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Bioequivalence of Samchundang Lercanidipine Tablet 10 mg to Zanidip Tablet (Lercanidipine Hydrochloride 10 mg) by Liquid Chromatography with Tandem Mass Spectrometry

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ABSTRACT – The purpose of the present study was to evaluate the bioequivalence of two lercanidipine hydrochloride tablets, Zanidip tablet (LG Life Sciences Ltd., Korea, reference drug) and Samchundang Lercanidipine tablet 10 mg (Sam Chun Dang Pharm. Co. Ltd., Korea, test drug), according to the guidelines of Korea Food and Drug Administration (KFDA). After adding an internal standard (amlodipine maleate) to human serum, serum samples were extracted using hexan-isoamyl alcohol (100 : 1, v/v). Compounds were analyzed by liquid chromatography/tandem mass spectrometry. This method showed linear response over the concentration range of 0.05 - 20 ng/mL with correlation coefficient of 0.9999. The lower limit of quantitation using 0.5 mL of serum was 0.05 ng/mL which was sensitive enough for pharmacokinetic studies. Thirty healthy male Korean volunteers received each medicine at the lercanidipine hydrochloride dose of 20 mg in a 2 × 2 crossover study. There was a one-week washout period between the doses. Serum concentrations of lercanidipine were monitored by an LC/MS/MS for over a period of 24 hr after the administration. AUC_t (the area under the serum concentration-time curve from time 0 to 24 hr) was calculated by the linear trapezoidal rule method. C_{max} (the maximum serum drug concentration) and T_{max} (the time to reach C_{max}) were compiled from the serum concentration-time data. Analysis of variance was carried out using logarithmically transformed AUC_t and C_{max}. No significant sequence effect was found for all of the bioavailability parameters, indicating that the crossover design was properly performed. The 90% confidence intervals of the AUC_t ratio and the C_{max} ratio for Samchundang Lercanidipine/Zanidip were log 0.9505 - log 1.2258 and log 0.9987 - log 1.2013, respectively. These values were within the acceptable bioequivalence intervals of log 0.80 - log 1.25. Thus, the criteria of the KFDA guidelines for the bioequivalence was satisfied, indicating Samchundang Lercanidipine tablet 10 mg and Zanidip tablet are bioequivalent.

Key words – Lercanidipine, Zanidip, Samchundang Lercanidipine, LC/MS/MS, Bioequivalence

Lercanidipine hydrochloride, (±)2-[(3,3-diphenylpropyl)methylamino]-1,1-dimethyl- ethyl methyl (4RS)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride, is a vasoselective dihydropyridine calcium channel antagonist used in the treatment of hypertension. It is a highly lipophilic drug that exhibits a slower onset and longer duration of action than other calcium channel antagonists.[1-4]

Lercanidipine is completely absorbed from the gastrointestinal tract following oral administration but undergoes extensive saturable first-pass metabolism. Peak plasma concentrations occur about 1 to 3 hr after oral administration. Lercanidipine is extensively metabolized mainly to inactive metabolites and about 50% of an oral dose is excreted in the urine. The terminal elimination half-life is about 2 to 5 hr.[4-6]

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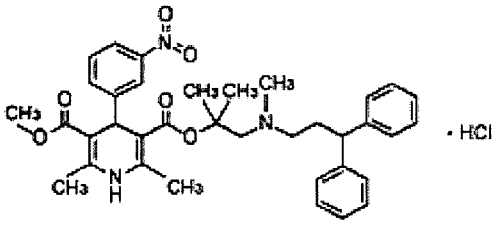
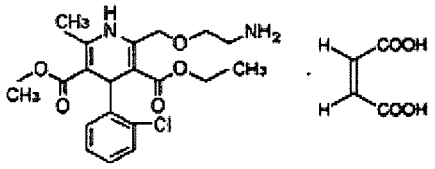
Lercanidipine can be measured in serum using liquid chromatography with tandem mass spectrometry (LC/MS/MS).[6-8] The method is sufficiently sensitive to allow the determination of the pharmacokinetics of the lercanidipine in biological fluids after administration of clinically useful oral doses. The LC/MS/MS method presented in this paper, was validated in the study of lercanidipine hydrochloride bioequivalence, and developed for the purpose of providing a rapid, simple and sensitive method for the determination of lercanidipine in human serum.[6] Thus, the aim of this study was to evaluate the bioequivalence of two lercanidipine hydrochloride tablets with the reported method, which was conducted in accord with KFDA guidelines.[9]

Materials and Methods

Materials and reagents



(A)



(B)

Figure 1—Chemical structures of (A) lercanidipine hydrochloride and (B) amlodipine maleate.

Lercanidipine hydrochloride (Figure 1A) was supplied from Sam Chun Dang Pharm. Co. Ltd. (Seoul, Korea). Amlodipine maleate (Figure 1B) was supplied from Hana Pharm Co. Ltd. (Seoul, Korea). Methanol (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA) and the other chemicals were of HPLC grade or highest quality available. A Milli-Q® (Millipore Co., Milford, MA, USA) water purification system was used to obtain the purified water for the HPLC. The test medication, Samchundang Lercanidipine (10 mg lercanidipine hydrochloride tablet, Sam Chun Dang Pharm. Co. Ltd., Korea) and the reference medication, Zanidip (10 mg lercanidipine hydrochloride tablet, LG Life Sciences Ltd., Korea) were supplied in the form of tablets.

Dissolution Test

The *in vitro* dissolution tests, as second equivalence criterion of two lercanidipine preparations, were carried out using the dissolution apparatus II (paddle method) of K.P. VIII at 37 ± 0.5°C, 50 rpm and 900 mL of the dissolution media, pH 1.2, 4.0, 6.8 buffer solution and deionized water (D.W.). Drug release testing should be conducted on 12 individual dosage units of the test and the reference preparations used in the bioequivalence (BE) studies. Samples were removed at 5, 10, 15, 30, 45, 60, 90 and 120 min (by 120 min at pH 1.2) filtered and assayed by HPLC method. Finally, the dissolved lercanidipine content was expressed as percent of stated amounts.

The acceptance criteria for assessment of equivalence of dissolution profiles between two preparations are as follows. When the average dissolution from reference preparation reaches 85% within 15 min, the average dissolution from test preparation should also reach 85% within 15 min. When the average dissolution from reference preparation reached 85% after more than 15 min, the average dissolution from test preparation should not be deviated by more than 15% from that of the reference preparation at two time points.[9)]

Selection of volunteers

The study population consisted of thirty healthy male Korean volunteers with an average age of 22.93 years and an average weight of 68.27 kg. The volunteers were selected after passing a clinical screening procedure including a physical examination and laboratory tests (blood analysis; hemoglobin, hematocrit, RBC, WBC, platelet, differential counting of WBC, total protein, albumin, sGOT, sGPT, alkaline phosphatase, total bilirubin, cholesterol, creatinine, blood urea nitrogen, and glucose fasting and urine analysis; specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and cast). The volunteers were excluded if there was any possibility of their being sensitive to this type of medication, had a history of any illness of the hepatic, renal or cardiovascular systems, or a history of excessive alcohol intake or other medications. This was done to ensure that the existing degree of variation would not be due to an influence of illness or other medications.

Blood sampling from volunteers

All of the volunteers avoided taking other drugs for at least one week prior to the study and until its completion. They also refrained from consuming xanthine-containing foods, alcoholic beverages and other beverages for 12 hr prior to each dosing and until the collection of the last blood sample. Each volunteer received a single dose of 20 mg of lercanidipine hydrochloride in a standard 2 × 2 crossover model in a randomized order. Since the half-life of 20 mg lercanidipine hydrochloride was reported as 2-5 hr[4-6)] after oral administration, we had a one-week washout period between the doses. All of the participants signed a written consent form after they had been informed of the nature and details of the study in accordance with the Korean Guidelines for Bioequivalence Test.[9)] Subjects fasted for at least 10 hr before each drug administration and continued to fast up to 4 hr. At 8:00 a.m., their median cubital vein was cannulated (JELCO™, 22G, Johnson & Johnson Medical, Pomezia, Italia) and 8 mL of blood samples were drawn into Vacutainer® (Becton Dickins

and Company, Franklin Lakes, NJ, USA). The doses (two lercanidipine tablets; 20 mg lercanidipine hydrochloride) were taken at 8:30 a.m. of each dosing day along with 240 mL of water. At 4 hr after the oral administration, standardized meals were given to all of the subjects. Approximately 8 mL of blood samples were collected via the cannula at the following times; predose, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr after the administration. On each occasion, the blood sample was centrifuged immediately, and serum sample was frozen at − 80°C until the LC/MS/MS analysis.

LC/MS/MS analysis of lercanidipine in serum samples

An LC/MS/MS method modified from the reported LC/MS/MS methods[6-8] was developed and validated for lercanidipine assay in serum samples. A Varian LC/MS/MS system (Palo Alto, CA, USA) consisted of a ProStar Dynamix autosampler, a ProStar-410 binary pump, and a 1200L triple quadrupole mass spectrometer equipped with an electrospray ionization source. Varian MS workstation (version 6.5 software) was used for data acquisition and processing.

The chromatographic separation was achieved on a Symmetry® MS C18 (2.1 × 50 mm, 3.5 μm) at 45°C. The mobile phase was prepared by mixing acetonitrile and aqueous formic acid (0.2%) in the ratio of 70:30 (v/v) at the flow rate of 0.32 mL/min.

The electrospray ionization (ESI) mass spectrometer was operated in the positive ion mode. The electrospray capillary voltage was set to 80 V and nitrogen was used as a drying gas for solvent evaporation. The scan time was 0.7 sec and the detector multiplier voltage was set to 1650 V. Multiple reaction monitoring (MRM) of the precursor-product ion transitions from *m/z* 612.3 to *m/z* 280.0 for lercanidipine and from *m/z* 409.0 to *m/z* 238.0 for amlodipine (as internal standard, I.S.) was used for quantitation (Figure 2).

The primary stock solution of lercanidipine was prepared at 1000 μg/mL in methanol and stored at 4°C. Lercanidipine stock solution was serially diluted with methanol and added to drug-free serum to obtain final concentrations of 0.05, 0.2, 0.5, 1, 5, 10 and 20 ng/mL for the preparation of calibration curve. In order to assess the intra-day coefficient of variation (C.V.) and accuracy for serum samples, the standard samples were analyzed. The lower limit of quantitation (LLOQ) was determined from S/N=5. The precision and accuracy for inter-day assay were assessed at the same concentration and repeated for five different days.

After thawing at room temperature and vortexing briefly, an aliquot of each sample (500 μL) was pipetted into a clean test tube and amlodipine solution (45 μL, 1 μg/mL) and 200 μL of

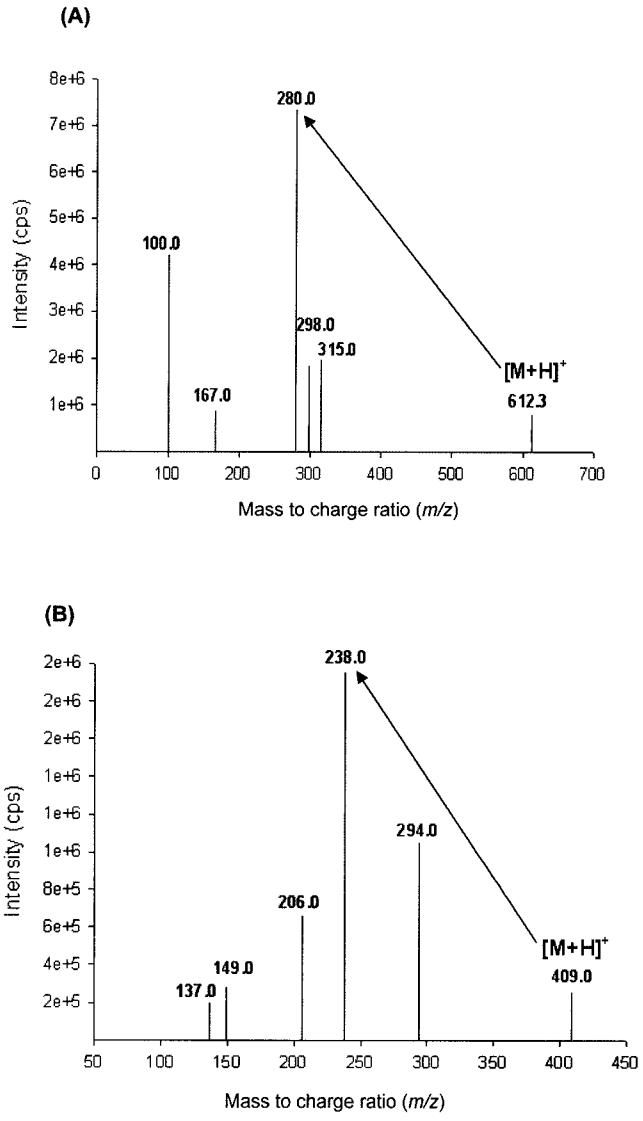


Figure 2—Product ion mass spectra used in multiple reaction monitoring for (A) lercanidipine and (B) amlodipine determinations.

0.1 M NaOH were added. After adding hexan-isoamyl alcohol (100:1, v/v) and vortexing for 20 sec, the mixture was centrifuged at 3000 rpm for 10 min and kept at − 70°C for 20 min. The organic layer was separated and evaporated to dryness under the nitrogen stream. The residue was reconstituted with 200 μL of mobile phase, then 15 μL of the solution was injected into the LC/MS/MS system, and the peak area and retention time of selected ions were recorded.

Statistical analysis of pharmacokinetic parameters

Each volunteer received an oral dose of 20 mg of lercanidipine hydrochloride in a standard 2 × 2 crossover model in a randomized order. Pharmacokinetic parameters such as AUC_t,

Table I – *Dissolution Data of Two Lercanidipine Preparations in Four Dissolution Media*

Dissolution media	pH 1.2		pH 4.0[a]		pH 6.8[a]		Water[a]	
Preparations	Ref.	Test	Ref.	Test	Ref.	Test	Ref.	Test
Mean	88.3	84.7	92.3	86.5	95.3	84.4	89.9	78.6
S.D.	1.6	2.5	2.0	2.2	2.5	3.7	0.7	3.5
C.V.	1.81	2.95	2.17	2.54	2.62	4.38	0.78	4.45

[a]Percent of dissolved lercanidipine content within 15 min.

C_{max} and T_{max} were calculated from serum concentration-time curves. C_{max} and T_{max} were recorded as actual measurement values and AUC_t was calculated by trapezoidal formula in 0-24 hr. Their ratios (test/reference) using log-transformed data, together with their means and 90.0% confidence intervals, were analyzed with two-way analysis of variance (ANOVA) that performed with the K-BE Test program® at a significant level of 0.05.[10] The bioequivalence of two lercanidipine hydrochloride tablets were estimated by AUC_t and C_{max}. T_{max} was used as a reference value.

Results and Discussion

Dissolution Test

Both preparations were released more than 85% of lercanidipine within 15 min in pH 4.0, pH 6.8 buffer solution and water, and 90 min in pH 1.2 buffer solution. The release profiles of two lercanidipine preparations were very similar at all dissolution media (Table I and Figure 3).

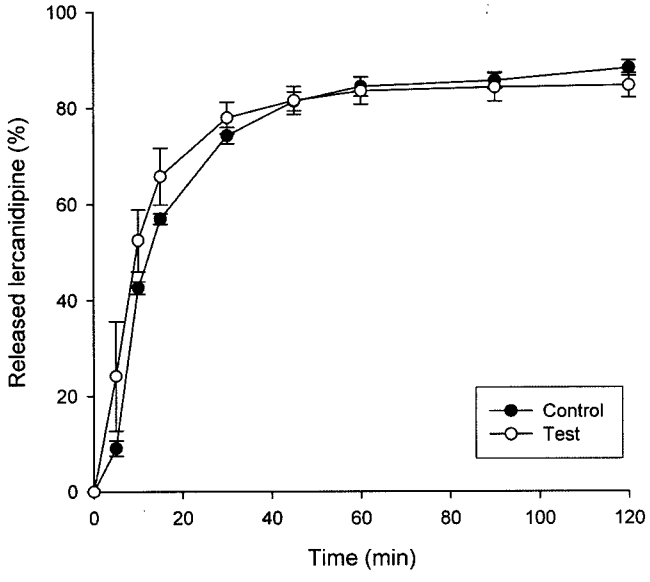


Figure 3–Dissolution profiles of lercanidipine from Zanidip (●, reference) and Samchundang Lercanidipine tablets (○, test) in pH 1.2 buffer. Data are presented as mean ± S.D..

LC/MS/MS analysis of lercanidipine in serum samples

The method was validated according to the FDA guidance and international guidelines.[11] In this LC/MS/MS method, lercanidipine and amlodipine were well separated from the biological background under the described chromatographic conditions at retention times of 0.5 - 0.7 min, respectively (Figure 4B). The peaks were of good shape, completely resolved one. No interference with constituents from serum was observed (Figure 4A).

The calibration curve was obtained by analyzing seven samples. The curve was linear in the whole range tested (0.05-20 ng/mL) and described by following equation: $Y = 0.1819X + 0.01046$ (X = lercanidipine concentration (ng/mL), Y = ratio of peak areas) with a correlation coefficient of 0.9999. In the range of 0.05 - 20 ng/mL, the accuracy percentage was ranged from 85.51 to 112.2%. The intra-day precision percentage was ranged from 6.45 to 15.4% with the inter-day precision from 3.56 to 13.1%. These results indicate that the present method has a satisfactory accuracy and precision.

Pharmacokinetic Analysis

The developed method was successfully used for a bioequivalence test in which serum concentrations of lercanidipine in thirty healthy male volunteers were determined up to 24 hr after the oral administration of 20 mg lercanidipine hydrochloride. Figure 5 shows the mean serum concentration-time

Table II – *Precision and Accuracy for the Determination of Lercanidipine in Human Serum (n = 5)*

Concentration (ng/mL)	Precision (C.V.%)		Accuracy% (n = 5)
	Intra-day (n = 5)	Inter-day (n = 5)	
0.05	15.4	7.20	87.53
0.2	11.4	4.89	85.51
0.5	12.9	13.1	91.75
1	6.45	3.56	93.69
5	12.3	3.76	112.2
10	7.13	8.76	103.6
20	11.1	8.32	104.9

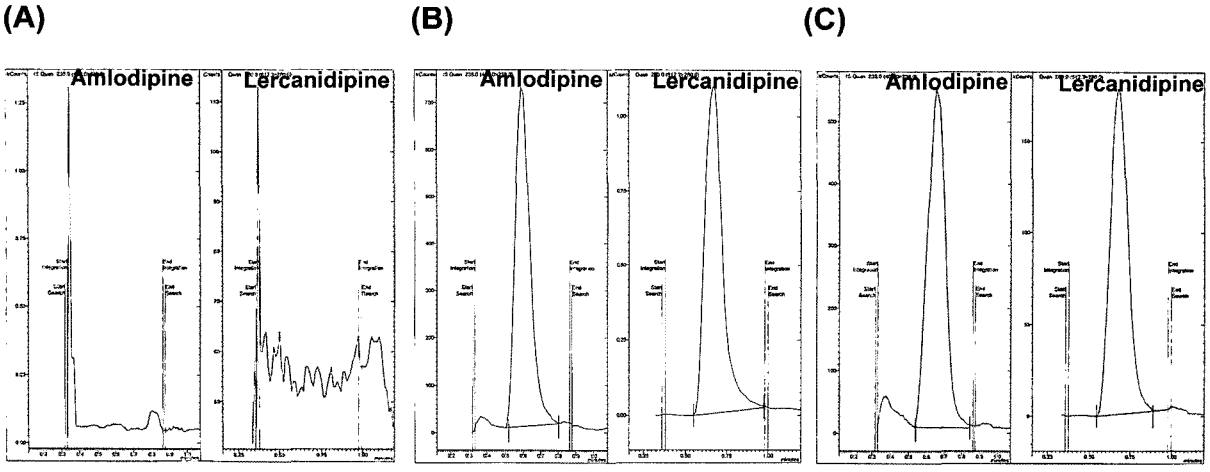


Figure 4—Chromatograms of (A) blank human serum, (B) blank human serum spiked with lercanidipine (10 ng/mL) and internal standard (I.S., amlodipine 1 μg/mL) and (C) serum sample 1 hr after oral administration of two 10 mg lercanidipine tablets (The serum concentration of lercanidipine corresponds to 1.90 ng/mL).

curves of lercanidipine following oral administration of Zanidip and Samchundang Lercanidipine tablets, and descriptive statistics of the derived pharmacokinetic parameters such as AUC_t, C_{max}, and T_{max} for two preparations are summarized in Table III.

AUC_t, C_{max}, and T_{max} for lercanidipine were 21.13±11.44 ng·hr/mL (Zanidip) and 22.35±11.49 ng·hr/mL (Samchundang Lercanidipine), 7.30±4.22 ng/mL (Zanidip) and 7.52±3.65 ng/mL (Samchundang Lercanidipine), and 1.78±0.97 hr (Zanidip) and 1.39±0.73 hr (Samchundang Lercanidipine), respectively (Table III). The differences of the means of the test to reference medication for AUC_t and C_{max} were 5.77% and 3.01% which are generally accepted if the differences of mean values for AUC_t and C_{max} lie within ±20%.

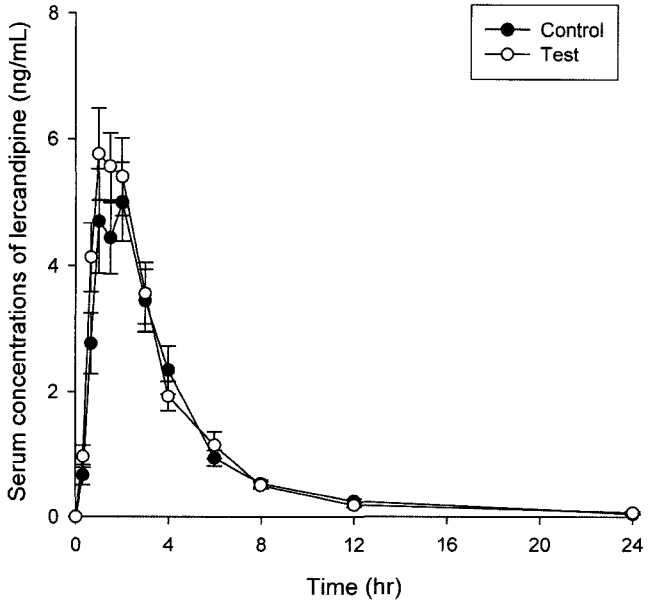


Figure 5—Mean (±S.E., n=30) serum concentration-time curves of lercanidipine following oral administration of Samchundang Lercanidipine (○) and Zanidip (●) tablets at the dose of 20 mg lercanidipine hydrochloride.

Bioequivalence test of lercanidipine hydrochloride products

No significant sequence effect was found for all of the bioavailability parameters indicating that the crossover design was properly performed. The parametric 90% confidence intervals for AUC_t and C_{max} (Table IV) were in the range of log 0.9505 - log 1.2258 and log 0.9987 - log 1.2013, respectively, which were satisfied with the accepted bioequivalence criteria of log 0.80 - log 1.25.[12] Geometric means of the parameters such as AUC_t and C_{max} of the test drug were similar to those of the reference drug, which proved that there was no significant difference between the bioavailability of Zanidip (reference drug) and Samchundang Lercanidipine (test drug).

Conclusions

This LC/MS/MS method was suitable for the analysis of lercanidipine in human serum samples collected for bioequivalence studies. Using this method, the bioequivalence of two different lercanidipine hydrochloride tablet formulations was examined at a dose of 20 mg in 30 healthy normal male volunteers. It can be concluded that Samchundang Lercanidipine is bioequivalent to Zanidip on the basis of the pharmacokinetic and statistical analysis results obtained in this study, and that the two formulations may be prescribed interchangeably in medical practice.

Table III – *Bioavailability Parameters in Normal and Logarithmic Scales for Each Subject Obtained after Oral Administration of Zanidip and Samchundang Lercanidipine Tablets at the Lercanidipine Hydrochloride Dose of 20 mg*

Subjects	Zanidip Tablet					Samchundang Lercanidipine Tablet				
	AUC_t (ng·hr/mL)	Ln AUC_t	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)	AUC_t (ng·hr/mL)	Ln AUC_t	C_{max} (ng/mL)	Ln C_{max}	T_{max} (hr)
A1	26.32	3.27	6.98	1.94	4.00	13.60	2.61	8.38	2.13	0.67
A2	10.43	2.34	6.20	1.82	1.00	15.48	2.74	5.40	1.69	2.00
A3	18.71	2.93	8.64	2.16	2.00	22.28	3.10	6.25	1.83	1.00
A4	15.06	2.71	5.31	1.67	2.00	12.53	2.53	4.68	1.54	1.00
A5	7.84	2.06	1.50	0.41	1.00	8.24	2.11	2.74	1.01	0.33
A6	43.01	3.76	10.50	2.35	3.00	21.75	3.08	8.51	2.14	1.00
A7	10.82	2.38	4.78	1.56	1.50	20.97	3.04	7.34	1.99	0.67
A8	10.44	2.35	1.90	0.64	1.00	10.80	2.38	2.11	0.75	0.67
A9	32.67	3.49	8.08	2.09	3.00	33.37	3.51	9.45	2.25	3.00
A10	21.85	3.08	9.00	2.20	1.50	47.31	3.86	10.47	2.35	3.00
A11	21.47	3.07	11.31	2.43	2.00	33.31	3.51	7.81	2.06	3.00
A12	9.05	2.20	3.73	1.32	0.67	15.58	2.75	4.56	1.52	1.00
A13	5.33	1.67	2.16	0.77	1.00	5.38	1.68	2.86	1.05	0.67
A14	36.03	3.58	11.36	2.43	1.00	27.28	3.31	12.24	2.50	1.00
A15	8.36	2.12	2.80	1.03	1.00	15.71	2.75	5.73	1.75	2.00
B1	27.15	3.30	8.14	2.10	2.00	21.17	3.05	6.92	1.93	1.00
B2	23.71	3.17	7.49	2.01	1.50	29.28	3.38	10.67	2.37	1.00
B3	11.15	2.41	2.00	0.69	3.00	8.67	2.16	2.23	0.80	1.00
B4	6.43	1.86	1.45	0.37	1.00	7.11	1.96	1.71	0.54	2.00
B5	11.94	2.48	4.29	1.46	1.00	12.00	2.48	6.52	1.87	0.67
B6	26.09	3.26	10.60	2.36	2.00	21.50	3.07	7.92	2.07	1.50
B7	45.94	3.83	18.07	2.89	1.00	40.21	3.69	13.91	2.63	1.00
B8	11.24	2.42	7.53	2.02	0.67	24.90	3.21	7.04	1.95	1.50
B9	24.83	3.21	9.55	2.26	1.50	18.41	2.91	7.48	2.01	1.00
B10	25.38	3.23	5.67	1.74	3.00	24.85	3.21	8.33	2.12	2.00
B11	33.59	3.51	17.75	2.88	1.00	45.81	3.82	18.59	2.92	1.00
B12	16.46	2.80	4.82	1.57	3.00	16.70	2.82	8.22	2.11	2.00
B13	36.70	3.60	9.91	2.29	2.00	20.46	3.02	7.73	2.05	1.50
B14	34.84	3.55	9.57	2.26	4.00	34.97	3.55	9.68	2.27	1.50
B15	21.15	3.05	7.89	2.07	1.00	40.79	3.71	10.18	2.32	2.00
Mean	21.13	2.89	7.30	1.79	1.78	22.35	2.97	7.52	1.88	1.39
±S.D.	11.44	0.60	4.22	0.69	0.97	11.49	0.56	3.65	0.57	0.73

Table IV–*Bioavailability Parameters for Each Volunteer Obtained After Oral Administration of Samchundang Lercanidipine and Zanidip Tablets at the Lercanidipine Hydrochloride Dose of 20 mg*

	Parameters*		
	AUC_t	C_{max}	T_{max}
Difference (%)	5.77	3.01	−21.91
F_G[a)]	1.7451	1.2789	0.0529
Test/Ref point estimate	1.0794	1.0954	−0.3887
Confidence interval (δ)[b)]	$\log 0.95 \le \delta \le \log 1.23$	$\log 1.00 \le \delta \le \log 1.20$	$-41.70 \le \delta \le -2.02$

*The AUC_t and C_{max} values were calculated on the basis of log-transformed data, and the T_{max} values on the basis of untransformed data.
a)$\alpha = 0.05, F(1, 28) = 4.200$, b)$\alpha = 0.05$.

Acknowledgements

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