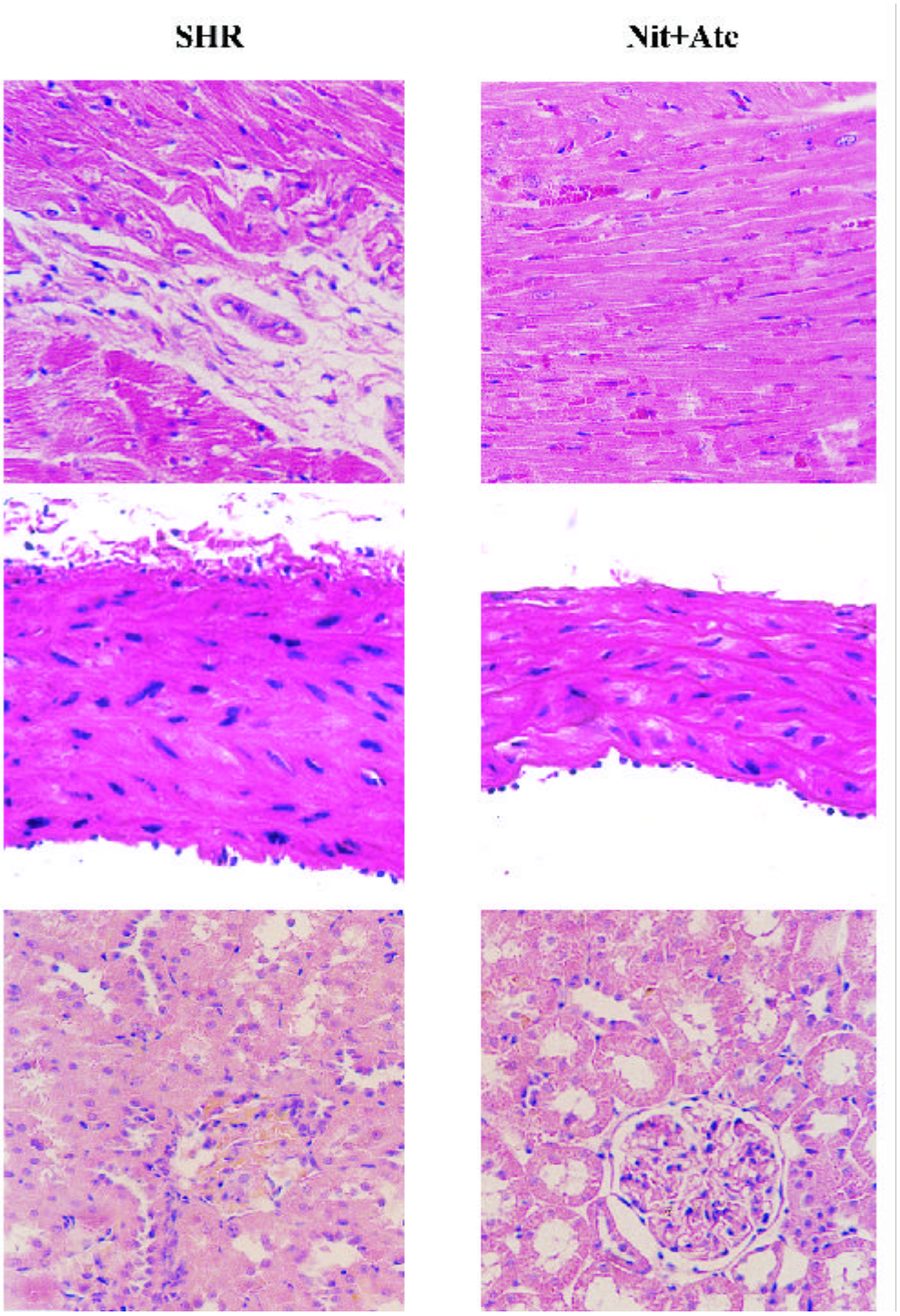

Fig 1. Effects of long-term treatment of Nile on histopathological changes in left ventricles, aortae, and kidney in SHR. SHR indicates spontaneously hypertensive rats; Nit+Ate, combination of nitrendipine and atenolol. Top row: left ventricular tissues with hematoxylin and eosin stain, ×60; Middle row: aortic tissues with hematoxylin and eosin stain, ×60; Bottom row: renal tissues with hematoxylin and eosin stain, ×60.

DISCUSSION

The present work clearly demonstrated that long-term treatment with Nile not only decreased BP level, but also decreased BPV and increased BRS, and Nile possessed obvious effects on organ protection in SHR. Clinically, preventing or reversing end-organ damage is an important objective in the treatment of hypertension. It is well known that high BP level induces organ dam-

Tab 3. Linear regression coefficient (*r*) between BP, BPV, BRS values, and organ damages in Nile-treated and untreated spontaneously hypertensive rats. *n*=14. [b]*P*<0.05, [c]*P*<0.01.

	LVW/BW	AW/length	GSS
SBP	0.771[c]	0.790[c]	0.746[c]
DBP	0.620[b]	0.634[b]	0.631[b]
SBPV	0.850[c]	0.801[c]	0.612[b]
DBPV	0.631[b]	0.634[b]	0.427
BRS	-0.678[c]	-0.763[c]	-0.826[c]

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; SBPV, systolic blood pressure variability; DBPV, diastolic blood pressure variability; BRS, baroreflex sensitivity; LVW, left ventricular weight; BW, body weight; AW, aortic weight; GSS, glomerulosclerosis score.

age and decreasing BP level prevents end-organ damage. In the present work, long-term treatment with Nile significantly decreased SBP and DBP in SHR. In the univariate correlation analysis, both SBP and DBP were positively related to EOD parameters. But both SBP and DBP were eliminated in the stepwise multiple-regression analysis. These results indicate that lowering BP may contribute to the organ protective action of Nile in SHR, but it is not the most important determinant for organ damages in Nile-treated and untreated SHR.

High BP level is not the unique factor determining hypertensive end-organ damage. Recently, it has been proposed that BPV may be important factor determining organ damage in hypertension. In 1987, Parati *et al* found that for nearly any levels of 24-h mean BP, patients in whom the BPV was lower had a lower prevalence and severity of organ damage than those with higher BPV[4]. Our previous study demonstrated that the severity of organ damage (EOD score) was positively related to BP levels (r=0.31-0.32, n=50, P<0.05), and to BPV (r=0.63-0.65, n=50, P<0.01) in 60-week-old SHR[6]. A similar result was obtained in our another study[17]. In the present work, long-term treatment with Nile markedly decreased SBPV and DBPV in SHR. In the univariate regression analysis, BPV was positively related to EOD parameters. Stepwise multiple-regression analysis showed that both LVW/BW and AW/length were independently associated with higher SBPV. These results indicate that decrease in BPV might be one of the major mechanisms for the organ protective action of Nile in SHR.

In addition, arterial baroreflex dysfunction is another feature of hypertension. It has been well recognized that BRS is impaired in hypertensive humans and animals[18-21]. Our previous study proposed that BRS was one of the independent variables related to EOD score, and BRS predicted the end-organ damage in hypertension[17]. In the present work, long-term treatment with Nile markedly enhanced BRS in SHR, and BRS was negatively related to LVW/BW, AW/length, and GSS. In the stepwise multiple-regression analysis, it was found that BRS was the most important determinant for GSS in Nile-treated and untreated SHR. These results suggest that the restoration of baroreflex function may contribute to the organ protective action of Nile in SHR.

In conclusion, long-term treatment with Nile possessed obvious organ protection in SHR. Besides the BP reduction, the decrease in BPV and the restoration of BRS may importantly contribute to this organ protection.

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血压波动性在尼群地平和阿替洛尔联合治疗自发性高血压大鼠的器官保护中的重要性[1]

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关键词　阿替洛尔；尼群地平；合并用药；压力感受性反射；血压；血压波动性；器官保护作用；高血压

目的：研究降低血压波动性(BPV)在尼群地平和阿替洛尔合用对高血压大鼠的器官保护中的重要作用．方法：自发性高血压大鼠食物中给予尼群地平(10 mg·kg^{-1}·d^{-1})和阿替洛尔(20 mg·kg^{-1}·d^{-1})12周．结果：尼群地平和阿替洛尔合用明显降低自发性高血压大鼠的血压和BPV，明显增高其动脉压力感受性反射敏感性(BRS)，并且有效减轻其终末器官损伤．左心室肥厚指数、主动脉肥厚指数和肾小球硬化积分都与血压和BPV呈正相关，而与BRS呈负相关．多重回归的结果显示，左心室肥厚和主动脉肥厚减轻主要与收缩期BPV的降低相关，而肾脏损伤的改善主要决定于BRS的增高．结论：尼群地平和阿替洛尔合用长期治疗能有效减轻自发性高血压大鼠的终末器官损伤．这种保护作用除与其降低血压有关外，还与其降低BPV和增高BRS有重要关系．

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