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Synergic Effects of Levamlodipine and Bisoprolol on Blood Pressure Reduction and Organ Protection in Spontaneously Hypertensive Rats

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SUMMARY

Aims: Stroke is a major cause of disability and death worldwide. Hypertension is one of the most important risk factors for stroke. The objective of this work was to study the synergic effects of levamlodipine and bisoprolol on blood pressure reduction and organ protection in spontaneously hypertensive rats (SHR). **Methods:** Blood pressure was continuously monitored in conscious SHR. For acute study, a single dose of drugs was administrated via an intragastric catheter. For chronic study (4 months), drugs were delivered via rat chow. **Results:** A single dose of levamlodipine (from 1 mg/kg), bisoprolol (from 0.125 mg/kg), and their combinations significantly decreased blood pressure. The levamlodipine-induced tachycardia and the bisoprolol-induced bradycardia were temporized by the combination of these two drugs. Upon chronic treatment, this combination also decreased blood pressure variability and reduced organ damage. **Conclusion:** Levamlodipine and bisoprolol produce synergic effects on blood pressure reduction and organ protection in SHR.

Introduction

Stroke is a major cause of disability and death worldwide. Preventive measures that modify risk factors are the most effective strategy to curb the stroke pandemic. Hypertension is one of the most important risk factors for stroke [1–7]. People with hypertension are three to four times more likely to develop stroke than those without hypertension [7]. Randomized controlled trials have shown that single-drug treatment is usually not adequate to achieve blood pressure goal in most hypertensive patients [8,9]. 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 [10,11].

Previous studies showed that combinations of β-blockers and calcium-channel blockers produce synergic effects on reducing and stabilizing blood pressure in hypertensive rats [12,13]. In those studies, atenolol and amlodipine were used as the representative drugs for ß-blockers and calcium-channel blockers, respectively. Amlodipine is a racemic mixture of (*R*)- and (*S*)-amlodipine isomers, but only (*S*)-amlodipine is active. As a result, (*S*)-amlodipine is twice as more potent than amlodipine and has fewer adverse events [14]. In the present work, we examined the synergic effects of (*S*)-amlodipine (levamlodipine) and bisoprolol.

Blood pressure variability (BPV) is an important factor that contributes to organ damage as well as pathogenesis of stroke [15,16]. We therefore, also measured BPV in the present work.

Methods

Animals and Chemicals

Levamlodipine besylate was provided by Shihuida Pharmaceutical Group (Baishan, Jilin, China). Bisoprolol fumarate was purchased from Aventis Pharma Group (Haikou, China). Male SHR rats (16 weeks of age) 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 (08:00–20:00 h light, 20:00–08:00 h dark). Food and tap water were available without restriction. All the animals used in this work received humane care in compliance with institutional animal care guidelines.

Intra-Arterial Blood Pressure Measurements

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart period (HP) were continuously recorded using as previously described [17]. Briefly, rats were anesthetized with ketamine (40 mg/kg, i.p.) and diazepam (6 mg/kg, i.p.). 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 [18]. The catheters were exteriorized through the interscapular skin. Upon monitoring, the aortic catheter was connected to a blood pressure transducer via a rotating swivel that allowed the animals to move freely. Blood pressure was recorded using a microcomputer after 4-h habituation. SBP, DBP, and HP values from every heart beat were determined on line. The mean values of these parameters during a period of 6 h for each rat were calculated.

Long-Term Treatment Study

Levamlodipine and bisoprolol were mixed in the rat chow. The daily doses were: levamlodipine (1 mg/kg/day), bisoprolol (0.25 mg/kg/day), combinations of levamlodipine and bisoprolol (1 + 0.25 mg/kg/day). After 16 weeks of treatment, SBP, DBP, and HP were continuously recorded for 24 h. The standard deviation over the mean was defined as the quantitative parameter for SBP variability (SBPV), DBP variability (DBPV), and HP variability (HPV).

Morphological Examination

The animals were euthanized with an overdose of sodium pentobarbital. The kidneys, aorta, and heart were excised and rinsed in cold physiological saline. Gross examination included kidney weight, renal cortical thickness, renal medullary thickness, heart weight, left ventricular weight, and left ventricular wall thickness. A 30-mm-long segment of thoracic aorta was harvested just below the branch of the left subclavicular artery, and weighted. Ratio of heart weight to body weight (HW/BW), aortic weight to the length of aorta (AW/length), and kidney weight to body weight (KW/BW) were calculated.

Probability Sum Test

Synergistic action was examined using a probability sum test (q test) [19]. A decrease of blood pressure for ≥20 mmHg from the baseline was defined as a response. Rats with a decrease of blood pressure 20 mmHg from the baseline were considered as nonresponders. The synergism was examined with the following equation: $q = P_{A+B}/(P_A+P_B-P_A\times P_B)$, where 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. A q value at <0.85 indicates antagonistic action. A q value at >1.15 indicates synergistic action. A q value between 0.85 and 1.15 indicates additive effects.

Statistical Analysis

Data are expressed as mean ± SD. The differences among groups were evaluated using analysis of variance followed by a two-tailed Student's t-test. The differences prior to and after drug administration were evaluated using Student's t-test of paired comparison.

Results

Acute Effects

Significant decreases in SBP and DBP were observed in all 15 groups of SHR treated with a single dose of levamlodipine (from 1 mg/kg), bisoprolol (from 0.125 mg/kg), and their combination (from 1 + 0.125 mg/kg), respectively. Levamlodipine significantly decreased HP (tachycardia). In contrast, bisoprolol increased HP (bradycardia). The levamlodipine-induced tachycardia and the bisoprolol-induced bradycardia were temporized by the combination of these two drugs (Table 1).

All q values were larger than 1.15 when levamlodipine was used at 1 mg/kg in the combination (Table 2). The largest q value (= 1.69) was obtained with the combination of levamlodipine 1 mg/kg + bisoprolol 0.25 mg/kg.

Effects of Long-Term Treatment

Chronic treatment with levamlodipine, bisoprolol, or their combination significantly decreased both SBP and DBP. HP was not affected with an exception of slight tachycardia in levamlodipine-treated rats (Table 3). Both levamlodipine and bisoprolol significantly decreased SBPV but not DBPV. The combination was more potent on SBPV as well as DBPV than monotherapies (Table 4). HPV was not significantly affected by any treatment.

Organ damage included HW/BW (reflecting cardiac hypertrophy), AW/length (reflecting aortic hypertrophy), and KW (reflecting kidney atrophy) (Table 5). Chronic treatment with levamlodipine or bisoprolol significantly reduced cardiac and aortic hypertrophies. This effect was more pronounced in rats receiving combination treatment. Chronic treatment increased the kidney weight (preventing kidney atrophy) in all three groups (Table 5).

Discussion

According to the reports published by the World Health Organization, about 15 million people fall victim to stroke per year, one-third of whom die and another third are left permanently disabled [20]. Therefore, prevention is the only possible way to curb the stroke pandemic [8,21,22]. Hypertension is the most important

Table 1 The effects of a single dose of levamlodipine (Lev), bisoprolol (Bis), and their combination on systolic (SBP) and diastolic blood pressure (DBP), and heart period (HP) in conscious freely moving spontaneously hypertensive rats. Before: before drug administration; After: after drug administration (average values during a period of 6 h). ** $P < 0.01$ versus Before

Dose (mg/kg)		SBP (mmHg)		DBP (mmHg)		HP (ms)	
Lev	Bis	Before	After	Before	After	Before	After
1	0	170±10.3	155±10.1**	112±14.5	96.0±11.9**	138±11.5	133±8.74**
2	0	165±13.6	139±10.2**	122±19.9	89.2±14.4**	145±13.3	138±10.3
4	0	162±12.2	130±9.23**	114±10.0	83.0±11.7**	141±17.6	126±6.46**
0	0.125	175±20.3	163±18.6**	122±25.8	111±24.3**	140±15.4	149±19.4*
0	0.25	173±7.60	155±7.67**	126±8.71	106±10.0**	139±12.1	162±11.3**
0	0.5	178±11.3	152±14.4**	128±19.0	99.0±19.7**	141±16.4	180±26.9**
1	0.125	174±9.53	160±9.36**	124±9.09	110±7.56**	139±16.4	136±10.5
1	0.25	176±14.1	152±8.03**	123±9.52	98.0±11.2**	143±22.6	155±20.1**
1	0.5	179±22.5	145±15.3**	123±17.6	100±12.2**	136±10.3	152±18.8
2	0.125	172±14.5	147±13.2**	124±19.6	97.0±14.9**	138±8.60	140±9.30
2	0.25	179±17.0	149±13.1**	126±14.5	101±9.20**	138±10.1	140±4.70
2	0.5	175±18.9	141±15.0**	126±17.7	93.0±13.3**	151±21.3	159±20.7**
4	0.125	177±15.9	138±15.9**	127±19.0	90.4±15.8**	153±11.0	155±5.10
4	0.25	176±18.1	135±19.8**	126±16.6	86.7±16.5**	137±8.30	142±12.6
4	0.5	168±13.1	126±11.0**	118±10.9	81.0±8.80**	144±11.8	153±14.5

Table 2 The result of probability sum test for the combination of levamlodipine (Lev) and bisoprolol (Bis)

Dose (mg/kg)			
Lev	Bis	Probability	*q*
1	0	0.1	–
2	0	0.5	–
4	0	0.9	–
0	0.125	0.1	–
0	0.25	0.5	–
0	0.5	0.8	–
1	0.125	0.3	1.32
1	0.25	0.6	1.69
1	0.5	0.8	1.28
2	0.125	0.6	0.90
2	0.25	0.8	0.94
2	0.5	1.0	–
4	0.125	0.9	0.77
4	0.25	1.0	–
4	0.5	1.0	–

modifiable risk for stroke and blood pressure level is an important determinant for stroke [22]. Blood pressure control is an important way to reduce the morbidity of stroke. Accordingly, expected benefits of blood pressure lowering for stroke risk reduction are broadly consistent across different population subgroups. To better control blood pressure, combination therapy is recommended [22,23].

In the acute experiments of the current study, we used an intragastric catheter for drug administration. This catheter was previously inserted into stomach via middle abdominal incision under

Table 3 The effects of long-term treatment with levamlodipine (Lev), bisoprolol (Bis), and their combination on systolic (SBP) and diastolic blood pressure (DBP), and heart period (HP) in conscious freely moving spontaneously hypertensive rats. Values are the means of 24 h. * $P < 0.05$, ** $P < 0.01$ versus Control

Dose (mg/kg)				
Lev	Bis	SBP (mmHg)	DBP (mmHg)	HP (ms)
0	0	182±10.6	129±10.3	199±9.26
1	0	165±6.22**	104±9.80**	190±7.36*
0	0.25	165±7.24**	109±7.29**	205±5.29
1	0.25	161±11.9**	104±14.5**	193±14.3

anesthesia [18]. The main advantages of this method include minimal level of stress the ability to correctly record the online changes of blood pressure during drug administration. In the chronic

Table 4 The effects of long-term treatment with levamlodipine (Lev), bisoprolol (Bis), and their combination on systolic (SBPV) and diastolic blood pressure variability (DBPV), and heart period variability (HPV) in conscious freely moving spontaneously hypertensive rats. * $P < 0.05$, ** $P < 0.01$ versus Control.

Dose (mg/kg)				
Lev	Bis	SBPV (mmHg)	DBPV (mmHg)	HPV (ms)
0	0	12.3±1.55	9.16±1.18	17.9±3.83
1	0	10.9±1.23*	8.50±1.23	20.3±4.67
0	0.25	10.7±1.15*	8.70±1.52	18.1±3.38
1	0.25	10.1±1.35**	7.87±1.18*	15.1±2.68

Table 5 The effects of long-term treatment with levamlodipine (Lev), bisoprolol (Bis), and their combination on hypertensive organ damages in spontaneously hypertensive rats. HW: heart weight; BW: body weight; AW: aorta weight, KW: kidney weight. * $P < 0.05$, ** $P < 0.01$ versus Control

Dose (mg/kg)		Index		
Lev	Bis	HW/BW	AW/Length	KW/BW
0	0	3.55±0.26	2.53±0.36	6.02±0.27
1	0	3.24±0.16**	2.11±0.36*	6.59±0.75*
0	0.25	3.33±0.17*	2.18±0.15*	6.46±0.51*
1	0.25	3.11±0.25**	2.01±0.34**	6.55±0.51**

experiments, we used a modified probability sum test to verify potential synergic action [18].

Previous studies demonstrated that calcium blocker and β-blocker produce maximum synergism on blood pressure reduction and stabilization as well as on organ protection. Specific combinations included nitrendipine + atenolol [24] and amlodipine + atenolol [13,19]. In the current work, we used levamlodipine and bisoprolol, two antihypertensive drugs with relatively fewer side effects, in comparison with amlodipine and atenolol, respectively [14,23,25].

Our results demonstrated the following advantages with the levamlodipine/bisoprolol combination therapy: (1) synergistic effects in blood pressure reduction and blood pressure stabilization; (2) a temporization of tachycardia and bradycardia induced by two drugs respectively; (3) a better organ protection. These advantages may contribute significantly to stroke prevention.

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Conflict of Interest

The authors declare no conflict of interest.

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