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Drug Synergism of Antihypertensive Action in Combination of Telmisartan with Lercanidipine in Spontaneous Hypertensive Rats

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To examine drug synergism between angiotensin II AT1-receptor blocker and Ca^{2+} channel blocker for lowering blood pressure (BP), telmisartan and lercanidipine were orally injected into to telemetered-spontaneous hypertensive rats and BP was monitored. The highest doses of both drugs (7.66 mg/kg of telmisartan and 1.92 mg/kg of lercanidipine) were clinically relevant at 80 and 20 mg human equivalent doses, respectively, and denoted as dose 1. After constructing the dose-response curve using 0 (vehicle-treated control), 1/16, 1/8, 1/4, 1/2 and 1 doses, all possible combinations of both drugs were tested. Drug synergism in combination therapy of telmisartan with lercanidipine was assessed by calculating the interaction index (γ) as evaluated by $\gamma < 1$. We found statistically significant drug synergism in the investigated (telmisartan:lercandipine) combinations of (1/8:1/4), (1/4:1) and (1/8:1). Our results suggest that the combination therapy of telmisartan and lercanidipine at lower doses are effective in lowering BP, and also reduce side effects caused by maximal doses of each drug. Therefore, drug combination of AT1-receptor blocker with Ca^{2+} channel blocker is a clinically important tool for the management of hypertension and hypertension-related cardiovascular risks.

Key words: Hypertension, Drug synergism, SHR, Telmisartan, Lercanidipine

INTRODUCTION

Hypertension is considered an important risk factor in cardiovascular diseases and complications resulting from hypertensive vascular disease (Guyton, 1991). The lowering of blood pressure (BP) is important for reducing the risk of cardiovascular events, and the combination of angiotensin II AT_1-receptor blocker (ARB, -sartans) and vasoselective dihydropyridine Ca^{2+} channel blocker (CCB, -dipines) is a rational approach for reducing BP in the treatment of hypertension and hypertension-related cardiovascular diseases. Previous studies have shown that a combination of candesartan and nifedipine increases capillary density and preserves the ultrastructure of the left ventricular myocardium in a rate model of isoproterenol-induced heart failure (Okuda et al., 2005). Cilnidipine was also applied in combination with an ARB to control blood pressure without any significant adverse effects. It also successfully reduces elevated heart rate, which is a possible risk factor for cardiovascular events (Nagahama et al., 2007). Telmisartan plus amlodipine effectively lowers BP at all clinically relevant doses up to −26.5 mmHg of systolic BP (SBP), and almost 9 out of 10 patients achieve diastolic BP (DBP) control (Littlejohn et al., 2009). The administration of olmesartan combined with azelnidipine strongly inhibits neointimal formation and reduces oxidative stress and inflammatory markers in the injured artery (Inaba et al., 2009). Moreover, this combination synergistically blunts oxidative stress at least partly through the inhibition of phosphatidy-

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linositol 3-kinase (PI3K)-linked protein kinase B (Akt/PKB) activity and enhances the beneficial effects of these drugs in atherosclerosis compared with monotherapy. Thus, ARB/CCB combination therapy could be more effective than CCB monotherapy in controlling BP.

Telmisartan and lercanidipine are approved for the treatment of hypertension either as monotherapy or in combination with other antihypertensive agents (Battershill and Scott, 2006). Telmisartan is widely used in the treatment of hypertension and hypertension-related cardiovascular end-organ damage (de Gasparo et al., 2000). Due to its similar structure to pioglitazone, telmisartan is also used as a unique, moderately potent, selective partial agonist of peroxisome proliferators-activated receptor γ (PPARγ), a nuclear hormone receptor that is a target for insulin sensitizing drugs (Benson et al., 2004; Schupp et al., 2004; Kota et al., 2005). Lercanidipine is a vasoselective dihydropyridine CCB used for the treatment of hypertension (Borghi, 2005; Prandin et al., 2007). Besides reducing BP, lercanidipine produces pleiotropic effects such as antioxidant effects via reducing matrix metalloproteinase (MMP) activity (Yue et al., 2004; Martinez et al., 2006). Taking into consideration that enhanced oxidative stress is a major factor that leads to the activation of MMPs (Grote et al., 2003; Nelson and Melendez, 2004; Ra and Parks, 2007) and reactive oxygen species (ROS) are involved in vascular remodeling of hypertension via MMP activation (Grote et al., 2003). Therefore, a combination therapy of telmisartan with lercanidipine might be beneficial for the treatment of hypertension and hypertension-related cardiovascular end-organ damage with metabolic disorders.

This study was designed to examine drug synergism between telmisartan and lercanidipine for lowering BP in spontaneous hypertensive rat (SHR), a genetically-manipulated hypertensive model that simulates human hypertension. Our results suggest that combination therapy of telmisartan with lercanidipine results in drug synergism to lower BP in SHR.

MATERIALS AND METHODS

Animals

Male SHRs (200-220 g; Charles River Lab.) were fed ad libitum with fresh tap water and standard chow diet (Ralston Purina Diet). Animals were housed one per cage and were raised in a room controlled for temperature (22 ± 2°C), humidity (50 ± 5%), and lighting (12 h dark-light cycle, lights on 7:00 AM.). After the 1-week acclimatization period, animals were used for experiments, and all efforts were made to minimize animal suffering. Protocols were approved and performed according to the Guide for the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by Chungnam National University Animal Care and Use Committee.

Measurement of blood pressure and heart rate using the telemetry system

SHRs were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and maintained at 37°C on a servo-controlled, heated rodent operating table. SHRs had a BP catheter attached to a telemeter inserted into their abdominal aorta as previously described (Shin et al., 2009). After the procedure, they were allowed to recover for 2 weeks (Morgan et al., 2000; Griffiths et al., 2001; Shin et al., 2009). Hemodynamic data were measured over a 10 s interval every 5 min.

Experimental protocol

Twenty-four SHRs were given vehicle, various doses of telmisartan, lercanidipine, or a combination of two drugs at 9:00 AM in a blinded and randomized order. The highest doses used for both drugs (7.66 mg/kg of telmisartan and 1.92 mg/kg of lercanidipine) were clinically relevant at 80 and 20 mg human equivalent doses (HEDs), respectively, and was denoted as dose 1. As shown in Table I, half of telmisartan or lercanidipine dose 1 was 3.83 or 0.96 mg/kg, respectively, and was denoted as 1/2 dose. Similarly, 1.92, 0.96 and 0.48 mg/kg of telmisartan or 0.48, 0.24 and 0.12 mg/kg of lercanidipine were denoted as 1/4, 1/8 and 1/16 doses. Dose 0 was used for vehicle-treated control. BP (mmHg) and heart rate (HR, beats/min) were measured for 3 days prior to drug administration, and the average of these responses was used as a baseline measurement (Webb et al., 1998; Shin et al., 2009). Each treatment by single injection (n=8) was performed with an interval of several days between each injection for washout and return to higher SBP (≈200 mmHg). Mean arterial pressure (MAP) and HR were recorded continuously in telemetered rats, and data were collected over 24 h after injection. Drugs were prepared in 0.15 N NaOH for telmisartan and ethanol : PEG 400 : Cremophor® EL : normal saline (0.5:1:0.2:98.3, v/v%) for lercanidipine and control rats received either solvent as a vehicle. The area over the curve (AOC) value of MAP was calculated as the change from baseline.

Calculation of interaction index

Drug synergism was evaluated by calculating the interaction index using PharmTools Pro software

(McCary Group). The index, denoted by γ, is defined by the isobolar relation as

$$\frac{a}{A}+\frac{b}{B}=\gamma$$

where A and B are the dose of drug A (alone) and B (alone), respectively, that give the specified effect and a and b are the combination doses that produce this effect level (Tallarida, 2002).

Statistics

The AOC values of drug-treated SHR were compared to vehicle-treated SHR and the statistical analysis was performed by two-way ANOVA and paired *t*-test. Statistical significance was indicated by $p < 0.05$.

RESULTS

Dose-response curve for telmisartan or lercanidipine

To construct a dose-response curve for either telmisartan or lercanidipine, various concentrations shown in Table I were applied to SHRs and MAP was measured. The data shown in Fig. 1 illustrates the dose-response curves for both drugs. The AOC values in both telmisartan- (Fig. 1A) and lercanidipine-treated (Fig. 1B) groups were increased in a dose-dependent manner. BP for 24 h after injection (mmHg × hour) at dose 1 of telmisartan (7.66 mg/kg) or lercanidipine (1.92 mg/kg) was significantly reduced to 668.2 ± 42.3 from 126.2 ± 50.6 of control and to 285.4 ± 63.4 from 15.8 ± 53.0 of control, respectively.

Table I. Dosing protocols for telmisartan and lercanidipine

Dose expressed in this study	Drugs			
	Telmisartan		Lercanidipine	
	Solvent	0.15 N NaOH	EtOH:PEG 400:Cremophor® EL:N/S = 0.5:1.0:0.2:98.3 (v/v%)	
	Dose			
	SHR (mg/kg)	Human Equivalent (mg)	SHR (mg/kg)	Human Equivalent (mg)
0	0	0	0	0
1/16	0.48	5	0.12	1.25
1/8	0.96	10	0.24	2.5
1/4	1.92	20	0.48	5
1/2	3.83	40	0.96	10
1	7.66	80	1.92	20

Animal dose (mg/kg) was calculated by human equivalent dose (HED, mg)/60 kg/0.174 where BSA-CF (body surface area-conversion factor) = 0.174 for 60 kg of human and 300 g of SHR.
EtOH, ethanol; PEG 400, polyethylene glycol 400; N/S, normal saline; SHR, spontaneous hypertensive rat

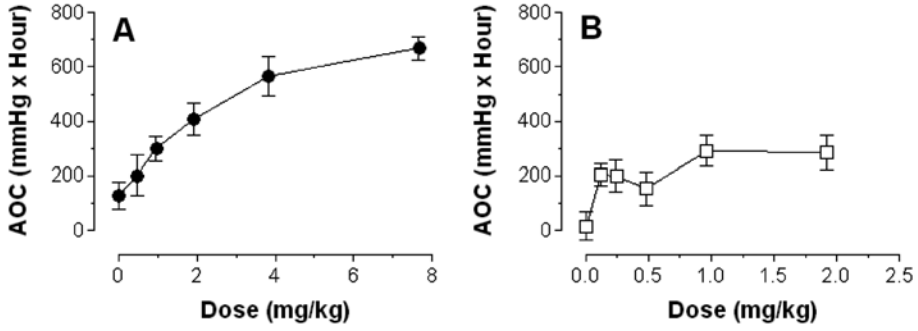

Fig. 1. Dose-response curve for telmisartan (**A**) or lercanidipine (**B**) on lowering blood pressure (BP) in spontaneous hypertensive rats (SHRs). Twenty-four conscious-telemetered SHRs that had similar decreases in mean arterial pressure (MAP) were selected and the average of BP values measured for 3 days prior to drug administration was used as the baseline measurement. According to the dosing protocol shown in Table I, various concentrations of telmisartan or lercanidipine were orally administered by a single injection and the area over the curve (AOC) values of MAP was calculated as the change from baseline. Values represent 24-h averages for each treatment group (n = 8) and are expressed as the mean ± S.E.M. Dose 0 indicates vehicle-treatment.

Effects of combination therapy of telmisartan with lercanidipine

To examine the effect of co-treatment of telmisartan and lercanidipine, all possible combinations of both drugs with various concentrations used in dose-response curve experiments were made and applied to SHRs, and MAP was measured. The results shown in Fig. 2 illustrates that the dose-response curve of telmisartan was shifted to the left with the addition of lercanidipine. All mean value changes of MAP and HR in SHR treated with telmisartan or lercanidipine alone or in combination were presented in Tables II and III, respectively. All AOC values for SHR treated with telmisartan or lercanidipine alone or in combination are presented in Table IV. The AOC value of dose 1 of telmisartan or lercanidipine was 668.2 ± 42.3 (mmHg × hour) or 285.4 ± 63.4, respectively, and 691.3 ± 47.4 at the (1:1) dose combination. In the case of the (1/4:1) combination of telmisartan and lercanidipine, the AOC value was 627.3 ± 58.1, which was significantly greater than that of the 1/4 dose for telmisartan (407.8 ± 59.3) or 1 dose for lercanidipine (285.4 ± 63.4), and comparable to that of the (1:1) combination. Moreover, the AOC value at (1/4:1) combination was similar to that of the telmisartan 1 dose (668.2 ± 42.3), despite reduction of the telmisartan dose from dose 1 (7.66 mg/kg) to dose 1/4 (1.92 mg/kg). All combinations reduced heart rate [HR; (beat/min) × hour)], and did not show any reflex tachycardia (Table V).

Interaction index of combination therapy

To investigate which combinations showed drug synergism, interaction indices were calculated. As shown in Table VI, the interaction indices at (1/8:1/4), (1/4:1) and (1/8:1) combinations of telmisartan with lercanidipine were 0.282, 0.318 and 0.325, respectively, which were less than 1. Thus, these three combinations were identified to exhibit drug synergism and these effects were statistically significant.

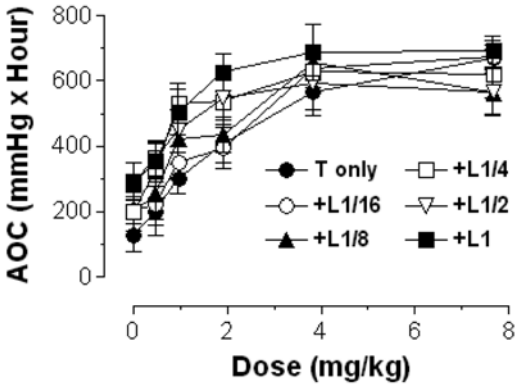

Fig. 2. Dose-response curve for the combined treatment of telmisartan with lercanidipine in lowering blood pressure (BP) in spontaneous hypertensive rats (SHRs). Twenty-four conscious-telemetered SHRs that had similar decreases in mean arterial pressure (MAP) were selected and the average of BP values measured for 3 days prior to drug administration was used as the baseline measurement. According to the dosing protocol as shown in Table I, various concentrations of telmisartan (0, 0.48, 0.96, 1.92, 3.83 and 7.66 mg/kg) combined with varying doses of lercanidipine (0, 0.12, 0.24, 0.48, 0.96 and 1.92 mg/kg) were orally administered by a single injection. The area over the curve (AOC) values of MAP was calculated as the change from baseline, and dose-response curves for telmisartan monotherapy and combination with lercanidipine were plotted. Values represent 24-h averages for each treatment group (n = 8) and are expressed as the mean ± S.E.M. Dose 0 means vehicle-treatment in lieu of telmisartan.

DISCUSSION

This study demonstrates that oral co-administration of lower doses of telmisartan and lercanidipine reduces BP in SHR to a similar extent as high-dose of telmisartan monotherapy and to a greater extent than high-dose lercanidipine monotherapy. The (1/8:1/4), (1/

Table II. Mean value changes of BP (mmHg) in SHR treated with telmisartan or lercanidipine alone or in combination

Dosage (mg/kg)		Telmisartan					
		0	0.48	0.96	1.92	3.83	7.66
Lercanidipine	0	172.8 ± 3.3[a] 177.6 ± 3.7[b]	175.2 ± 2.5	173.2 ± 1.1	168.8 ± 3.4	157.6 ± 3.0	154.9 ± 3.6
	0.12	179.3 ± 2.4	177.5 ± 2.2	172.5 ± 1.6	165.6 ± 3.8	158.7 ± 3.3	155.9 ± 4.1
	0.24	178.2 ± 3.7	174.7 ± 2.4	171.1 ± 1.2	165.3 ± 2.7	158.0 ± 2.8	154.3 ± 4.8
	0.48	176.4 ± 3.1	172.4 ± 2.4	165.9 ± 1.0	160.1 ± 2.9	157.5 ± 2.2	152.4 ± 3.7
	0.96	173.1 ± 2.7	170.1 ± 2.1	169.5 ± 2.7	160.6 ± 3.6	156.4 ± 3.4	154.8 ± 1.6
	1.92	173.4 ± 1.7	169.9 ± 2.0	167.1 ± 2.7	156.1 ± 2.9	153.2 ± 2.5	150.7 ± 2.9

[a]Values when used 0.15 N NaOH as a vehicle for telmisartan.
[b]Values when used EtOH:PEG400:Cremophor® EL:NS=0.5:1.0:0.2:98.3 (v/v %) for lercanidipine.
Vaules represent the mean ± S.E.M. for each treatment group (n=8). Mean value changes of BP (mmHg) of mean arterial pressure (MAP) for 24 h were calculated.

Table III. Mean value change of HR (beat/min) in SHR treated with telmisartan or lercanidipine alone or in combination

Dosage (mg/kg)		Telmisartan					
		0	0.48	0.96	1.92	3.83	7.66
Lercanidipine	0	329.4 ± 9.0[a] 241.7 ± 12.5[b]	328.6 ± 3.3	343.4 ± 9.1	341.6 ± 11.3	342.9 ± 12.6	354.6 ± 6.3
	0.12	313.8 ± 3.2	326.1 ± 4.9	341.4 ± 8.3	341.1 ± 7.2	336.8 ± 12.5	359.3 ± 8.0
	0.24	315.6 ± 4.8	320.7 ± 6.0	336.6 ± 8.7	351.9 ± 10.7	333.0 ± 11.3	357.9 ± 13.8
	0.48	314.7 ± 3.7	316.8 ± 6.2	326.1 ± 8.5	330.1 ± 7.6	336.4 ± 7.9	350.2 ± 8.2
	0.96	324.9 ± 7.6	321.1 ± 3.5	340.3 ± 11.7	327.0 ± 6.8	347.2 ± 12.0	344.1 ± 8.8
	1.92	327.6 ± 10.1	327.1 ± 6.1	332.7 ± 7.9	334.8 ± 6.0	337.4 ± 7.8	352.3 ± 7.2

[a]Values when used 0.15 N NaOH as a vehicle for telmisartan.
[b]Values when used EtOH:PEG400:Cremophor® EL:NS=0.5:1.0:0.2:98.3 (v/v %) for lercanidipine.
Vaules represent the mean ± S.E.M. for each treatment group (n = 8). Mean value changes of HR (beat/min) for 24 h were calculated.

Table IV. AOC values (mmHg×hour) for BP in SHR treated with telmisartan or lercanidipine alone or in combination

Dosage (mg/kg)		Telmisartan					
		0	0.48	0.96	1.92	3.83	7.66
Lercanidipine	0	126.2 ± 50.6[a] 15.8 ± 53.0[b]	201.4 ± 76.6	298.7 ± 45.0	407.8 ± 59.3	567.0 ± 72.6	668.2 ± 42.3
	0.12	204.3 ± 40.7	223.2 ± 64.7	350.3 ± 49.5	393.6 ± 62.6	637.9 ± 34.1	674.7 ± 51.3
	0.24	197.9 ± 59.6	253.2 ± 78.4	422.1 ± 42.7	433.2 ± 53.3	656.3 ± 46.4	563.3 ± 69.7
	0.48	197.8 ± 69.8	324.7 ± 86.1	527.9 ± 64.5	536.2 ± 80.7	630.4 ± 49.1	621.6 ± 73.2
	0.96	290.7 ± 55.6	362.4 ± 53.5	446.8 ± 64.5	548.0 ± 69.8	594.1 ± 84.6	567.6 ± 71.9
	1.92	285.4 ± 63.4	353.0 ± 54.8	504.3 ± 69.5	627.3 ± 58.1	687.4 ± 85.8	691.3 ± 47.4

[a]Values when used 0.15 N NaOH as a vehicle.
[b]Values when used EtOH:PEG 400:Cremophor® EL:N/S = 0.5:1.0:0.2:98.3 (v/v %) as a vehicle.
Values represent the mean ± S.E.M. for each treatment group (n = 8). Baseline values were the average of blood pressure values measured for 3 days prior to drug administration. The area over the curve (AOC) values of mean arterial pressure (MAP) for 24 h were calculated by change from baseline.

Table V. AOC values [(beat/min)×hour] for heart rate in SHR treated with telmisartan or lercanidipine alone or in combination

Dosage (mg/kg)		Telmisartan					
		0	0.48	0.96	1.92	3.83	7.66
Lercanidipine	0	807.8 ± 214.2[a] 623.4 ± 270.7[b]	784.4 ± 87.7	558.7 ± 221.1	595.7 ± 211.7	519.0 ± 270.2	324.0 ± 170.5
	0.12	1228.5 ± 78.7	825.5 ± 124.8	616.3 ± 200.2	435.3 ± 239.5	860.1 ± 178.9	360.1 ± 221.4
	0.24	1119.5 ± 113.4	923.0 ± 141.4	694.4 ± 220.1	578.9 ± 227.7	982.8 ± 117.6	295.7 ± 300.4
	0.48	1202.0 ± 110.2	1032.4 ± 152.2	957.1 ± 254.0	938.0 ± 224.4	770.1 ± 210.7	327.9 ± 173.7
	0.96	1047.9 ± 144.9	918.6 ± 109.9	636.4 ± 270.2	854.3 ± 153.1	539.7 ± 278.3	566.6 ± 225.1
	1.92	930.9 ± 183.6	793.8 ± 163.0	817.2 ± 210.2	737.5 ± 133.0	807.4 ± 172.6	305.6 ± 176.0

[a]Values when used 0.15 N NaOH as a vehicle.
[b]Values when used EtOH:PEG 400:Cremophor® EL:N/S = 0.5:1.0:0.2:98.3 (v/v %) as a vehicle.
Values represent the mean ± S.E.M. for each treatment group (n = 8). Baseline values were the average of heart rate values measured for 3 days prior to drug administration. The area over the curve (AOC) values of heart rate for 24 h were calculated by change from baseline.

4:1) and (1/8:1) combinations of telmisartan plus lercanidipine were identified to exhibit drug synergism, indicating that the drug efficacy at these combinations is similar in lowering BP to one induced by a fixed-dose (1:1) combination. Therefore, the combined therapy of telmisartan and lercanidipine with lower doses might have two advantages as compared with full doses of each individual drug: 1) full pharmacological

Table VI. Interaction index for telmisartan + lercanidipine

Method	Model	Dose		Interaction Index	95% Confidence Limits	ANOVA	p-value	Interpretation
		Telmisartan	Lercanidipine					
Isobole	Linear regression	1/8	1/4	0.282	0.233~0.331	Significant	$p < 0.05$	Synergism
		1/4	1	0.318	0.244~0.392	Significant	$p < 0.05$	Synergism
		1/8	1	0.325	0.270~0.380	Significant	$p < 0.05$	Synergism

efficacy and 2) lower incidence of adverse effects induced by high doses.

The strategy of ARB/CCB fixed-dose combination therapy has been widely applied to the clinical management of hypertension. In a randomized, double-blind, placebo-controlled, factorial study involving 1940 patients with a mean baseline seated BP level of 164/102 mmHg (SBP/DBP), the combination of olmesartan medoxomil (40 mg/day) and amlodipine besylate (10 mg/day) was associated with a mean SBP reduction of 30.1 mmHg after 8 weeks of treatment (Chrysant et al., 2008; Oparil and Weber, 2009). A greater reduction in SBP (35.8 mmHg) was observed with a combination therapy consisting of valsartan (160 mg/day) and amlodipine (5-10 mg/day) for 6 weeks in patients with a mean baseline BP of approximately 171/112 mmHg. This effect was greater than that (31.8 mmHg) for lisinopril plus hydrochlorothiazide administered in combination (Poldermans et al., 2007). In another clinical trial involving patients with less severe hypertension at a baseline BP level of 156.7/99.1 mmHg), the combination therapy of valsartan (160 mg/day) and amlodipine (10 mg/day) for 8 weeks resulted in a mean SBP reduction of 27.8 mmHg, and this dose was equivalent to the maximal marketed dose of the drug when administered as a fixed-dose combination in Europe (Philipp et al., 2007; Mourad, 2008). Thus, the results of these recent clinical trials with ARB/CCB combination therapy suggest that this therapeutic strategy potentially lowers BP, especially SBP, and provides a useful tool in the management of hypertension and hypertension-related cardiovascular risk.

Various animal models have been used to study to simulate human hypertension. These animal models include non-genetic and genetic models. Genetic models of hypertension such as SHR are suitable for identifying factors involved in the development of inherited human hypertension (Okamoto and Akoi, 1963). Although further interpretation of experimental data is needed to apply these findings to humans, experimental investigation using animal models provides useful guidelines and the establishment of therapeutic strategies to manage hypertension. In these small animal models, reliable measurement of BP is critical to estimate the efficacies of drugs and drug candidates. Since the telemetry method for direct measurement of BP used in this study can be performed in conscious and freely moving animals, this is accepted as the most reliable tool for monitoring the dynamic nature of BP (Kurtz et al., 2005). Therefore, our results suggest the pharmacological efficacies and drug synergism for the combined administration of telmisartan and lercanidipine for the management of hypertension.

In SHR, a circadian rhythm in HR traced by telemetry showed the great changes of HR ranging from 300 to 400 beats/min (Lemmer et al., 2004; Witte et al., 2004). Our results demonstrated that all drug-treatment groups do not show any significant changes in HR as compared with control (Tables III and V). In particular, (1/8:1/4), (1/4:1) and (1/8:1) combinations observed to exhibit drug synergism did not show any significant changes in HR. Therefore, the combination therapy of telmisartan and lercanidipine with lower doses did not cause any significant reflex tachycardia in SHR.

In conclusion, the combination therapy of telmisartan with lercanidipine showed drug synergism to lower BP in SHR, suggesting that the combination therapy of ARBs with CCBs may be beneficial for the management of hypertension. Therefore, the low dose combination of telmisartan and lercanidipine may reduce side effects caused by maximal doses of each drug, and this combination therapy could be a clinically important tool to lower BP in patients with hypertension and hypertension-related cardiovascular risks.

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