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# Effects of combination therapy with atenolol and amlodipine on blood pressure control and stroke prevention in stroke-prone spontaneously hypertensive rats[1]

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## Key words

stroke; hypertension; stroke-prone; spontaneouslyhypertensive rats; atenolol; amlodipine; blood pressure; blood pressure variability

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## Abstract

**Aim:** To test the effects of atenolol and amlodipine, either alone or in combination, on blood pressure, blood pressure variability (BPV), baroreflex sensitivity (BRS), and the prevalence of stroke in stroke-prone spontaneously hypertensive rats (SHR-SP). **Methods:** In the first set of the study, 24 8-month-old, female SHR-SP rats were randomly divided into 3 groups. Blood pressure, heart period, and BRS were determined before and after the intragastric administration of atenolol (10 mg/kg) and amlodipine (1.0 mg/kg), either alone or in combination. In the second set of the study, 40 male and 40 female rats were randomly assigned to 1 of the following 4 groups: control, atenolol (10 $mg \cdot kg^{-1} \cdot d^{-1}$), amlodipine (1.0 $mg \cdot kg^{-1} \cdot d^{-1}$), and both (10 male and 10 female in each group). The stroke incident and survival time were recorded. **Results:** Atenolol and amlodipine, either alone or in combination, significantly decreased blood pressure, with the exception of the amlodipine-induced effect on diastolic blood pressure. Meanwhile, only the combination treatment significantly decreased the BPV levels for the same period. The *q*-values calculated by the probability sum analysis were 1.17 and 2.67 for systolic and diastolic blood pressure, respectively, and were 2.48 and 2.10 for systolic and diastolic BPV, respectively, following administration. Neither drug exhibited any significant effect on BRS. Atenolol and amlodipine, either alone or in combination, significantly increased the lifespan of SHR-SP, with the best effect elicited by the combination therapy. **Conclusion:** A significant synergism exists between atenolol and amlodipine in lowering and stabilizing blood pressure in SHR-SP. Combination therapy may be an optimal strategy for the prevention of stroke in hypertension.

## Introduction

Stroke is the third leading cause of death, only preceded by heart disease and cancer in many countries[1]. According to recent estimates published by the World Health Organization, about 15 million people per year fall victim to stroke worldwide. Therefore, prevention is the only possible way to curb the stroke pandemic[2]. Blood pressure level is one of the most consistent and powerful predictors for stroke. As a result, blood pressure control is an important way to reduce the morbidity of stroke[3–5].

Recently, the importance of combination therapy has been well recognized in the treatment of hypertension. Clinically, combination therapy against hypertension using 2 or more drugs from different classes can produce better drug efficacy[6,7]. Both β-adrenergic blockers and dihydropyridine calcium antagonists are widely used in the treatment of hypertension. Atenolol and amlodipine are prototypes of the long-acting drugs from these 2 classes. Several works within our Department (Second Military Medical University, Shanghai, China) have demonstrated that there is significant synergism between atenolol and amlodipine in

lowering and stabilizing blood pressure. For an acute setting, the synergistic effect is at its highest when the dose proportion of the 2 drugs is 10:1 in hypertensive rats[8, 9].

A number of reports have shown that the combination therapy of antihypertension has overt effects on organ protection in hypertensive rats[10]. However, to the best of our knowledge, little information is available regarding the impact of the combination therapy of atenolol and amlodipine on the prevalence of stroke. Given that stroke-prone spontaneously hypertensive rats (SHR-SP) are the most widely used animal models for experimental stroke[11], this study employed this animal model to investigate the synergism between atenolol and amlodipine on blood pressure reduction and stroke prevention.

## Materials and methods

**Drugs and animals** Amlodipine was purchased from Nanjing Pharmaceutical Co (Nanjing, China) and atenolol was purchased from Shanghai Second Pharmaceutical Co (Shanghai, China). SHR-SP of either sex were provided by the Animal Center of our University. They 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 standard food and drinking water. All the animals used in this experiment received humane care and the study was in compliance with the institutional guidelines for the health and care of experimental animals.

**Blood pressure measurements** Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart period were continuously recorded using the previously described technique[12,15]. Briefly, the rats were anesthetized with a combination of ketamine and diazepam. A polyethylene catheter was inserted into the lower abdominal aorta via the left femoral artery for blood pressure measurement and another catheter was inserted into the left femoral vein for phenylephrine administration. A third catheter was placed into the stomach via a mid-abdominal incision for drug administration. After a 2 d recovery period, the animals were placed in individual cylindrical cages. 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 habituation, the blood pressure signal was digitized by a microcomputer. SBP, DBP, and heart period values were determined online. The mean values and standard deviation of SBP and DBP were calculated. The standard deviation of both values obtained was denoted as the quantitative parameter of variability, that is, systolic blood pressure variability (SBPV) and diastolic blood pressure (DBPV) for each rat.

**Determination of baroreflex sensitivity** In the earlier-mentioned blood pressure recording condition, baroreflex sensitivity (BRS) was measured in the conscious rat, by using the previously described method[14,15]. Briefly, a bolus injection of phenylephrine was used to induce a blood pressure elevation. The dose of phenylephrine (5–10 μg/kg) was adjusted to raise the SBP to about 30 mmHg. The slope with the largest correlation coefficient (r) of heart period/SBP was expressed as BRS (ms/mmHg). The mean of the 2 measurements with the proper dose was taken as the final result.

**Stroke symptom observation and morphological examination** To detect the stroke symptoms, the movement of limbs, respiration, diet, fur, and consciousness of all SHR-SP, they were observed twice daily (at 8:00 and 18:00). When the rats died, the brains were removed, and signs of hemorrhage, edema, or infarction were examined and then photographed.

**Probability sum test** To determine whether the drugs were acting synergistically, we used the probability sum test according to previous reports[10,16]. Briefly, compared with the mean values from the control rats, the 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 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 and 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 were 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 was antagonistic, when $q>1.15$, the combination was synergistic, and when $q$ was between 0.85 and 1.15, the combination was additive.

**Experimental protocols**

Acute studies The experiment was performed in 8-month-old, female SHR-SP. They were randomly divided into 3 groups ($n$=8 in each group) and received 10 mg/kg atenolol, 1.0 mg/kg amlodipine, and a 10+1.0 mg/kg combination of these 2 drugs (intragastrically) respectively. The drugs were dissolved in 0.8% carboxymethylcellulose sodium. After about 4 h habituation (from 8:00 to 12:00), blood pressure was recorded during a period of 60 min (from 12:00 to 13:00) and BRS was measured using the earlier-mentioned methods. These values were taken as baseline before administration. After 30 min habituation, a single dose of these drugs was given via an intragastric catheter. About 30 min after drug administration, blood pressure was recorded

for another 60 min (from 15:00 to 16:00 ) and BRS was determined again. These values were defined as after administration values.

Chronic studies The experiment was performed in 6-week-old, SHR-SP of either sex. Forty male and 40 female rats were randomized into 4 groups: the control group and 3 groups treated with 10 $mg \cdot kg^{-1} \cdot d^{-1}$ atenolol, 1.0 $mg \cdot kg^{-1} \cdot d^{-1}$ amlodipine, and a 10+1.0 $mg \cdot kg^{-1} \cdot d^{-1}$ combination of these 2 drugs (intragastrically), respectively ($n$=20 in each group, 10 male and 10 female). The drugs were mixed into the food and administrated consecutively. The survival time was recorded (from birth to death).

**Statistical analysis** The investigators were blind to the procedures during blood pressure and heart period recording, BRS determination, and morphological examination. Statistical analysis data are expressed as the mean±SD. Comparisons between pre- and post-drug were made by paired $t$-tests. Comparisons among 4 or 3 groups were made by one-way ANOVA. In the chronic experiment, the Kaplan-Meier analysis was used to estimate survival probabilities. Log-rank testing was used to evaluate the equality of survival curves. $P$<0.05 was considered statistically significant.

## Results

**Effects of atenolol and amlodipine on blood pressure, heart period, and BRS** The effects of a single dose of atenolol and amlodipine, either alone or in combination, on blood pressure, heart period, and BRS in conscious SHR-SP are shown in Figure 1. Compared with the baseline value, SBP was significantly decreased in all 3 groups (atenolol: 171±33.2 *vs* 190±27.9 mmHg, $P$<0.01; amlodipine: 175±56.8 *vs* 189±51.5 mmHg, $P$<0.05; and in combination: 156±23.4 *vs* 189±23.4 mmHg, $P$<0.01). Compared with the baseline, DBP also significantly decreased in 2 groups ($P$<0.05), but not in the amlodipine group ($P$>0.05). The mean heart period value after administration was markedly higher only in the rats treated with atenolol alone. Compared with the baseline value, BRS did not exhibit any significant changes in all the groups tested.

**Effects of atenolol and amlodipine on SBPV and DBPV** Compared with the baseline value, there was a significant decrease in SBPV and DBPV in the combination group ($P$< 0.05), but not in the groups of atenolol and amlodipine alone ($P$>0.05; Figure 2).

**Synergistic interaction of atenolol and amlodipine on blood pressure and blood pressure variability** Based on the results presented in Figure 1, the effectiveness of the decrease in blood pressure was calculated for the rats individually. Compared with the baseline, the rats with a decrease in blood pressure >20 mmHg were defined as responders and those with a decrease in blood pressure ≤20 mmHg were defined as non-responders. The results of probability testing are presented in Table 1. We arrived at $q$-values of 1.17 for SBP and 2.67 for DBP for the combination of atenolol and amlodipine. Compared with the baseline value, the rats with a decrease in BPV >2 mmHg were defined as responders. According to this criterion, the $q$-values were 2.48 for SBPV and 2.10 for DBPV for the combination of atenolol and amlodipine.

**Effects of atenolol and amlodipine on survival time** Among the 80 rats studied, all the animals that died from stroke were confirmed to display neurological symptoms of stroke and/or brain pathological examination. The survival time expressed by the Kaplan-Meier survival curve is shown

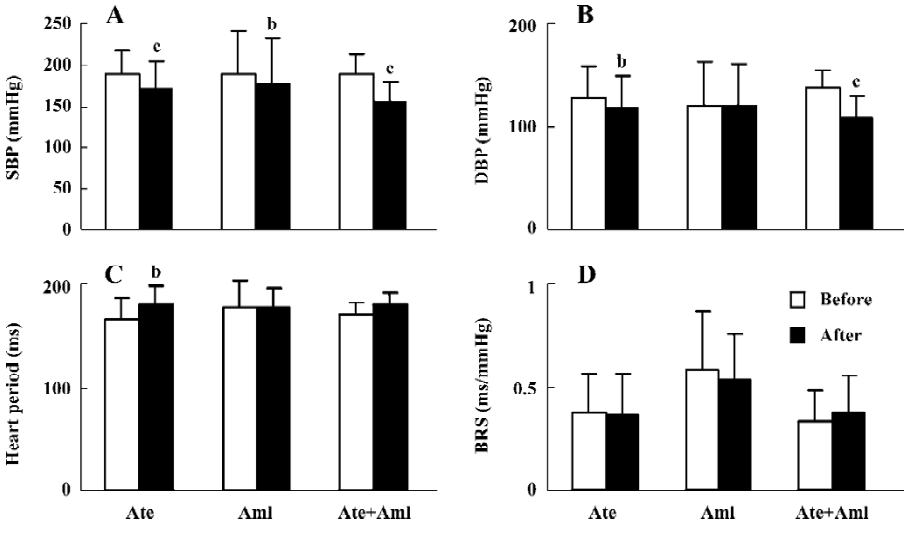


**Figure 1.** Effects of a single dosage of atenolol (Ate, 10 mg/kg) and amlodipine (Aml, 1 mg/kg) alone and in combination (Ate+Aml, 10+1 mg/kg) on systolic blood pressure (A) and diastolic blood pressure (B), heart period (C), and BRS (D) in SHR-SP. Open bars, before intragastric administration; solid bars, after intragastric administration. Values are expressed as mean±SD. $^bP$<0.05, $^cP$< 0.01 *vs* before administration. $n$=8 in each group.

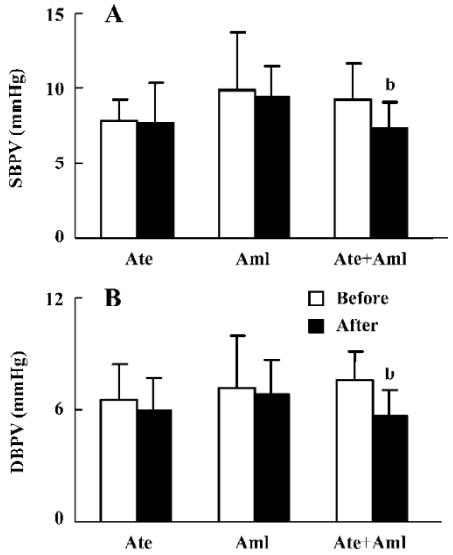


**Figure 2.** Effects of a single dosage of atenolol (Ate, 10 mg/kg) and amlodipine (Aml, 1 mg/kg) alone and in combination (Ate+Aml, 10+1 mg/kg) on systolic (A) and diastolic (B) blood pressure variability in SHR-SP. SBPV and DBPV are the standard deviations of SBP and DBP during the 1 h test period, respectively, Open bars, before intragastric administration; solid bars, after intragastric administration. Values are expressed as mean±SD. $^{b}P<0.05$ *vs* before administration. $n=8$ in each group.

**Table 1.** Results of the probability sum test in female SHR-SP treated with a single dosage of atenolol and amlodipine alone and in combination. *P*-values are the percentages of animals possessing an effective decrease in SBP, DBP (20 mmHg), and BPV (2 mmHg) produced by atenolol and amlodipine alone and in combination. $q>1.15$ means synergism.

| Parameter | *P*-value (%) | *q*-value |
|---|---|---|
| SBP | | |
| Atenolol | 62.5 | |
| Amlodipine | 33.3 | 1.17 |
| Atenolol+amlodipine | 87.5 | |
| DBP | | |
| Atenolol | 12.5 | |
| Amlodipine | 12.5 | 2.67 |
| Atenolol+amlodipine | 62.5 | |
| SBPV | | |
| Atenolol | 12.5 | |
| Amlodipine | 14.3 | 2.48 |
| Atenolol+amlodipine | 62.5 | |
| DBPV | | |
| Atenolol | 25 | |
| Amlodipine | 14.3 | 2.1 |
| Atenolol+amlodipine | 75 | |

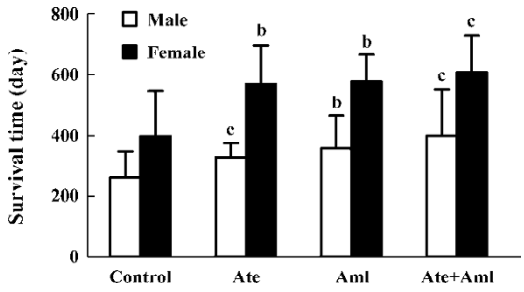


**Figure 3.** Effects of atenolol (Ate) and amlodipine (Aml) alone and in combination on the stroke death in SHR-SP. Eighty rats were randomly divided into 4 groups: the control group and 3 groups treated with atenolol (10 mg·kg$^{-1}$·d$^{-1}$) and amlodipine (1 mg·kg$^{-1}$·d$^{-1}$) alone and in combination (atenolol+amlodipine, 10+1 mg·kg$^{-1}$·d$^{-1}$). Open bars, values of male rats; solid bars, values of female rats. Survival times are expressed as mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ for male treatment group *vs* male control group; female treatment group *vs* female control group. $n=20$, including 10 males and 10 females in each group.

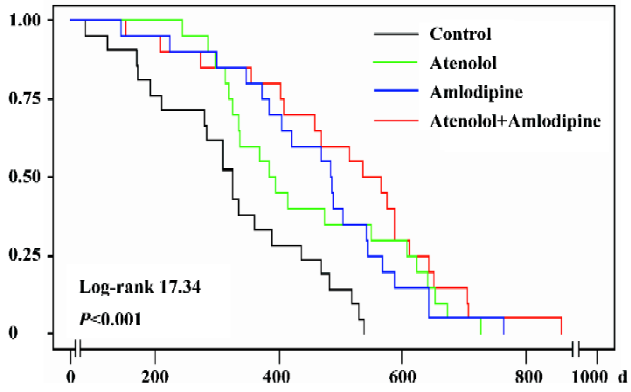


**Figure 4.** Effects of atenolol and amlodipine alone and in combination on the stroke death in SHR-SP. Eighty rats were randomly divided into 4 groups: the control group and 3 groups treated with atenolol (10 mg·kg$^{-1}$·d$^{-1}$) and amlodipine (1 mg·kg$^{-1}$·d$^{-1}$) alone and in combination (atenolol+amlodipine, 10+1 mg·kg$^{-1}$·d$^{-1}$). Kaplan-Meier survival curves by these 4 subgroups. $n=20$, including 10 males and 10 females in each group.

in Figure 4. A significant difference was detected between the control and the 3 drug treatment groups (Log-rank testing $\chi^2=17.34$, $P<0.001$). Compared with the control group, the lifespan of SHR-SP in either sex was both significantly increased by atenolol and amlodipine alone and in combination (male: 328±46.7 d, 357±109 d, and 399±152 d *vs* 262±85.7 d; female: 569±127 d, 576±89.4 d, and 608±121*vs* 397±149 d). In the male rats, survival time was 1.25-fold greater in the atenolol group, 1.36-fold greater in the amlodipine group, and 1.50-fold greater in the combination treatment group. In

the female rats, similar results were obtained. Compared with the control group, the survival time was 1.43-fold greater in the atenolol group, 1.45-fold greater in the amlodipine group, and 1.53-fold greater in the combination group.

## Discussion

Blood pressure level is an important determinant for the prevalence of stroke. The risk of stroke is increased by about 25% with each 10 mmHg increase in SBP[3,4]. Therefore, the use of antihypertensive drugs is an important way of reducing the morbidity of stroke. Combination treatment of different antihypertensive drugs may be an ideal and more effective method for blood pressure control and stroke prevention. The proper combination of different drugs may produce synergy of drug responses, for example, improved blood pressure control and compliance compared with using a single drug alone[17]. In this work, we selected atenolol and amlodipine as the combination with a proportion of 10:1 according to our previous report[9]. Our results confirmed that the combination between atenolol and amlodipine was synergistic on blood pressure control in SHR-SP rats. An obvious decrease in SBP and DPB was found in SHR-SP treated by this combination, with a maximal decrease of SBP (33 mmHg) and DBP (27 mmHg). These values were significantly higher than the treatment of atenolol and amlodipine alone. Meanwhile, the *q*-values for SBP and DBP after administration were higher than 1.15, the threshold value for synergistic effects.

Both β-adrenergic blockers and dihydropyridine calcium antagonists are widely used in antihypertensive therapy. The combination of a β-adrenergic blocker and a dihydropyridine calcium antagonist is a logical choice[18]. Theoretically, calcium antagonists are vasodilators and tend to increase plasma rennin levels, which may be offset by β-blockers[19]. A combination of these compounds can also neutralize the side-effects of both, for example, the initial heart rate decreases induced by atenolol, and the rise in peripheral resistance elicited by some β-blockers[20]. Our results confirmed these benefits. In the atenolol group, the heart period was significantly increased, but not in the combination treatment group.

Elevated blood pressure is not the unique factor determining the occurrence of stroke in hypertension. It has been proposed that BPV may play an important role in determining the prognosis of hypertension. 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 end-organ damage than those with higher BPV levels [21]. Our previous study also confirmed this in hypertensive rats and proposed that BPV might be a new strategy for the treatment of hypertension[22,23], even including its most important complication, stroke. Therefore, it is very important to emphasize the role of BPV in antihypertensive therapy and stroke prevention. In the present work, we found that in SHR-SP, 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 were 2.48 and 2.10, respectively. These findings demonstrate that the combination has an overt synergistic effect on stabilizing BPV in SHR-SP rats.

The most important aspect of this study may be the significantly prolonged lifespan following combination therapy of atenolol and amlodipine, as opposed to either atenolol or amlodipine alone. In the setting of the acute studies, we found that the combination therapy displayed a maximal decrease of SBP and DBP associated with a significant stabilization in blood pressure, which suggests that a decrease of blood pressure and BPV may contribute concurrently to stroke-preventive action in SHR-SP.

In conclusion, atenolol and amlodipine in combination have a synergistic effect in lowering and stabilizing blood pressure in SHR-SP. Combination therapy is likely to be the optimal way of preventing stroke in hypertension.

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