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Effects of Combination Therapy with Levamlodipine and Bisoprolol on Stroke in Rats

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SUMMARY

Aim: To examine the effects of combination with levamlodipine and bisoprolol on stroke in rats. **Methods:** For acute study, Systolic blood pressure (SBP) and heart period (HP) were monitored in conscious stroke prone-spontaneously hypertensive rats (SHR-SP) and sino-aortic denervation (SAD) rats before and after intragastric administration of either drug at a single dose. Rats were subjected to middle cerebral arterial occlusion (MCAO) half an hour after drug administration; sacrificed 24 h later to measure the infarct size. For long-term study, drugs (either alone or in combination) were delivered via food to SHR-SP. The survival time was recorded. **Results:** SBP was significantly reduced by combination therapy both in SHR-SP and SAD rats. Neutralization on heart rate (HR) was observed in combination. The drug combination increased baroreflex sensitivity (BRS) and reduced SBP variability (SBPV). In chronic experiments, the lifespan of SHR-SP rats exposed to the drug combination was longer than that in rats exposed to either drug alone. The infarct area was the smallest in subjects receiving drug combination in SD rats both with and without SAD. **Conclusion:** Combined use of levamlodipine and bisoprolol produced better protection against stroke.

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The first two authors contributed equally to this work.

Introduction

Stroke is the second leading cause of death worldwide with high rate of disability in survivors who often require lifelong assistance [1]. Hypertension is one of the most important risk factors for stroke [2]. Antihypertensive treatment could reduce the stroke risk. Combination of two or more antihypertensive drugs from different classes could achieve identical efficacy with lower doses, and thus decreasing dose-related adverse effects caused by individual drug [3,4]. A previous study from this laboratory in spontaneously hypertensive rats (SHR) suggested that the best ration of the calcium-channel blocker levamlodipine to the β-blocker bisoprolol is 4:1 in terms of lowering blood pressure [5]. In the present study, we examined the effects of this combination on stroke in rats.

Increased blood pressure variability (BPV) and impaired baroreflex sensitivity (BRS) contribute to organ damage as well as the pathogenesis of hypertension and stroke [6,7]. We therefore also examined the effects of the combination on BPV and BRS.

Materials and Methods

Drugs and Animals

Levamlodipine besylate was provided by Shihuida Pharmaceutical Group (Baishan, Jilin, China). Bisoprolol fumarate was purchased

from Aventis Pharma Group (Haikou, China). Male stroke prone-SHR (SHR-SP) and Sprague-Dawley (SD) rats were obtained from the animal center of the Second Military Medical University and housed in a facility with controlled temperature (23–25°C) and lighting (on: 08:00–20:00). Food and tap water were available without restriction. All the animals received humane care in compliance with the institution guidelines for the health and care of experimental animals.

Measurement of Blood Pressure

Systolic blood pressure (SBP) and heart period (HP) were continuously recorded as previously described [8]. Briefly, rats were anesthetized with ketamine and diazepam. 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 stomach via middle abdominal incision for drug administration [9]. The catheters were exteriorized through the interscapular skin. After a 2-day recovery period, rats were placed in individual cages with the aortic catheter connected to a pressure transducer via a rotating swivel that allowed the animals to move freely. Blood pressure was recorded after 4-h habituation. SBP and HP were determined on line for every heartbeat. The standard deviation of SBP was used as a quantitative parameter of SBP variability (SBPV). The heart rate (HR) was calculated based on HP using the following equation: HR = 60000 ms / HP. The mean values of these parameters during a period of 6 h for each rat were used.

Determination of BRS

BRS was measured in conscious rats as previously described [10] prior to and after drug administration. Briefly, blood pressure was raised by about 30 mmHg with a proper dose of phenylephrine. The slope with the largest correlation coefficient (r) of HP/SBP was expressed as BRS (ms/mmHg). The mean of two measurements was taken as the final result.

Sinoaortic Denervation (SAD)

SAD was carried out in male SD rats (12 weeks of age) as previously described [11,12]. Briefly, the rats were anesthetized with a combination of ketamine (50 mg/kg) and diazepam (5 mg/kg) intraperitoneally and then received atropine sulfate (0.5 mg/kg, ip) and procaine benzylpenicillin (60 000 U, im). A midline neck incision was made to denervate aortic baroreceptors bilaterally. The superior laryngeal nerves were cut near the vagi. The superior cervical ganglia were removed. The aortic depressor nerves were sectioned. The carotid sinus baroreceptors were denervated bilaterally by stripping the carotid bifurcation and its branches followed by the application of 10% phenol (in 95% ethanol) to the external, internal, common carotid arteries, and the occipital artery. Sham operation consisted of the midline neck incision and isolation of the neck muscles. Rats were allowed to recover for 1 month prior to the experiments.

Middle Cerebral Arterial Occlusion (MCAO) and Morphological Examination

MCAO was carried out in SAD rats using a suture-occluded method [13–15] at 30 min after drug administration. Briefly, rats were anesthetized with 3.5% chloral hydrate. A monofilament nylon suture (0.26 mm in diameter and 40 mm in length; Beijing Sunbio Biotech Co. Ltd, Beijing, China) was introduced through an incision of the left common carotid artery in the internal carotid artery to occlude the origin of the left middle cerebral arterial for 120 min. Body temperature was maintained at a physiological level during surgery.

For morphological examination, the brain was removed and stored into cold 0.1 M phosphate buffer (pH 7.4) for a few minutes, sliced transversely into seven sections (from the anterior to the posterior), stained with 2% 2,3,5-triphenyl tetrazolium chloride (TTC) for 30 min, and fixed with 4% paraformaldehyde. The infarct area in each section was measured using a computerized image analysis system. Total infarct area of the brain was estimated by adding the infarct area of each section.

Long-Term Treatment in SHR-SP

SHR-SP (4 months of age) randomly received levamlodipine (1 mg/kg/day), bisoprolol (0.25 mg/kg/day), a combination of levamlodipine and bisoprolol (1+0.25 mg/kg/day), or vehicle control via rat chow. The survival time was recorded.

Statistical Analysis

Data are expressed as mean ± SD. The differences prior to and after drug administration were evaluated using paired t-test. One-way analysis of variance (ANOVA) with Tukey's posttest for multiple comparisons was used to analyze the potential difference among drug treatments. 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 Levamlodipine and Bisoprolol in SHR-SP

SBP was significantly decreased by levamlodipine (160 ± 8.6 vs. 177 ± 12.6 mmHg, $P < 0.01$), bisoprolol (163 ± 10.7 vs. 180 ± 8.3 mmHg, $P < 0.01$), or the combination (156 ± 9.6 vs. 175 ± 12.2 mmHg, $P < 0.01$). In comparison with the baseline, the HR was increased by bolus injection of levamlodipine and decreased by bisoprolol. The levamlodipine-induced tachycardia and the bisoprolol-induced bradycardia were temporized by the combination of the two drugs (Figure 1B). SBPV was not affected by any treatment (Figure 1C). BRS was increased by levamlodipine alone (0.53 ± 0.069 vs. 0.32 ± 0.034, $P < 0.01$) as well as the drug combination (0.57 ± 0.077 vs. 0.3 ± 0.045, $P < 0.01$), but not affected by bisoprolol alone (0.28 ± 0.0283 vs. 0.34 ± 0.05) (Figure 1D).

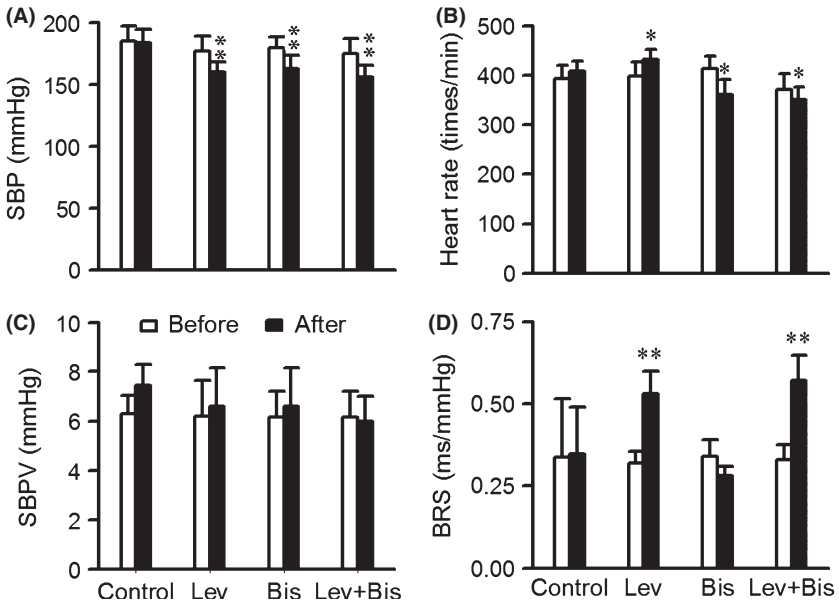

Figure 1 Effects of a single dose of Lev (1 mg/kg), Bis (0.25 mg/kg) and Lev+Bis (1 + 0.25 mg/kg) on SBP (**A**), HR (**B**), SBPV (**C**) and BRS (**D**) in SHR-SP. Open bars, before intragstric administration; solid bars, after intragstric administration. Values are expressed as mean ± SD. **P* < 0.05, ***P* < 0.01 versus before administration, n = 10 in each group. Lev, levamlodipine; Bis, bisoprolol; SBP, systolic blood pressure; HR, heart rate; SBPV, SBP variability; BRS, baroreflex sensitivity; SHR-SP, stroke prone-spontaneously hypertensive rats.

Effects of Levamlodipine and Bisoprolol in SAD Rats

SBP was decreased by levamlodipine or bisoprolol alone, as well as the drug combination in SAD rats (levamlodipine: 137 ± 12.8 vs. 144 ± 12.6 mmHg, $P < 0.05$; bisoprolol: 138 ± 7.92 vs. 146 ± 10.4 mmHg, $P < 0.05$; and in combination: 129 ± 9.54 vs. 144 ± 12.2 mmHg, $P < 0.01$; Figure 2A). In comparison with the baseline, the HR was increased by levamlodipine and decreased by bisoprolol. The levamlodipine-induced tachycardia and the bisoprolol-induced bradycardia were temporized by the combination of these two drugs. SBPV was significantly decreased only by the combination treatment, and not by either drug alone (Figure 2C).

Effects of Levamlodipine and Bisoprolol on Survival of SHR-SP

The lifespan of SHR-SP was prolonged by levamlodipine alone as well as by the drug combination (Figure 3A,B). Bisoprolol alone did not affect the survival time.

Effects on MCAO-Induced Brain Injury in SD Rats

The infarct area caused by MCAO was decreased by levamlodipine alone and by the drug combination (Figure 4). The infarct area in rats receiving the drug combination was smaller than in rats receiving levamlodipine alone. The percentage infarct area relative to the total area was 34.9 ± 6.84% in the vehicle control, 28.0 ± 6.00% in rats receiving levamlodipine alone, 26.6 ± 12.8% in rats receiving bisoprolol alone, and 22.8 ± 10.9% in rats receiving both drugs.

Effects on MCAO-Induced Brain Injury in SAD Rats

The infarct area is response to MCAO was decreased by bisoprolol alone and by the combination treatment (Figure 5). The percentage infarct area was 36.7 ± 4.95% in the vehicle control, 30.5 ± 10.4% in rats receiving levamlodipine alone, 26.1 ± 4.40% in rats receiving bisoprolol alone, and 24.4 ± 5.56% in rats receiving both.

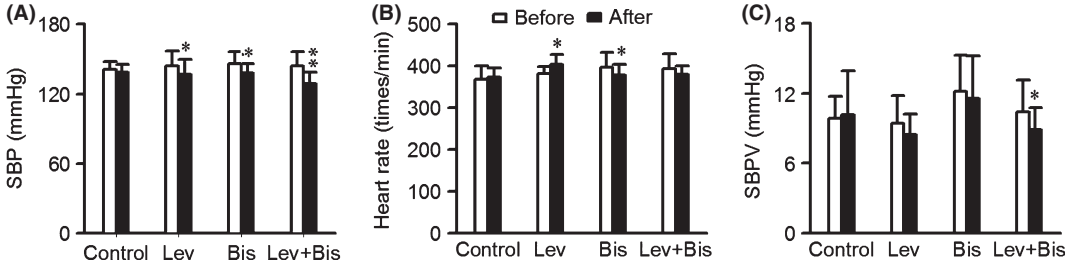

Figure 2 Effects of a single dose of Lev (1 mg/kg), Bis (0.25 mg/kg) and Lev+Bis (1 + 0.25 mg/kg) on SBP (**A**), HR (**B**) and SBPV (**C**) in SAD rats. Values are expressed as mean ± SD. **P* < 0.05, ***P* < 0.01 versus before administration, n = 10 in each group. SAD, sinoaortic denervation.

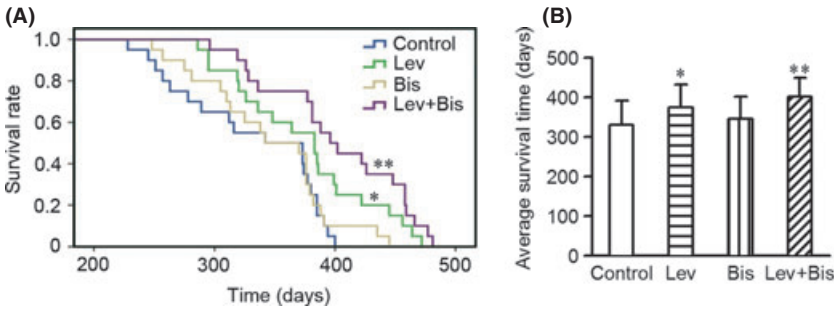

Figure 3 Effects of Lev (1 mg/kg/day), Bis (0.25 mg/kg/day) and Lev+Bis (1 + 0.25 mg/kg/day) on the stroke death in SHR-SP. (**A**) Survival curves of all the rats in four groups. Blue for Control, Green for Lev, Yellow for Bis and Purple for Lev+Bis. (**B**) Histogram for average survival time of SHR-SP in each group. Values are expressed as mean ± SD, n = 20 in each group, **P* < 0.05, ***P* < 0.01 versus Control.

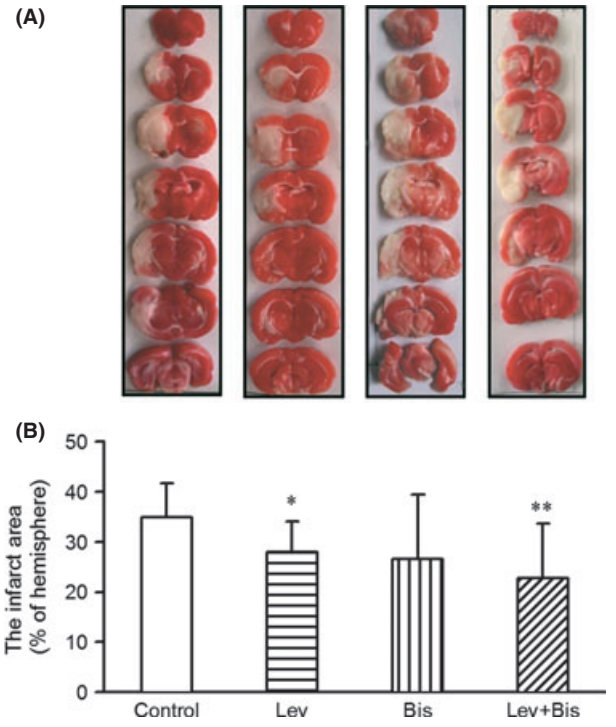

Figure 4 Effects of Lev (1 mg/kg), Bis (0.25 mg/kg) and Lev+Bis (1 + 0.25 mg/kg) on the cerebral injury induced by MCAO in SD rats. (**A**) TTC staining of samples in each group. (**B**) Infarct area (%) of each group. Values are expressed as mean ± SD, n = 10 per group, **P* < 0.05, ***P* < 0.01 versus Control. MCAO, middle cerebral arterial occlusion; SD, Sprague–Dawley; TTC, 2,3,5,-triphenyl tetrazolium chloride.

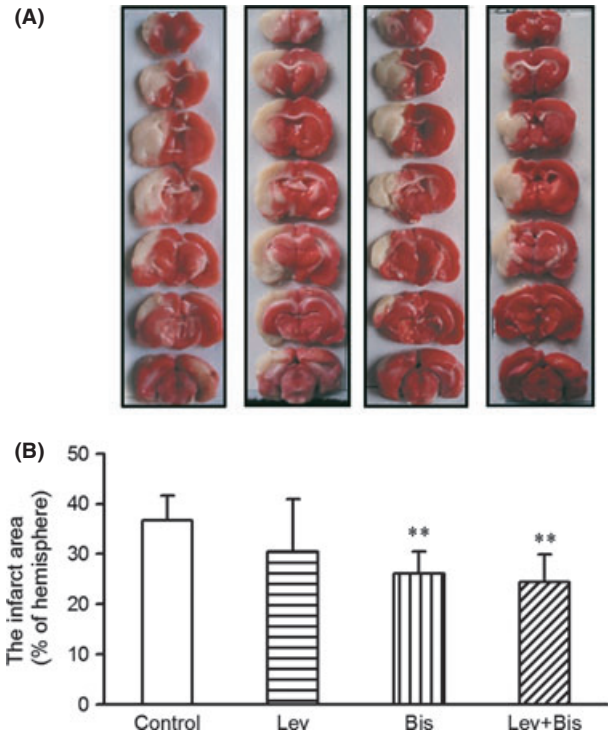

Figure 5 Effects of Lev (1 mg/kg), Bis (0.25 mg/kg) and Lev+Bis (1 + 0.25 mg/kg) on the cerebral injury induced by MCAO in SAD rats. (**A**) TTC staining of samples in each group. (**B**) Infarct area (%) of each group. Values are expressed as mean ± SD, n = 10 per group, **P* < 0.05, ***P* < 0.01 versus Control.

Discussion

In clinical practice, combination therapy with two or more classes of antihypertensive drugs was recommended for hypertension. The advantage of combination regimen includes more rapid blood pressure control and more effective blood pressure reduction. In most cases, the dose could be reduced. As a result, side effects could be decreased. The combination of a β-adrenergic blocker and a calcium antagonist is a common choice [16]. Calcium antagonists are vasodilators and tend to increase HR, while β-blockers decrease HR. Theoretically, the combination of these compounds produces less impact on HR. Here, we showed that the HR is increased by levamlodipine alone and decreased by bisoprolol alone, but no altered in subject receiving both agents in SHR-SP and SAD rats.

Baroreflex function is an independent prognostic factor for acute ischemic stroke [17]. Other factors, including vascular remodeling, inflammation, and oxidative stress, increase the risk of stroke in patients with hypertension [18]. SBPV and BRS contribute to organ damage more than high SBP in SHR rats [7,19].

Stabilizing blood pressure and enhancing arterial baroreflex function may become new strategies in prevention of stroke [20–22].

A variety of chemicals, such as clonidine, moxonidine, folic acid, mecobalamin [23], and gastrodin [24], could enhance arterial baroreflex function in rats. Previous studies also found ketanserin, a selective 5-HT_2A receptor antagonist, could reduce the incidence of fetal stroke in SHR-SP through restoring the impaired BRS in SHR-SP [20]. Another report showed that SAD aggravated damage caused by MCAO and that acetylcholine-alpha 7nAchR participates in the protection of arterial baroreflex against ischemic stroke [25]. In the present study, BRS was enhanced by levamlodipine alone and its combination with bisoprolol in SHR-SP. In SAD rats, SBPV was significantly decreased in rats receiving the combination treatment, but not by rather drug alone. The combination of levamlodipine and bisoprolol has better effects on stabilizing SBP and enhancing BRS than either drug alone. Consistent with these data, the lifespan of SHR-SP was significantly prolonged by the combination treatment with levamlodipine and bisoprolol. In the chronic experiments, we did not measure SBP, HP, SBPV, and BRS to avoid impact on survival. Nevertheless, this represents a limitation in the present study.

In acute experiments, single treatment with both agents markedly reduced the infarct area in SD rats both with and without SAD. In SAD rats, the infarct area was also decreased by bisoprolol alone, which needs further investigation.

Taken together, these results suggest that the combination of levamlodipine and bisoprolol could prevent stroke in rats by decreasing and stabilizing blood pressure as well as enhancing BRS.

Conflict of Interest

The authors declare no conflict of interest.

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