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The combination of atenolol and amlodipine is better than their monotherapy for preventing end-organ damage in different types of hypertension in rats

Ping Han [a], Fu-Ming Shen [a], He-Hui Xie [a], Yuan-Yuan Chen [a], Chao-Yu Miao [a], Jawahar L. Mehta [b], Jean Sassard [c], Ding-Feng Su [a, *]

[a] Department of Pharmacology, Second Military Medical University, Shanghai, China
[b] Department of Cardiovascular Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[c] Department of Physiology and Clinical Pharmacology, Faculty of Pharmacy, University Lyon-1, Lyon, France

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Abstract

Combinations therapy is often used in hypertensive patients whether combination therapy is necessary for preventing end-organ damage is not known. The objective of this study was to determine in four different hypertensive animal models the necessity of adding the calcium channel blocker amlodipine to therapy with the ß-blocker atenolol to modulate end-organ damage. Spontaneously hypertensive rats, DOCA-salt hypertensive rats, two-kidney, one-clip renovascular hypertensive rats and Lyon genetically hypertensive rats were used to study this objective. These animal models have different sensitivities to atenolol and amlodipine. The dosages of therapy employed were 10 mg/kg atenolol alone, 1 mg/kg amlodipine, 10 mg atenolol + 1 mg/kg amlodipine and 5 mg/kg atenolol+0.5 mg/kg amlodipine. BP was continuously recorded in all animals. After determination of baroreflex sensitivity, rats were sacrificed for end-organ damage evaluation. The combination of amlodipine and atenolol had a synergistic inhibitory effect on blood pressure and blood pressure variability, and end-organ damage as compared with monotherapy with atenolol or amlodipine in all animal models. Baroreflex sensitivity also improved with the combination therapy more than with monotherapy. In conclusion, atenolol and amlodipine combination exerts a superior effect on blood pressure, blood pressure variability, baroreflex sensitivity and end-organ damage. The superior effect of the combination was observed in all four models of hypertension.

Keywords: amlodipine • atenolol • combination therapy • hypertension • hypertensive rats

Introduction

Randomized controlled trials have shown that single drug treatment usually is not adequate to achieve blood pressure goal in most hypertensive patients [1]. Initiating therapy with more than one agent offers the potential advantages of achieving blood pressure control more rapidly and avoiding dose-related adverse effects of individual drugs by producing greater blood pressure reduction at lower doses of the component agents [2].

The sensitivity to an anti-hypertensive drug varies among hypertensive patients. For example, young people or those with high plasma renin activity are sensitive to ß-blockers, angiotensin-converting enzyme inhibitors or angiotensin II type 1 receptor blockers, while elderly or those with low plasma renin activity are sensitive to diuretics or calcium channel blockers [3, 4]. It is not clear if it is necessary to add a calcium channel blockers to patients with high plasma renin activity and treated with ß-blockers. Similarly, it is not clear if addition of β-blockers confers advantage to monotherapy with a calcium channel blocker. We hypothesized that a combination therapy would be superior to therapy with either a β-blocker or a calcium channel blocker in most types of hypertension in terms of blood pressure reduction and prevention of end-organ damage. Therefore, the present work was designed to test this hypothesis in rats.

Atenolol and amlodipine, the representative drugs for ß-blockers and calcium channel blockers, respectively, were used as anti-hypertensive drugs in this study. Spontaneously hypertensive rats (SHR), deoxycorticosterone acetate (DOCA)-induced hypertensive

*Correspondence to: Ding-Feng Su,
Department of Pharmacology, Second Military Medical University,
325 Guo He Road, Shanghai 200433, China.
Tel.: (86-21) 2507 0323
Fax: (86-21) 6549 3951
E-mail: dfsu@citiz.net

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rats, two-kidney, one-clip renovascular hypertensive rats (2K1C) and Lyon hypertensive rats (LH) were used as models for four different types of hypertension. SHR and LH are genetically hypertensive rats [5] and 2K1C and DOCA are models of experimentally induced hypertension. The 2K1C and SHR are models of angiotensin-dependent hypertension [6, 7] while LH and DOCA are models of low-renin hypertension [8].

Methods

Animals and chemicals

Amlodipine was provided by Beijing Shuanghe Pharmaceutical Co. Ltd. (Beijing, China) and atenolol by Shanghai Sanwei Pharmaceutical Co. Ltd. (Shanghai, China). DOCA was purchased from Sigma (St. Louis, MO, USA). Male Spargue-Dawley rats (used for preparation of hypertensive models) were purchased from the Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China). Male SHR and LH rats with 16 weeks were provided by the animal centre of our university. The animals were housed with controlled temperature (23–25°C) and lighting (08:00–20:00 hrs light, 20:00–08:00 hrs dark) and with free access to food and tap water. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

Preparation of 2K1C hypertensive rats

Male Sprague-Dawley rats weighing 180–200 g were anaesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg). The right renal artery of each animal was isolated through a flank incision, as described previously [9], and a silver clip (0.2-mm internal gap) was placed on the right renal artery. All animals were fed standard rat chow and tap water *ad libitum*. On day 21 after operation, blood pressures were measured by tail-cuff plethysmography and the rats with systolic blood pressure >150 mmHg were used for this study. Five weeks after placement of the clip, these rats received the treatment with different drugs.

Preparation of DOCA hypertensive rats

DOCA hypertensive rats were prepared as previously described [10]. Male Sprague- Dawley rats weighing 110–130 g were anaesthetized with a combination of ketamine (40 mg/kg) and diazepam (6 mg/kg) and underwent a right nephrectomy *via* a flank incision. Rats were given daily subcutaneous injections of DOCA (50 mg/kg) and 0.9% saline to drink for 5 weeks. Systolic blood pressure was measured by tail-cuff plethysmography before the anti-hypertensive drug administration and the rats with Systolic blood pressure >150 mmHg were used for this study.

Intra-arterial blood pressure measurements

Systolic blood pressure, diastolic blood pressure and heart period were continuously recorded using previously described technique [11]. Briefly, rats were anaesthetized by 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 blood pressure measurement and another catheter was inserted into left femoral vein for phenylephrine injection. The catheters were exteriorized through the interscapular skin. After a 2-day recovery period, the animals were placed in individual cages containing food and water. The aortic catheter was connected to a blood pressure transducer *via* a rotating swivel that allowed the animals to move freely in the cage. After about 4-hrs habituation, the blood pressure signal was digitized by a microcomputer. Systolic blood pressure, diastolic blood pressure and heart period values from every heartbeat were determined on line. The mean values of these parameters during a period of 4 hrs for each rat were calculated. The standard deviation of the mean was calculated and defined as the quantitative parameter of variability; that is systolic blood pressure variability, diastolic blood pressure variability and heart period variability.

Determination of baroreflex sensitivity

After a 4-hrs blood pressure recording, baroreflex sensitivity was measured in the conscious rat by using our previously described method [12]. A bolus of phenylephrine was injected to induce a blood pressure elevation. The dose of phenylephrine (5–10 mg/kg) was adjusted to raise systolic blood pressure in the range of 20–40 mmHg. There exists a delay (about 1 sec.) between the elevation of blood pressure (stimulus) and the prolongation of HP (response) for arterial baroreflex. In rats, the heart period is about 5 or 6/sec. So, heart period was plotted against systolic blood pressure for linear regression analysis for 2–8 shifts (calculated by computer); the slope with the best close correlation coefficient of heart period/systolic blood pressure was expressed as baroreflex sensitivity (ms/mmHg). The mean of two measurements with proper dose served as the final result.

Morphological examination

The animals were weighted and euthanized with intraperitoneal sodium pentobarbital. 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 and the heart were gently blotted for gross detection, including kidney weight, renal cortical thickness, renal medullary thickness, heart weight, left ventricular weight and left ventricular wall thickness. 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 weighted. Ratio of left ventricular weight to body weight (LVW/BW), left ventricular thickness (LVT), aortic weight to the length of aorta (AW/length) and right cortical thickness to right medullar thickness (RCT/RMT) were calculated.

For semi-quantitative evaluation of glomerular damage, the glomerular sclerosis score (GSS) of the right kidney was determined according to the previously published criteria [13]. Approximately 50 glomeruli from the outer cortex and the same number of glomeruli from the inner cortex for each kidney were graded based on the degree of sclerosis; grade 0, if no mesangial expansion; grade 1, if mild mesangial expansion (less than 30% of a glomerular area); grade 2, if moderate mesangial expansion (30–60% of a glomerular area); grade 3, if marked mesangial expansion (more than 60% of a glomerular area); and grade 4, if the sclerosis was global. A composite sclerosis score was then calculated for each kidney according to the

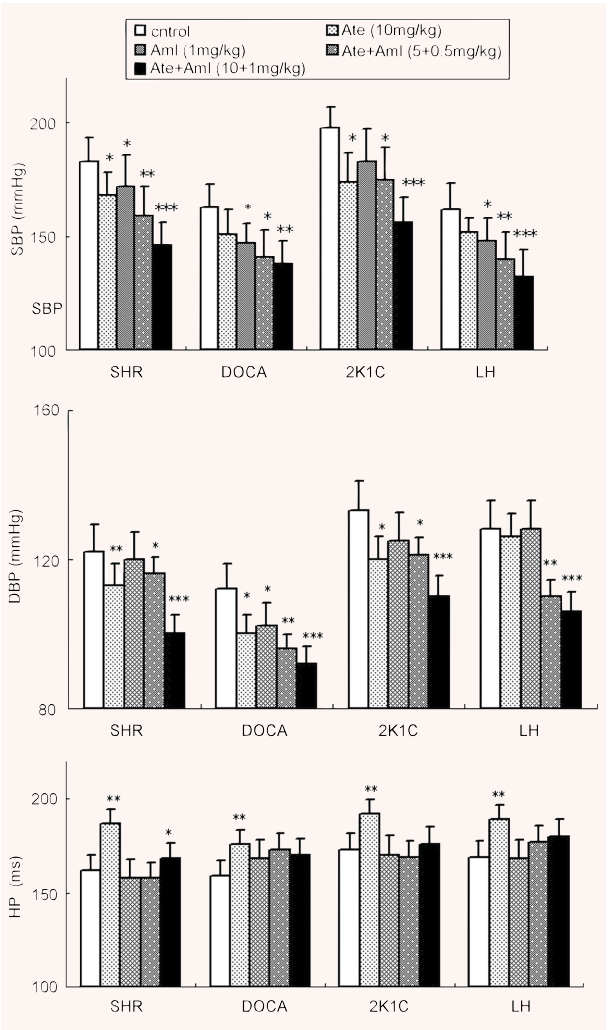

Fig. 1 Effects of long-term treatment with combination of amlodipine and atenolol on blood pressure (BP) and heart period (HP) in spontaneously hypertensive rats (SHR), Deoxycorticosterone Acetate-Salt rats (DOCA), 2-kidney, 1-clip rats (2K1C) and Lyon genetically hypertensive rats (LH). $n = 10$, mean ± S.D. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control.

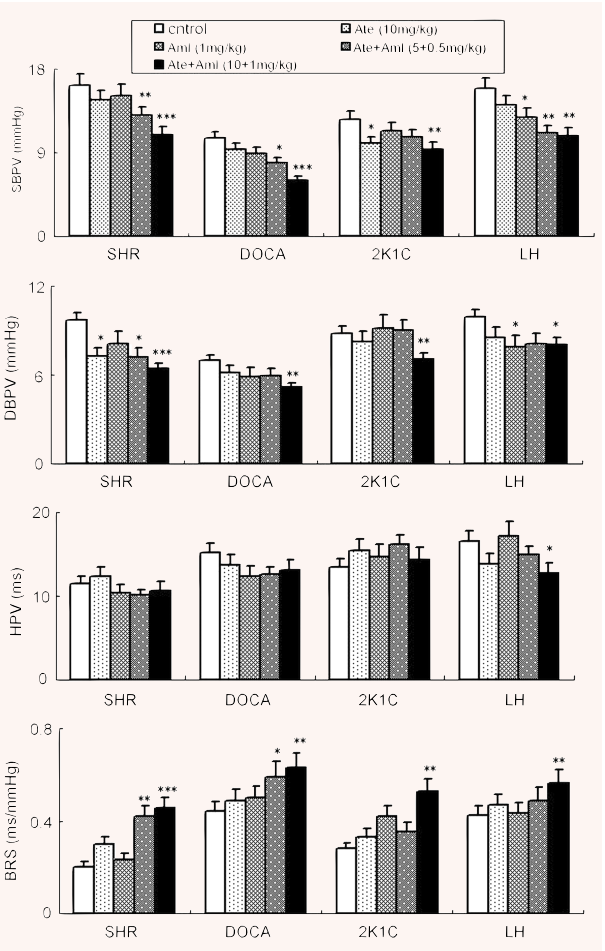

Fig. 2 Effects of long-term treatment with combination of amlodipine and atenolol on blood pressure variability (BPV), heart period variability (HPV) and Baroreflex sensitivity (BRS) in SHR, DOCA, 2K1C and LH. $n = 10$, mean ± S.D. See Figure 1 for abbreviations. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control.

following formula: GSS = [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).

Experimental protocols

At least 50 rats in each hypertension model were randomly divided into five groups. Amlodipine and atenolol were mixed in the chow. The content of drugs in the rat chow was calculated according to consumption. The daily-ingested doses were as follows: atenolol (10 mg/kg/day), amlodipine (1 mg/kg/day), combinations of atenolol and amlodipine (5+0.5, and 10+1 mg/kg/day). After 16 weeks of drug administration, systolic blood pressure, diastolic blood pressure and HP were continuously recorded in the conscious state for 4 hrs. Then, the blood pressure variability was calculated and the baroreflex sensitivity was determined in conscious freely moving rats.

Probability sum test

To determine if the drugs were acting synergistically, we used the probability sum test (q test) [14]. Compared with the mean values in the control group of rats, treated rats with a decrease in blood pressure >20 mmHg were defined as responders and rats with a decrease in blood pressure 20 mmHg were defined as non-responders. For blood pressure

variability, the criterion was 2 mmHg. The formula used is as follows: $q = P_{A+B}/(P_A+P_B-P_A\times P_B)$. Here, A and B indicate drug A and drug B; P is the percentage of responders in each group. P_{A+B} is the real percentage of responders and $(P_A+P_B-P_A\times P_B)$ is the expected response rate. (P_A+P_B) is the sum of the probabilities when drug A and drug B are used alone. $P_A\times P_B$ is the probability of rats responding to both drugs when they were used alone. When q was <0.85, the combination was thought to be antagonistic, when $q > 1.15$, the combination was thought to be synergistic, and when q was between 0.85 and 1.15, the combination was thought to be additive.

Statistical analysis

Data are expressed as mean ± S.D. The differences among groups were evaluated using analysis of variance followed by a two-tailed Student's unpaired t-test. The relationship between morphological and haemodynamic parameters was assessed by univariate regression analysis. The correlation coefficients and their 95% confidence intervals were calculated for haemodynamic and morphological data.

Results

Effects of combination therapy on blood pressure and heart period in four types of hypertensive rats

After 4 months of treatment, atenolol therapy alone was found to significantly reduce systolic blood pressure in SHR and 2K1C rats and had less effects in LH and DOCA rats. Compared to atenolol, amlodipine therapy decreased systolic blood pressure more effectively in LH and DOCA rats, but less effectively in SHR and 2K1C rats. The combination of atenolol and amlodipine (10 + 1 mg/kg) produced the largest effect on blood pressure reduction in all four types of hypertensive rats. Systolic blood pressure fell ($P < 0.001$) in SHR (–37 mmHg), DOCA (–25 mmHg), 2K1C (–42 mmHg) and LH (–30 mmHg). Even in rats treated with half-dose of the combination (atenolol + amlodipine = 5 + 0.5 mg/kg), the decrease in systolic blood pressure was greater than with monotherapy with either atenolol or amlodipine in SHR, DOCA and LH and similar to that induced by atenolol in 2K1C rats. The effects of combination therapy on diastolic blood pressure were similar to those on systolic blood pressure. Finally, we observed that atenolol when used alone increased heart period in all four types of hypertensive rats. No significant change in heart period was found in rats treated with amlodipine or with combination therapy (Fig. 1).

Effects of combination therapy on blood pressure variability and baroreflex sensitivity

As shown in Figure 2, monotherapy had little effect on systolic blood pressure variability. Atenolol significantly ($P < 0.05$) decreased systolic blood pressure variability in 2K1C rats and diastolic blood pressure variability in SHR; amlodipine significantly ($P < 0.05$) decreased systolic blood pressure variability and diastolic blood pressure variability in LH. Both systolic blood pressure variability and diastolic blood pressure variability decreased significantly in all four types of hypertension models, with full dose combination therapy. The reduction in systolic blood pressure variability was 33% in SHR ($P < 0.001$), 43% in DOCA ($P < 0.001$), 25% in 2K1C ($P < 0.01$) and 32% in LH ($P < 0.01$). Half-dose combination therapy (0.5+5 mg/kg/d) also significantly reduced systolic blood pressure variability in SHR, DOCA and LH rats and diastolic blood pressure variability in SHR. No significant changes in heart period variability was observed in any group of animals (Fig. 2), with the exception of LH rats treated with full dose of combination in which the heart period variability was significantly decreased ($P < 0.05$). In this study, baroreflex sensitivity fell in all animals groups, particularly in SHR (0.204 ms/mmHg) and 2K1C (0.281 ms/mmHg) groups of rats in the conscious state.

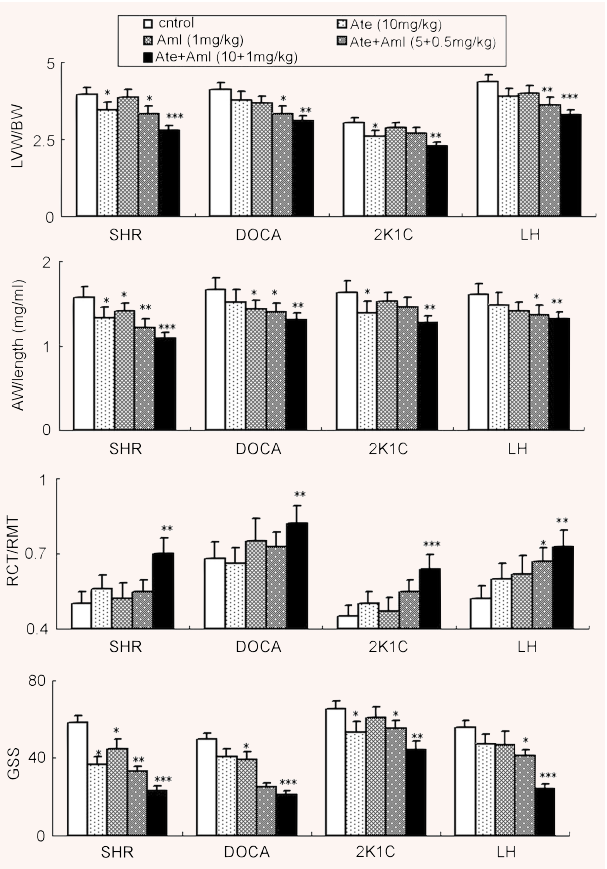

Fig. 3 Effects of long-term treatment with atenolol, amlodipine alone and in combination on pathological changes in ventricles, kidneys and aorta in SHR, DOCA, 2K1C and LH. $n = 10$, mean ± S.D. See Table 2 and Figure 1 for abbreviations. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *versus* control.

Table 1 The result of probability sum test on haemodynamics in four hypertensive rat models treated with long-term atenolol and amlodipine

	Drug (mg/kg/d)		SHR	DOCA	2K1C	LH
SBP	Ate (10)	P_{Ate}	4/10	2/10	4/10	1/10
	Aml (1)	P_{Aml}	3/10	3/10	2/10	3/10
	Comb (10+1)	$P_{Ate+Aml}$	8/10	8/10	8/10	6/10
		q	1.38	1.81	1.54	1.62
DBP	Ate (10)	P_{Ate}	2/10	1/10	3/10	2/10
	Aml (1)	P_{Aml}	3/10	3/10	2/10	2/10
	Comb (10+1)	$P_{Ate+Aml}$	7/10	6/10	6/10	5/10
		q	1.59	1.62	1.36	1.39
SBPV	Ate (10)	P_{Ate}	3/10	3/10	3/10	2/10
	Aml (1)	P_{Aml}	3/10	3/10	2/10	2/10
	Comb (10+1)	$P_{Ate+Aml}$	9/10	7/10	7/10	6/10
		q	1.76	1.37	1.59	1.67
DBPV	Ate (10)	P_{Ate}	2/10	2/10	2/10	2/10
	Aml (1)	P_{Aml}	2/10	3/10	2/10	4/10
	Comb (10+1)	$P_{Ate+Aml}$	6/10	6/10	5/10	8/10
		q	1.67	1.36	1.38	1.54
BRS	Ate (10)	P_{Ate}	2/10	2/10	3/10	1/10
	Aml (1)	P_{Aml}	3/10	3/10	0	2/10
	Comb (10+1)	$P_{Ate+Aml}$	7/10	6/10	6/10	4/10
		q	1.25	1.36	2	1.43

Ate, atenolol; Aml, amlodipine; Ate+Aml, combination of atenolol and amlodipine; SBP, systolic blood pressure; DBP, diastolic blood pressure; SBPV, systolic blood pressure variability; DBPV, diastolic blood pressure variability; BRS, baroreflex sensitivity.

Importantly, long-term treatment with the combination improved baroreflex sensitivity significantly in all four types of hypertensive rats (Fig. 2).

Effects of combination therapy on organ damage in four types of hypertensive rats

Some representative parameters of end-organ damage are shown in Figure 3. The parameters shown are LVW/BW (reflecting left ventricular hypertrophy), AW/length (reflecting aortic hypertrophy) and RCT/RMT and GSS (reflecting glomerular damage). We observed that atenolol alone reduced left ventricular hypertrophy, aortic hypertrophy and glomerular damage in SHR and 2K1C rats. Amlodipine alone reduced aortic hypertrophy and glomerular damage in SHR and DOCA rats. When animals were treated with full dose combination, all four parameters of end-organ damage were markedly less ($P < 0.01$ or $P < 0.001$ *versus* monotherapy) in all four types of hypertension models. Most of the end-organ damage parameters were less pronounced when half dose of the combination was used (Fig. 3).

Synergism between amlodipine and atenolol

Table 1 shows the results of the probability sum test for haemodynamic data from SHR, DOCA, 2K1C and LH treated with amlodipine (1 mg/kg/d), atenolol (10 mg/kg/d) and a combination

Table 2 The result of probability sum test on pathological changes in four different hypertensive rat models treated with long-term atenolol and amlodipine

	Drug (mg/kg/d)	P or q	SHR	DOCA	2K1C	LH
LVW/BW	Ate (10)	P_{Ate}	3/10	0	5/10	2/10
	Aml (1)	P_{Aml}	1/10	3/10	2/10	3/10
	Comb (10+1)	$P_{Ate+Aml}$	6/10	5/10	9/10	7/10
		q	1.62	1.67	1.5	1.59
AW/length	Ate (10)	P_{Ate}	4/10	1/10	4/10	0
	Aml (1)	P_{Aml}	2/10	4/10	3/10	3/10
	Comb (10+1)	$P_{Ate+Aml}$	8/10	7/10	8/10	5/10
		q	1.54	1.52	1.38	1.67
RCT/RMT	Ate (10)	P_{Ate}	2/10	3/10	5/10	2/10
	Aml (1)	P_{Aml}	1/10	3/10	2/10	2/10
	Comb (10+1)	$P_{Ate+Aml}$	5/10	8/10	9/10	6/10
		q	1.79	1.57	1.5	1.67
GSS	Ate (10)	P_{Ate}	2/10	0	3/10	1/10
	Aml (1)	P_{Aml}	3/10	3/10	1/10	2/10
	Comb (10+1)	$P_{Ate+Aml}$	8/10	6/10	8/10	5/10
		q	1.82	2.0	2.16	1.79

BW, body weight; LVW, left ventricular weight; AW, aortic weight; RCT, right cortical thickness; RMT, right medullar thickness; GSS, glomerulosclerosis score.

of these two agents (1+10 mg/kg/d). All *q* values were larger than 1.15, implying that the combination of amlodipine and atenolol exerted a significant synergistic effect on blood pressure and blood pressure variability reduction and baroreflex sensitivity enhancement in SHR, DOCA, 2K1C and LH. Table 2 shows the results of the probability sum test for end-organ damage data in all four hypertension models. All *q* values were larger than 1.15, again implying that the combination of amlodipine and atenolol exerted a synergism protective effect against end-organ damage in all four hypertension models.

Relationships between blood pressure, blood pressure variability, baroreflex sensitivity and end-organ damage

The relative dependence of end-organ damage on haemodynamic improvement was assessed by linear regression analysis (Table 3). In all four different hypertension models, end-organ damage correlated systolic blood pressure, diastolic blood pressure and systolic blood pressure variability and lower baroreflex sensitivity. The role of diastolic blood pressure variability in determination of end-organ damage parameters seemed less important than that of systolic blood pressure variability.

Discussion

The main findings of the present work are that: (1) there is a synergistic interaction between atenolol and amlodipine on blood pressure reduction, blood pressure variability reduction, baroreflex sensitivity enhancement and protection of end-organs. Even half-dose of the combination produces a greater effect than either single drug; (2) the synergism interaction between atenolol and amlodipine is seen in all four types of hypertension models.

Table 3 Linear regression coefficient (r) between BP, BPV, BRS values and organ damages in treated and untreated four different hypertensive rat models (n = 50)

SHR				
	LVW/BW	**AW/length**	**RCT/RMT**	**GSS**
SBP	0.621**	0.536**	–0.601**	0.677**
DBP	0.514**	0.477**	–0.422**	0.399*
SBPV	0.503*	0.671**	–0.401*	0.439*
DBPV	0.375*	0.451*	–0.269	0.286
BRS	–0.546**	–0.483**	0.467*	–0.413*
DOCA				
	LVW/BW	**AW/length**	**RCT/RMT**	**GSS**
SBP	0.532**	0.504**	–0.647**	0.521**
DBP	0.423**	0.379*	–0.467**	0.367*
SBPV	0.511**	0.595**	–0.421*	0.368*
DBPV	0.342*	0.497**	–0.306*	0.272
BRS	–0.583**	–0.574**	0.592**	–0.477*
2K1C				
	LVW/BW	**AW/length**	**RCT/RMT**	**GSS**
SBP	0.645**	0.547**	–0.572**	0.702**
DBP	0.425*	0.377*	–0.423**	0.543**
SBPV	0.589**	0.544**	–0.462**	0.518**
DBPV	0.379*	0.476**	–0.331*	0.432*
BRS	–0.523**	–0.422*	0.425*	–0.574**
LH				
	LVW/BW	**AW/length**	**RCT/RMT**	**GSS**
SBP	0.557**	0.513**	–0.572**	0.543**
DBP	0.312*	0.378*	–0.413*	0.376*
SBPV	0.549**	0.417**	–0.532**	0.499**
DBPV	0.287	0.354	–0.402*	0.231
BRS	–0.502**	–0.544**	0.485*	–0.532**

*P < 0.05; **P < 0.01. Abbreviations are the same as those in Tables 1 and 2.

A number of medications are available for the treatment of hypertension, and all can reduce blood pressure to normal or near-normal level [15, 16]. The present work showed that the combination of two commonly used drugs atenolol and amlodipine produced effect on blood pressure that was superior to that of either agent given alone. A major aim of anti-hypertensive therapy is to reduce cardiovascular events such as stroke, heart failure, renal failure and acute myocardial infarction that are often lethal. In this study, the synergistic interaction between the two drugs extended to a reduction in end-organ damage. The parameters of end-organ damage in this work included those reflecting left ventricular hypertrophy, aortic hypertrophy and renal injury. Both aortic hypertrophy and left ventricular hypertrophy are the typical pathological changes following hypertension. Regression of hypertrophy has been a major goal of clinical trials and of hypertension research [17]. Left ventricular weight/body weight (LVW/BW) and aortic weight/length (AW/Length) can reflect distinguished left ventricular hypertrophy and aortic hypertrophy directly. Kidney is one of the most important

target organs in hypertension. Glomerulosclerosis and tubulointerstitial fibrosis lead to renal dysfunction. Right cortical thickness/right medullar thickness (RCT/RMT) and glomerulosclerosis score (GSS) reflect the degree of glomerulosclerosis and tubulointerstitial fibrosis [18]. In addition, blood pressure variability and baroreflex sensitivity were also studied and both were improved considerably by the combination therapy. There is increasing evidence showing that high blood pressure variability and low baroreflex sensitivity contribute to end-organ damage in hypertension [19–26]. Interestingly, the present work clearly demonstrates a synergism between atenolol and amlodipine not only with regard to reduction in blood pressure, but also with regard to reduction in blood pressure variability, enhancement of baroreflex sensitivity and protection against end-organ damage.

The determination of synergistic interaction between drugs is not easy in practice. Recently, the probability sum test (q test) was successfully introduced to study the phenomenon of synergism of two anti-hypertensive drugs [27]. Using this test, we observed that the synergism between atenolol and amlodipine was highly significant. It is to be noted that the minimal q value was 1.38 for the effects on AW/length in 2K1C rats; the synergism begins when q exceeds 1.15. The probability sum test was calculated based on the results obtained from the treatment with full doses of the drugs. We also examined the effects of half-dose combination, and observed potentially salutary effects of half-dose combination. We found that half-dose combination produced a greater effect than either single drug or a similar effect to the 'sensitive' drug used alone. The latter finding also implies that it is not necessary to use large dose of an agent to which the animal is 'sensitive'. Among the synergistic interaction between different anti-hypertensive drugs, the synergism between atenolol and amlodipine seems to be most pronounced (unpublished data).

The rapid availability of SHR has made it possible not only to identify numerous cardiovascular abnormalities in this model but also to evaluate their role in the pathogenesis of hypertension by means of various interventions. It is well accepted that SHR is the best animal model for human essential hypertension. 2K1C model of hypertension shows high renin activity [28]. DOCA model of hypertension is associated with markedly depressed plasma renin activity [29]. LH rats exhibit low renin; and it is possible that the enhanced renal sensitivity to angiotensin II plays a primary role in the pathogenesis of hypertension in this model [30]. Our study showed that compared with amlodipine, atenolol was more effective in SHR and 2K1C rats, but less effective in LH and DOCA rats. Interestingly, a very significant synergism between atenolol and amlodipine was seen on all the parameters studied in all four types of hypertension models. Theoretically, this combination will be suitable for almost all hypertensive patients as it has a large coverage. The hypertensive patients with coronary artery diseases and/or with over-activity of sympathetic nervous system may benefit the most from this combination.

Individualization is another principle in the treatment of hypertension; however, identifying the best agent may take time. With combination therapy, it is obviously easier and quicker to control hypertension. Combination therapy may also lead to fewer side effects as larger doses of a single agent are avoided. This opinion is also supported by meta-analysis of data from randomized, controlled clinical trials that showed no significant difference in total major cardiovascular events between regimens based on major first-line anti-hypertensive medication used alone *versus* combination therapy although there were some differences in cause-specific outcomes [1]. So, the choice of drug may be decided by tolerability rather than long-term safety or efficacy [31]. In our recent unpublished data, we have observed a synergism on blood pressure and blood pressure variability reduction and end-organ protection not only with the combination of atenolol and amlodipine, but also with the combination of atenolol and nitrendipine [32]; hydrochlorothiazide and enalapril; amlodipine and candesartan; and amlodipine and irbesartan. Among these combinations we studied, it was found that the synergism of the combination between atenolol and amlodipine was the largest.

In conclusion, we have demonstrated a synergistic interaction between atenolol and amlodipine with regard as not only blood pressure reduction, but also blood pressure variability reduction, baroreflex sensitivity enhancement and end-organ protection. The synergism of atenolol and amlodipine was found in all models of hypertension.

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