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Synergistic effects of atenolol and amlodipine for lowering and stabilizing blood pressure in 2K1C renovascular hypertensive rats[1]

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Key words

atenolol; amlodipine; blood pressure; blood pressure variability; hypertension

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Abstract

Aim: To test the synergistic effects of atenolol and amlodipine on lowering blood pressure (BP) and reducing blood pressure variability (BPV) in 2-kidney, one-clip (2K1C) renovascular hypertensive rats. **Methods:** Forty-eight 2K1C renovascular hypertensive rats were randomly divided into 6 groups. They were respectively given 0.8% carboxymethylcellulose sodium (control), atenolol (10.0 mg/kg), amlodipine (1.0 mg/kg), and combined atenolol and amlodipine (low dose: 5.0+0.5 mg/kg; intermediate dose: 10.0+1.0 mg/kg; high dose: 20.0+2.0 mg/kg). The drugs were given via a catheter in a gastric fistula. BP was recorded for 25 h from 1 h before drug administration to 24 h after administration. **Results:** Compared with BP before medication, all 3 doses of combined atenolol and amlodipine significantly decreased the BP at 24 h after administration, except for the low dose on diastolic BP. Compared with the control group, all 3 doses of combined atenolol and amlodipine significantly reduced the average BP levels for the 24 h period after administration; furthermore, the high and intermediate doses also significantly decreased the BPV levels for the same period. The *q* values calculated by probability sum analysis for systolic and diastolic BP for the 24 h period after administration were 2.29 and 1.45, respectively, and for systolic and diastolic BPV for the same period they were 1.41 and 1.60, respectively. **Conclusion:** There is significant synergism between atenolol and amlodipine in lowering and stabilizing BP in 2K1C renovascular hypertensive rats.

Introduction

Blood pressure (BP) is not constant, and can vary spontaneously. This variation is defined as blood pressure variability (BPV). BPV is increased in hypertensive humans and animals[1-3]. Furthermore, BPV is positively related to the severity of organ damage in hypertensive humans and rats[4–6]. In other words, increased BPV can produce organ damage. Therefore, it has been proposed that antihypertensive drugs with a BP-stabilizing effect would act to protect organs in the treatment of hypertension. We have demonstrated that long-term treatment with ketanserin, candesartan, nitrendipine , a combination of nitrendipine and atenolol, and a hydrochlorothiazide mixture not only decrease BP, but also decrease BPV, and have obvious effects on organ protection in spontaneously hypertensive rats as well. Importantly, organ protection was attributed to the decrease in BPV[7–10].

Clinically, combination therapy against hypertension using 2 or more drugs from different classes can produce better drug efficacy[11]. Furthermore, the use of such synergistic therapy is also recommended for the initial treatment of hypertension[12]. Both β-blockers and dihydropyridine calcium antagonists are widely used in the treatment of hypertension. Atenolol and amlodipine are examples of long-acting drugs from these 2 classes. We hypothesized that these 2 drugs combined would produce a synergistic effect in the treatment of hypertension. Therefore, this study was designed to investigate the synergistic effects of atenolol and amlodipine on both lowing and stabilizing BP in 2K1C renovascular hypertensive rats (RVHR).

Materials and methods

Drugs and drug administration Amlodipine (Nanjing Pharmaceutical Co, Nanjing, China), atenolol (Shanghai Second Pharmaceutical Co, Shanghai, China) and a combination of these 2 drugs were dissolved in 0.8% carboxymethylcellulose sodium (CMC). The doses used were as follows: atenolol 10.0 mg/kg , amlodipine 1.0 mg/kg and 5.0+0.5 mg/kg, 10.0+1.0 mg/kg, and 20.0+2.0 mg/kg combinations of the 2 drugs. Five groups of rats received antihypertensive drugs and one group of rats received 0.8% CMC as control. Drugs were administered by a catheter in a gastric fistula implanted 3 days before treatment.

Animals and RVHR preparation Male Sprague–Dawley rats (160–180 g), purchased from the Sino-British SIPPR/BK Lab Animal Ltd (Shanghai, China), were anesthetized 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[13], and a silver clip (0.2 mm internal gap) was placed on the renal artery. Five weeks after placement of the clip, the systolic blood pressure (SBP) of rats was measured by using the tail-cuff method (CB10; Alcott Biotech Co, Shanghai, China). In total, 48 RVHR whose SBP was greater than 160 mmHg were used in this study. Rats were kept in a controlled temperature (23 °C–25 °C) and lighting (light 08:00–20:00, dark 20:00–08:00) environment, and had free access to food and tap water. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

BP and BPV measurement SBP, diastolic blood pressure (DBP) and heart period (HP) were continuously recorded using a previously described technique[10]. Briefly, rats were anesthetized by injection (ip) 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 stomach via a mid-abdominal incision for drug administration. 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 approximately 14-h habituation, at 9:00 o'clock the BP signal was begun to be digitized by a microcomputer. One hour later, at 10:00 o'clock the drug was given via the catheter in the gastric fistula. SBP, DBP, and HP values were recorded beat-to-beat for 25 h, up to 10:00 o'clock on the second day. The mean values of these parameters during a designated period were calculated and served as SBP, DBP and HP values. The standard deviation of all values obtained over 24 h was denoted as the quantitative parameter of variability; that is, SBP variability (SBPV), DBP variability (DBPV), and HP variability (HPV) for each rat.

Probability sum test To determine whether the drugs were acting synergistically, we used the probability sum test. This test comes from classic probability analysis and is useful for evaluating the synergistic interactions between 2 drugs (q test)[10,14,15].

In the present work, we used the following criteria. Compared with the mean values from control rats, treated rats with a decrease in BP (SBP or DBP) >20 mmHg were defined as responders and rats with a decrease in BP≤20 mmHg were defined as non-responders. For BPV (SBPV or DBPV), 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 (probability) 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 $q<0.85$, the combination is antagonistic, when $q>1.15$, the combination is synergistic, and when q is between 0.85 and 1.15, the combination is additive.

Statistical analysis Data were expressed as mean±SEM. Comparisons between values obtained in the same group before and after drug administration were made using the paired t-test. Comparisons among groups were carried out using ANOVA followed by Duncan's test. $P<0.05$ was considered statistically significant.

Results

Effects of atenolol and amlodipine on BP and HP at 24 h after treatment The effects of atenolol and amlodipine alone and in combination on BP and HP at 24 h after administration in 2K1C RVHR are shown in Table 1. The mean values of these variables 1 h before administration serve as the control values and are defined as "before"; the mean values at 24 h after administration are defined as "after". We found that SBP was significantly decreased in all 3 groups treated with both atenolol and amlodipine when compared with "before". A significantly lower DBP was also found in 2 groups of rats treated with both atenolol and amlodipine (10.0+1.0 mg/kg and 20.0+2.0 mg/kg) when compared with "before". Relative to "before" , the mean HP value at 24 h after administration was higher only in rats treated with atenolol (10.0

Table 1. Effects of atenolol (Ate) and amlodipine (Aml) alone and in combination on 1-h blood pressure and heart period (HP) in 2K1C renovascular hypertensive rats. n=8. Mean±SEM. ^{b}P<0.05, ^{c}P<0.01 *vs* before administration. ^{e}P<0.05, ^{f}P<0.01 *vs* Ate+Aml (5+0.5 mg/kg).

Groups	Dose/mg·kg^{-1}	SBP/mmHg		DBP/mmHg		HP/ms	
		Before	After	Before	After	Before	After
Control	0	170.0±3.2	172.0±4.2	99.0±2.8	100.0±3.9	172.0±5.7	178.0±4.2
Ate	10	172.0±3.9	161.0±4.2	101.0±3.5	94.0±3.5	167.0±3.9	207.0±6.7^{c}
Aml	1	172.0±3.9	162.0±3.5	102.0±3.2	92.0±3.9	168.0±7.1	172.0±4.9
Ate+Aml	5+0.5	174.0±4.2	159.0±3.9^{b}	102.0±3.9	89.0±4.6	175.0±6.0	180.0±7.0
Ate+Aml	10+1	171.0±3.2	146.0±4.2^{ce}	98.0±2.8	83.0±5.0^{b}	178.0±3.2	179.0±4.2
Ate+Aml	20+2	172.0±3.2	141.0±2.5^{cf}	104.0±3.2	82.0±4.2^{c}	176.0±5.3	170.0±5.7

SBP, systolic blood pressure; DBP, diastolic blood pressure; Before, the mean value of 1-h blood pressure and HP before administration; After, the mean blood pressure and HP value at 24 h after administration.

mg/kg) alone. Of the 3 groups treated with both atenolol and amlodipine, we found that both the intermediate and the high doses reduced SBP at 24 h after administration more effectively than the low dose.

Effects of atenolol and amlodipine on BP and HP for the 24-h period after administration We found that the average SBP in control rats during the 24 h after administration was 171.0±3.5 mmHg. The average SBP over this time was significantly lower in all 5 groups treated with either atenolol, or amlodipine, or both when compared with the control group (Figure 1). The minimal decrease (-13.0 mmHg) was found in rats treated with amlodipine alone and the maximal decrease (-39.0 mmHg) was found in rats treated with both atenolol and amlodipine at a high dose (20.0+2.0 mg/kg). Compared with control, the decrease in average DBP in the experimental groups during the 24 h after administration was also significant but not so profound. The mean value of HP during this time was only increased in the group treated with atenolol (10.0 mg/kg) alone. Of the 3 groups treated with both atenolol and amlodipine, both the intermediate and the high doses reduced SBP more obviously than the low dose (Figure 1).

Effects of atenolol and amlodipine on BPV and HPV for the 24-h period after administration Compared with the control group (13.00±0.53 mmHg for SBPV and 9.40±0.64 mmHg for DBPV), a significant decrease in BPV was found in groups treated with both atenolol and amlodipine at an intermediate (8.80±0.85 mmHg for SBPV and 7.60±0.53 mmHg for DBPV) and high dose (8.50±0.71 mmHg for SBPV and 7.30±0.53 mmHg for DBPV) (Figure 2).

Synergistic interaction of atenolol and amlodipine on BP and BPV for the 24-h period after administration Based on the results presented in Figure 1, the effectiveness of the decrease in BP was calculated for rats individually. Rats with a decrease in BP >20 mmHg (relative to controls) were defined as responders and those with a decrease in BP ≤20 mmHg as non-responders. The results of probability testing are presented in Table 2. We arrived at q values of 2.29 for SBP and 1.45 for DBP for the combination of both atenolol and amlodipine (10.0+1.0 mg/kg). Compared with the mean values for control rats, rats with a decrease in SBPV or DBPV >2 mmHg were defined as responders. According to this criterion, the q values were 1.41 for SBPV and 1.60 for DBPV for the combination of both atenolol and amlodipine (10.0+1.0 mg/kg).

Table 2. Results of probability sum tests for the combination of atenolol (Ate; 10.0 mg/kg) and amlodipine (Aml; 1.0 mg/kg) on blood pressure and blood pressure variability for the 24-h period after administration in 2K1C renovascular hypertensive rats. n=8.

Groups	Dose/ mg·kg^{-1}	SBP P	DBP P	SBPV P	DBPV P
Ate	10	2/8	1/8	2/8	1/8
Aml	1	2/8	2/8	3/8	1/8
Ate+Aml	10+1	8/8	4/8	6/8	3/8
q values		2.29	1.45	1.41	1.60

SBP P, percentage of responders for systolic blood pressure; DBP P, percentage of responders for diastolic blood pressure; SBPV P, percentage of responders for systolic blood pressure variability; DBPV P, percentage of responders for diastolic blood pressure variability. $q=P_{Ate+Aml}/(P_{Ate}+P_{Aml}-P_{Ate}\times P_{Aml})$. If q<0.85, the combination is antagonistic, if q>1.15, the combination is synergistic, if q is between 0.85 and 1.15, the combination is additive.

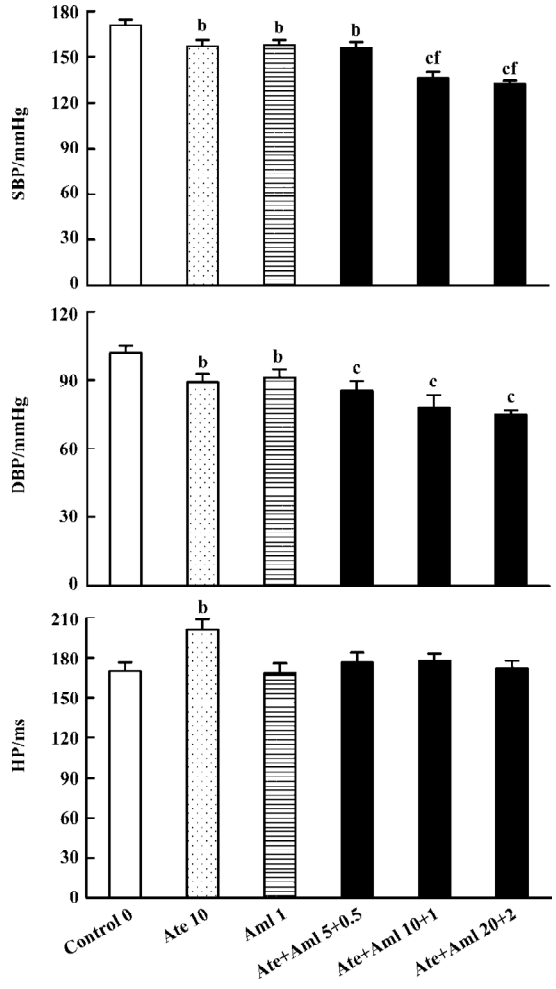

Figure 1. Effects of atenolol (Ate) and amlodipine (Aml) alone and in combination on blood pressure and heart period (HP) in 2K1C renovascular hypertensive rats for the 24-h period after administration. SBP, DBP, and HP are the mean values of systolic blood pressure, diastolic blood pressure, and heart period, respectively, and were calculated from each beat during the 24-h test period. The numbers shown after the compounds are dosages (mg/kg). n=8 rats. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control. $^{f}P<0.01$ *vs* Ate+Aml (5+0.5 mg/kg).

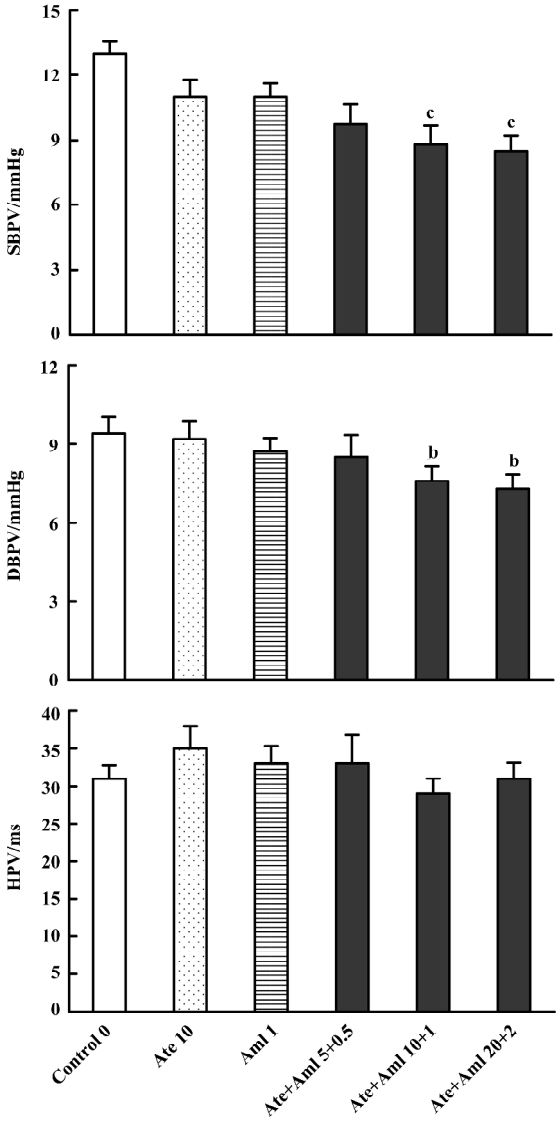

Figure 2. Effects of atenolol (Ate) and amlodipine (Aml) alone and in combination on blood pressure variability and heart period variability (HPV) in 2K1C renovascular hypertensive rats for the 24-h period after administration. SBPV, DBPV, and HPV are the standard deviations of systolic blood pressure, diastolic blood pressure, and heart period, respectively, and were calculated from each beat during the 24-h test period. The numbers shown after the compounds are dosages (mg/kg). n=8 rats. Mean±SEM. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control.

Discussion

The purpose of using fixed-dose combinations of 2 different kinds of antihypertensive drugs in the treatment of hypertension is to obtain increased BP control and to enhance compliance by using a single tablet that is taken once or twice daily[16]. Furthermore, by combining 2 different agents at lower doses, the clinical and metabolic side effects that would be produced by either drug at higher doses can be minimized[17]. Therefore, fixed-dose combinations of antihypertensive drugs could potentially increase BP control, simplify dosage regimens, improve compliance, decrease dose-dependent side effects, and reduce costs as the first-line treatment for hypertension[18]. These advantages make combination antihypertensive therapy the recommended initial treatment, particularly in patients with end-organ damage (EOD) or more severe initial hypertension[19,20]. However, high BP is not a unique factor determining hypertensive EOD. Parati *et al* found that for patients with similar mean hypertension levels for the 24 h after treatment, those whose BPV

levels were lower had less severe organ damage than those with higher BPV levels[4]. Our previous study found that in 60-week-old spontaneously hypertensive rats , the severity of organ damage was positively related to BP (r=0.31–0.32, n=50, P<0.05) and BPV levels (r=0.63–0.65, n=50, P<0.01), and BPV was positively related to the extent of organ damage, so BPV might be a new determinant of EOD[6,21,22].

Therefore, it seems very important to emphasize the role of BPV in antihypertensive therapy. However, it is not clear how BPV can be controlled in the treatment of hypertension. We have previously proposed 2 ways to reduce BPV in antihypertensive therapy[6]: (1) find antihypertensive drugs that decrease BPV even at a dose that does not affect BP, for example ketanserin; and (2) treat patients with long-acting antihypertensive drugs, for example candesartan or amlodipine. In the present work, we investigated a third way to control BPV in the treatment of hypertension, that is, combination therapy.

Both β-blockers and dihydropyridine calcium antagonists are widely used in antihypertensive therapy. The combination of a β-blocker and a dihydropyridine calcium antagonist is a logical choice[23], and is effective for treating hypertensive patients with chronic renal insufficiency and left ventricular hypertrophy[24]. Theoretically, calcium antagonists are vasodilators and tend to increase plasma renin, therefore combining them with β-blockers is a good idea[25]. A combination of these compounds can also neutralize the side effects of both, for example the initial heart rate increases induced by dihydropyridine calcium antagonists, and the rise in peripheral resistance elicited by some β-blockers[26].

In terms of the synergistic effects of atenolol (10.0 mg/kg) and amlodipine (1.0 mg/kg) on BP reduction, the findings of the present work are: (1) the combination of atenolol and amlodipine significantly decreases BP 24 h after administration, whereas neither drug alone had an obvious influence; (2) both atenolol or amlodipine alone reduced the average BP for the 24 h period after administration by less than 15 mmHg, but in combination they decreased SBP by up to 35 mmHg and DBP by approximately 25 mmHg. The q values for SBP and DBP for the 24 h period after administration were 2.29 and 1.45, respectively, which are higher than 1.15, the threshold value for synergistic effects. Concerning the synergism of combined atenolol and amlodipine on the reduction in BPV for the 24 h period after administration, we found that BPV was not influenced by treatment with atenolol or amlodipine alone, but was markedly reduced when they were used in combination. The q values for SBPV and DBPV for the 24 h period after administration were 1.41 and 1.60, respectively. These findings demonstrate that the combination of atenolol and amlodipine has a significant synergistic effect on lowing BP and stabilizing BPV in RVHR.

Our previous studies have demonstrated that long-term (4 months) treatment with a hydrochlorothiazide mixture, which consisted of 5 drugs at low doses, markedly reduced BP and BPV in spontaneously hypertensive rats[9]. Long-term (3 months) treatment with atenolol and nitrendipine had a significant protective effect on organs in spontaneously hypertensive rats[8].

We conclude that atenolol and amlodipine in combination have a synergistic effect in lowering and stabilizing BP in RVHR. Combination therapy is likely to be the optimal way to control BP and reduce BPV in the treatment of hypertension.

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