TCP Transl Clin Pharmacol
2014;22(2):102-105
http://dx.doi.org/10.12793/tcp.2014.22.2.102

A bioequivalence study of two levofloxacin tablets in healthy male subjects

Inbum Chung[1], Seonghae Yoon[1], SoJeong Yi[1], Bo-Hyung Kim[3], Sung-Vin Yim[3], In-Jin Jang[1] and Howard Lee[1,2]*

[1]Department of Clinical Pharmacology and Therapeutics, Seoul National University Hospital and College of Medicine, Seoul 110-744, Korea, [2]Department of Transdisciplinary Studies, Graduate School of Convergence Science and Technology, Seoul National University, Seoul 110-744, Korea, [3]Department of Clinical Pharmacology and Therapeutics, Kyung Hee University College of Medicine and Hospital, Seoul 130-872, Korea

*Correspondence: H. Lee; Tel: +82-2-3668-7602, Fax: +82-2-2072-4081, E-mail: howardlee@snu.ac.kr, leehwd@gmail.com

ORIGINAL ARTICLE

Received 17 Dec 2014
Revised 18 Dec 2014
Accepted 19 Dec 2014

Keywords
bioequivalence,
levofloxacin,
pharmacokinetics

pISSN: 2289-0882
eISSN: 2383-5427

Levofloxacin is a bactericidal broad spectrum antibiotic against Gram-positive and Gram-negative pathogens. A randomized, two-treatment, two-period, two-way crossover study was conducted to evaluate the bioequivalence of Lectacin 250 mg tablet, a generic levofloxacin, to its reference drug, Cravit 250 mg tablet. Each period was separated by a 7-day washout. Serial blood samples were collected until 24 h after dosing and plasma levofloxacin concentrations were determined using a high performance liquid chromatography. Pharmacokinetic parameters were analyzed using K-BE Test 2007 and BA calc 2007 (Ministry of Food and Drug Safety, Cheongju-si, South Korea). The peak concentration (C_{max}) and the area under the plasma concentration versus time curve from 0 to the last measurable concentration (AUC_{0-t}) for the generic and reference levofloxacin were 4.48±0.89 mg/L and 4.46± 0.95 mg/L, and 25.33±4.12 mg*h/L and 25.77±4.01 mg*h/L, respectively, leading to a geometric mean ratio (90% confidence interval) of the generic to the reference levofloxacin of 1.0060 (0.9339-1.0842) and 0.9810 (0.9476-1.0159), respectively, for C_{max} and AUC_{0-t}. Lectacin 250 mg tablet is bioequivalent to Cravit 250 mg tablet.

Introduction

Levofloxacin is the active S-enantiomer (L-isomer) of the racemic drug ofloxacin, an oral fluoroquinolone antimicrobial agent, and is approximately two times as potent as ofloxacin.[1] Levofloxacin possesses a broad spectrum of bactericidal activity against Gram-positive and Gram-negative pathogens such as Chlamydia, Legionella and Mycobacterium, which has made levofloxacin effective for the treatment of infectious diseases such as community-acquired pneumonia and acute exacerbation of chronic bronchitis.[2-5]

In South Korea, levofloxacin has been approved to treat uncomplicated or complicated urinary tract infection, chronic bacterial prostatitis, community-acquired pneumonia, nosocomial pneumonia, and skin and/or subcutaneous tissue infections. For these indications, a daily dose of levofloxacin between 250 mg and 750 mg can be used. Among the dose strengths approved in South Korea, 250 mg is the most frequently used one given the dosage recommendations.

UNIMED Pharm Inc. (Seoul, South Korea) has developed a new formulation of levofloxacin, Lectacin 250 mg tablet, containing the same amount of active ingredient and excipients as the reference levofloxacin drug, Cravit 250 mg tablet (Jeil Pharmaceutical Co. Ltd., Seoul, South Korea), which was approved by the Ministry of Food and Drug Safety (MFDS) for the indications mentioned above.

The aim of the present study was to compare the pharmacokinetic profile of Lectacin 250 mg tablet (test drug) with that of Cravit 250 mg tablet (reference drug) and to assess the bioequivalence of the two formulations.

Methods

This study was conducted at the Clinical Trials Center (CTC), Kyung Hee University Hospital (KHUH), Seoul, South Korea. The protocol was approved by the institutional review board at KHUH. Participants voluntarily provided written informed

consent before any screening test or procedure for eligibility was performed.

Study products

The test and reference drugs were Lectacin 250 mg tablet (batch number 08001, manufacture by UNIMED Pharm Inc., South Korea) and Cravit 250 mg tablet (batch number CRHB02, manufactured by Jeil Pharmaceutical Co., Ltd. in South Korea), respectively.

Study volunteers

Healthy Korean male volunteers aged 19 to 55 years were enrolled into this study. Eligible volunteers had to present no abnormalities based on screening, physical examination, vital signs, and routine clinical laboratory tests (hematology, clinical chemistry, and urinalysis). Volunteers were excluded if they had a history of hepatic, renal, pulmonary, cardiac, gastrointestinal, neurologic, or hematologic disorders. Eligible volunteers were instructed to abstain from taking alcohol, cigarette, and any drug for at least 72 hours prior to and during the study period. The study was conducted in accordance with the bioequivalence study guidelines published by the Ministry of Food and Drug Safety (MFDS) in South Korea.[6]

Study design

The study was conducted as a single dose, randomized, two-treatment, two-period, two-sequence, crossover design. Each period was separated by a 1-week washout, which was more than five times the half-life of levofloxacin.[5,7] A total of 26 volunteers were randomized into 2 sequences (A: test to reference, B: reference to test). After an overnight fasting of at least 12 hour, all volunteers were given a single oral dose of a test or reference levofloxacin with 240 mL of water in the morning on day 1 and 8 according the randomized sequence. Regular standardized meals were provided 4 hour after dose administration. Dietary, smoking, and drug-herbal product restrictions were maintained throughout the study period.

Pharmacokinetic samples collection

Blood samples (7 mL each) were drawn at 0 (i.e., pre-dose), 0.33, 0.67, 1, 1.5, 2, 2.5, 4, 6, 8, 12 and 24 hours after drug administration. A heparin-locked catheter was placed into the volunteer's forearm vein before drug administration and was left in place until the 24 hour blood sample was collected. Collected blood samples were centrifuged at 1,811 g for 10 minutes. The plasma was decanted in coded polypropylene tubes and stored at -70°C until analysis.

Quantification of levofloxacin concentration in plasma

Plasma concentrations of levofloxacin were determined by a validated method using high performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies, Santa Clara, CA, USA) coupled with tandem mass spectrometry (MS/MS, API3200, Applied Biosystems/MDS SCIEX, Foster City, CA, USA). A Luna 3u column (100 x 2.0 mm, Phenomenex, Torrance, CA, USA) was used with gradient mobile phase consisting of acetonitrile: 0.2% formic acid (50:50 v/v) and a flow rate of 0.3 mL/min. One hundred μL of plasma was added to 900 μl of enoxacin (internal standard, 1 μg/ml). After agitating for 30 second, the samples were centrifuged at 12,000 rpm for 8 minutes, and 1 μL of supernatant was injected into the HPLC system for analysis. The lower limit of quantification for levofloxacin was 0.1 μg/mL and the calibration curve was linear over the concentration range of 0.1-10 μg/mL with a mean correlation coefficient of 0.9972. The intra-batch and inter-batch accuracy was 87.22-101.66% and 87.22-101.66%, respectively, over the entire range of the calibration curve using known levofloxacin concentrations (Table 1), indicating that the bioanalytical method was accurate. Likewise, the intra-batch and inter-batch coefficient of variation ranged from 1.707 to 3.436% and from 2.865 to 7.587%, respectively, indicating that the method was precise.

Table 1. Accuracy and precision of levofloxacin concentration measurement by LC-MS/MS method (n=5)

Concentration (μg/mL)	Accuracy (%)		Precision (% Coefficient of Variance)	
	Intra-batch (n=5)	Intra-batch (n=5)	Intra-batch (n=5)	Inter-batch (n=5)
0.1	87.22	3.335	3.335	85.95
0.25	101.66	3.436	3.436	96.38
5	100.48	3.379	3.379	100.52
10	96.14	1.707	1.707	95.68

Pharmacokinetic analysis

The peak plasma concentration (C_{max}) and the time to C_{max} (t_{max}) were taken directly from the observed values. The pharmacokinetic parameters were calculated and compared using K-BE Test 2007 ver 1.1.0 and BA calc 2007 ver 1.0.0 (MFDS, Seoul, South Korea, both software programs). The area under the plasma concentration versus time curve from 0 to the last measurable concentration (AUC_{0-t}) was calculated with the linear trapezoidal rule.

Statistical analysis

Statistical analysis was performed using K-BE Test 2007 ver 1.1.0 (MFDS, Seoul, South Korea) or SPSS® (SPSS Korea, Seoul, South Korea). Descriptive statistics were used to summarize baseline demographics such as age, height, and body weight, and PK parameters. The geometric mean ratio (GMR) and its 90% confidence interval (CI) of the test to referecne formulations of levofloxacin were estimated for C_{max} and AUC_{0-t} using a linear-mixed effect model, in which natural logarithm-transformed C_{max} and AUC_{0-t} were the dependent variables,

treatment, sequence, and period were fixed effects, and subject nested for sequence was random effect. The two formulation were considered bioequivalent if the 90% confidence intervals for GMR fell entirely within the range of 0.80-1.25.

Results

Subjects

Twenty six healthy Korean adult male volunteers were enrolled and there was no dropout during the study. All the volunteers completed the study according to the study protocol and were included in the PK and safety analysis populations. The demographic characteristics of the volunteers are presented in Table 2.

Pharmacokinetics

The mean plasma concentration-time profiles of levofloxacin after a single oral administration of the two formulations were almost superimposable (Fig. 1). Both levofloxacin formulations were rapidly absorbed (t_{max}: 0.33 - 2.5 h, Table 3), and they were eliminated mono-exponentially (Fig. 1). C_{max} and AUC_{0-t} of levofloxacin was similar between the two formulations (Table 3), leading to a geometric mean ratio of 0.981 and 1.006, respectively. The 90% confidence interval for GMR of C_{max} and AUC_{0-t} were [0.9339-1.0842] and [0.9476-1.0159], respectively, falling entirely within the conventional bioequivalence range of [0.8-1.25], which also included unity (Table 3).

Tolerability evaluation

Both levofloxacin formulations were well tolerated by all the volunteers. There was no unexpected adverse event. None of the changes in laboratory tests and vital signs during the study were considered clinically important.

Discussion

The present study showed that the two formulations of levofloxacin (i.e., Lectacin 250 mg tablet and Cravit 250 mg tablet)

Table 2. Demographic characteristics of the volunteers

Characteristics	Values
Age (years)	24.0±2.2
Height (cm)	176.8±5.1
Body weight (kg)	70.2±8.6

All values are presented as mean ± SD.

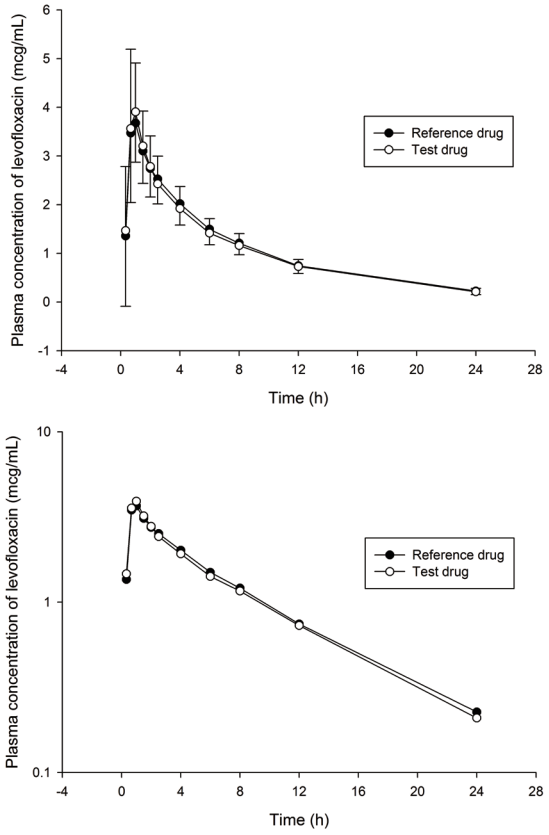

Figure 1. Mean plasma concentration - time curves of levofloxacin after a single oral dose of levofloxacin 250 mg. The error bars represent the standard deviations (Upper: linear scale, Lower: log scale)

Table 3. Pharmacokinetic parameters of levofloxacin after a single oral dose administration of levofloxacin at 250 mg

Parameters	Test (n=26)	Reference (n=26)	Geometric mean ratio (90% confidence interval)
t_{max} (h)	1.0 (0.33–2.5)	0.84 (0.67–2.5)	
C_{max} (μg/mL)	4.48±0.89 [4.39]	4.46±0.95 [4.36]	1.0060 (0.9339–1.0842)
AUC_{0-t} (h·μg/mL)	25.33±4.12 [24.99]	25.77±4.01 [25.47]	0.9810 (0.9476–1.0159)
$t_{1/2}$ (h)	6.5±0.8	6.7±0.8	
CL/F (mL/h)	9328.5±1552.3	9011.2±1369.5	

All values are presented as mean±SD [geometric mean], except for t_{max}, for which median (range) is presented. AUC_{0-t} = area under the plasma concentration-time curve (AUC) from time 0 to 24 hours post dose; C_{max} = peak plasma drug concentration; t_{max} = time to reach peak plasma concentration; Test = Lectacin 250 mg tablet; Reference = Cravit 250 mg tablet

are bioequivalent. Evidence is that the point estimate of the GMR of the test to the reference was close to unity (i.e., 1) for both the peak concentration and the area under the concentration-time curve, and its 90% confidence interval was contained entirely within the conventional bioequivalence range of [0.8-1.25] (Table 3), which has been also used by MFDS as the regulatory standard for approving the generic formulation.[6] Furthermore, other pharmacokinetics parameters including half-life ($t_{1/2}$) and apparent clearance were also similar to each other (Table 3), resulting in the almost overlapping concentration-time profiles between the reference and test formulations of levofloxacin. Moreover, the two formulations of levofloxacin were well tolerated. Because the pharmacokinetics of levofloxacin is linear over the dose range in the approved regimens,[8] the test formulation of levofloxacin can be also effectively used for daily doses of multiples of 250 mg.

Levofloxacin was rapidly absorbed after a single oral administration. In this study, the time to peak concentration (t_{max}) ranged between 0.33 and 2.5 h, which was consistent with not only what has been reported previously,[7,9] but the one in the drug label.[8] Orally administered levofloxacin is almost completely absorbed as indicated by its high bioavailability of ~99%.[5,8] Therefore, the low inter- and intra-individual variability in the exposure to levofloxacin, which has been also shown in the present study (i.e., <17%, Table 3), could be also due to its high bioavailability. Likewise, the half-life of levofloxacin was ~6.5 h (Table 3), which was in full agreement with the previously reported values of 6 to 8 h following a single or multiple oral doses.[1,5,9,10] Thus, one week was sufficient and appropriate as wash-out as employed in the present study.

In conclusion, the test formulation of levofloxacin (Lectacin 250 mg tablet) is bioequivalent to the reference formulation (Cravit 250 mg tablet). The two formulations of levofloxacin were well tolerated.

Acknowledgments

This study was supported by a research grant from UNIMED Pharm Inc. (Seoul, South Korea).

Conflict of Interest

All authors have nothing to declare.

References

1. North DS, Fish DN, Redington JJ. Levofloxacin, a second-generation fluoroquinolone. Pharmacotherapy 1998;18:915-935.
2. Croom KF, Goa KL. Levofloxacin: a review of its use in the treatment of bacterial infections in the United States. Drugs 2003;63:2769-2802.
3. Rafat C, Debrix I, Hertig A. Levofloxacin for the treatment of pyelonephritis. Expert Opin Pharmacother 2013;14:1241-1253. doi: 10.1517/14656566.2013.792805.
4. Torres A, Liapikou A. Levofloxacin for the treatment of respiratory tract infections. Expert Opin Pharmacother 2012;13:1203-1212. doi: 10.1517/14656566.2012.688952.
5. Anderson VR, Perry CM. Levofloxacin: a review of its use as a high-dose, short-course treatment for bacterial infection. Drugs 2008;68:535-565.
6. Guidance Document for Bioequivalence Study. In: MFDS, editor. 2010.
7. Tsaganos T, Kouki P, Digenis P, Giamarellou H, Giamarellos-Bourboulis EJ, Kanellakopoulou K. Pharmacokinetics of levofloxacin after single and multiple oral doses in patients undergoing intermittent haemodialysis. Int J Antimicrob Agents 2008;32:46-49. doi: 10.1016/j.ijantimicag.2008.02.011.
8. Levaquin® (levofloxacin tablets, oral solution, injection): US prescribing information. In: Ortho-McNeil Pharmaceutical I, editor. 2008.
9. Chien SC, Rogge MC, Gisclon LG, Curtin C, Wong F, Natarajan J, et al. Pharmacokinetic profile of levofloxacin following once-daily 500-milligram oral or intravenous doses. Antimicrob Agents Chemother 1997;41:2256-2260.
10. Gao X, Yao G, Guo N, An F, Guo X. A simple and rapid high performance liquid chromatography method to determine levofloxacin in human plasma and its use in a bioequivalence study. Drug Discov Ther 2007;1:136-140.

Appendix. Literature search strategies

1)PubMed Database (Date, searched: 2013. 06. 15)

#	Search strategy	Results
1	"Crohn Disease"[Mesh] OR "Crohn's disease" OR "Crohn Disease" OR crohn*[TW] CD[TW]	6,350
2	"Arthritis, Rheumatoid"[MeSH] OR "Rheumatoid arthritis*" OR "arthritis rheumatoid" OR "arthritis rheumatic" OR "chronic rheumatic arthritis" OR RA[TW]	136,215
3	1 OR 2	142,408
4	smok*[TW] OR tobacco[TW] OR cigarette*[TW] OR "polycyclic aromatic hydrocarbon"[TW] OR nicotin*[TW]	311,946
5	smoke[Mesh] OR nicotine[Mesh] OR tobacco[Mesh] OR "Polycyclic Hydrocarbons, Aromatic"[Mesh] OR "Tobacco Products"[Mesh]	435,186
6	4 OR 5	687,201
7	TNF-Alpha[TW] OR TNF-α OR TNF-a OR "TNF a" OR "tumor necrosis factor-alpha"[TW] OR "tumor necrosis factor-alpha"[Mesh]	157,355
8	Etanercept[TW] OR Enbrel[TW] OR rhu-TNFR:Fc OR TNR-001 OR "185243-69-0" OR "TNFR-Fc fusion protein" [Supplementary Concept]	5,049
9	Adalimumab[TW] OR Humira[TW] OR D2E7[TW] OR adalimumab [Supplementary Concept]	3,214
10	Infliximab[TW] OR Remicade[TW] OR "infliximab" [Supplementary Concept]	8,286
11	"Certolizumab pegol"[TW] OR Cimzia[TW] OR "CDP-870" OR "certolizumab pegol" [Supplementary Concept]	340
12	golimumab[TW] OR Simponi[TW] OR CNTO-148[TW] OR golimumab [Supplementary Concept]	263
13	7 OR 8 OR 9 OR 10 OR 11 OR 12	163,153
14	3 AND 6 AND 13	240
15	Limit 14 to Humans	194

2)Ovid Medline Database (Date, searched: 2013. 06. 15)

#	Search strategy	Results
1	exp Crohn Disease/	29,507
2	"Crohn's disease$".mp. OR "Crohn$ Disease$".mp. OR "Disease$ Crohn$".mp. OR CD.mp.	165,421
3	exp Arthritis, Rheumatoid/	98,838
4	"Rheumatoid$ adj arthritis$".mp. OR "arthritis$ rheumatic".mp. OR "chronic rheumatic arthritis$".mp. OR RA.mp.	49,641
5	or/1-4	287,109
6	(smok$ OR tobacco OR cigarette$ OR "polycyclic aromatic hydrocarbon" OR nicotin$).mp.	318,471
7	exp Smoke/	16,050
8	exp smoking/	118,351
9	exp tobacco/	22,965
10	exp Tobacco Products/	2,917
11	exp Polycyclic Hydrocarbons, Aromatic/	394,382
12	exp Nicotine/	20,833
13	or/6-12	705,205
14	TNF-Alpha.mp. OR tumor necrosis factor-alpha.tw. OR "Tumor Necrosis Factor Blocking Agent" OR tnf-a.mp. OR "tnf a".mp.	87,532
15	exp Tumor Necrosis Factor-alpha/	95,035
16	Etanercept.mp. OR Enbrel.mp. OR rhu-TNFR: Fc OR TNR-001	4,555
17	Adalimumab.mp. OR Humira.mp. OR Humira Pen.mp. OR D2E7.mp.	5,268
18	Infliximab.mp. OR Remicade.mp. OR Infliksimab	9,563
19	Certolizumab pegol.mp. OR Cimzia.tw. OR CDP-870	555
20	golimumab.mp. OR Simponi.mp. OR CNTO-148	299
21	or/14-20	135,991
22	5 AND 13 AND 21	478
23	limit 22 to humans	380

3) Ovid Embase Database (Date, searched: 2013. 06. 15)

#	Search strategy	Results
1	exp Crohn Disease/	53,194
2	"Crohn's disease$".mp. OR "Crohn$ Disease$".mp. OR "Disease Chrone$".mp. OR CD.tw.	146,829
3	exp rheumatoid arthritis/	142,136
4	"Rheumatoid arthritis$".mp. OR "arthritis$ rheumatoid".mp. OR "arthritis$ rheumatic".mp. OR "chronic rheumatic arthritis".mp. OR RA.tw.	180,939
5	or/1-4	324,342
6	(smok$ OR tobacco OR cigarette$ OR "polycyclic aromatic hydrocarbon" OR nicotin$).mp.	526,233
7	exp smoke/	6,962
8	exp smoking/	171,413
9	exp tobacco/	30,531
10	exp polycyclic aromatic hydrocarbon/	60,713
11	or/6-10	558,755
12	exp Tumor Necrosis Factor-alpha/	141,345
13	TNF-Alpha.mp. OR tumor necrosis factor-alpha.tw. OR "Tumor Necrosis Factor Blocking Agent" OR tnf-a.mp. OR "tnf a".mp.	122,577
14	exp etanercept/	16,315
15	etanercept.mp. OR enbrel.mp. OR "Enbrel SureClick" OR Etanerceptum OR Etanersept OR "rhu-TNFR: Fc" OR TNR-001	17,148
16	exp adalimumab/	12,295
17	adalimumab.mp. OR Humira.mp. OR "Humira pen" OR Trudexa.mp. OR D2E7 OR LU-200134	12,,553
18	exp infliximab/	25,481
19	infliximab.mp. OR Remicade.mp. OR Remsima.mp. OR Infliksimab.mp.	26,010
20	exp certolizumab pegol/	2,233
21	"certolizumab pegol".mp. OR Cimzia.mp. OR CDP-870 OR PHA-738144	2,277
22	exp golimumab/	1,431
23	golimumab.mp. OR Simponi.mp. OR CNTO-148	1,478
24	or/12-23	202,438
25	5 AND 11 AND 24	1,045
26	Limit 25 to humans	894

4) Cochrane Library Database (Date, searched: 2013. 06. 15)

#	Search strategy	Results
1	MeSH descriptor: [smoke] explode all trees	224
2	MeSH descriptor: [nicotine] explode all trees	1439
3	MeSH descriptor: [tobacco] explode all trees	201
4	(smok* OR tobacco OR cigarette* OR "polycyclic aromatic hydrocarbon" OR nicotin*):ti,ab,kw	15502
5	#1 OR #2 OR #3 OR #4	15502
6	MeSH descriptor: [Tumor Necrosis Factor-alpha] explode all trees	2064
7	(TNF-α OR "TNF a" OR "tumor necrosis factor-alpha" OR "Tumor Necrosis Factor Blocking Agent" OR "TNF-a"):ti,ab,kw	2979
8	(Etanercept OR Enbrel OR 185243-69-0):ti,ab,kw	464
9	(Adalimumab OR Humira OR D2E7):ti,ab,kw	270
10	(Infliximab OR Remicade):ti,ab,kw	550
11	("Certolizumab pegol" OR Cimzia):ti,ab,kw	42
12	(golimumab OR simponi OR CNTO-148):ti,ab,kw	48
13	#6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12	3806
14	#5 AND #13	54
15	limit #14 to humans	5