EVALUATION OF COMPARATIVE PHARMACOKINETICS AND BIOEQUIVALENCE OF A NEW ACECLOFENAC FORMULATION

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Abstract

Afenac® is a new generic formulation of aceclofenac, a commonly used nonsteroidal anti-inflammatory drug (NSAID) for treating pain of various origins, including inflammatory, degenerative, joint, and musculoskeletal diseases. We compared the pharmacokinetic (PK) properties and evaluated the bioequivalence of two formulations of aceclofenac 100 mg tablets. A single-dose, randomized, 2 × 2 crossover study was performed with 23 healthy Korean male subjects. The subjects randomly received 100 mg of either the test formulation (Afenac®, Korea Arlico Pharm, Seoul, Korea) or the reference formulation (Airtal®, Dae Woong Co., Ltd., Seoul, Korea). After a 1-week washout period, the subjects received the other formulation. Aceclofenac plasma concentrations were determined using the validated high-performance liquid chromatography coupled with tandem mass spectrometry, and pharmacokinetic (PK) parameters were calculated using a noncompartmental method. Geometric mean ratios (GMRs) of the test and reference formulations with 90% confidence intervals (90% CI) were estimated for PK parameters using a generalized linear mixed-effects model. The GMRs (with 90% CI) of the test and reference formulations for the maximum concentration of aceclofenac and the area under the plasma concentration versus time curve from 0 to the last measurable concentration were 0.994 (0.917-1.077) and 0.895 (0.859-0.932), respectively. Both formulations were well tolerated. The test formulation of aceclofenac was bioequivalent to the reference formulation and can be considered a therapeutic option for patients requiring aceclofenac treatment.

Key words: *aceclofenac, bioequivalence, pharmacokinetics*

Introduction

Aceclofenac, (2-{2-[(2,6-Dichlorophenyl)amino]phenyl}acetoxy)acetic acid, is an orally administered nonsteroidal anti-inflammatory drug (NSAID) used to treat painful rheumatic conditions (Reginster *et al.*, 2001) such as rheumatoid arthritis and ankylosing spondylitis. It alleviates pain and reduces inflammation by inhibiting cyclooxygenase enzymes (Dugowson and Gnanashanmugam, 2006; Gonzalez *et al.*, 1994), which prevents the production of prostaglandins (lipid compounds that cause pain, swelling, and inflammation at sites of injury or damage). Among all the NSAIDs, aceclofenac is particularly well tolerated (Pareek and Chandurkar, 2013), with a lower incidence of gastrointestinal adverse effects such as indigestion, heartburn, nausea, diarrhea, bleeding, and ulcers (Legrand, 2004; Raza *et al.*, 2014).

The recommended dosage of aceclofenac for the symptomatic treatment of pain and inflammatory or degenerative arthropathies is 100 mg twice daily (Moore *et al.*, 2009; Scheen, 1999). The time to reach maximum plasma concentration for aceclofenac (T_{max}) is 1.25 to 3 hours and the half-life ($t_{1/2}$) is 3.5 to 6.2 hours after oral administration, which necessitates dosing every 12 hours to maintain optimum levels of analgesia (Bae *et al.*, 2012). The primary route of elimination of aceclofenac is the liver, with only one percent of the administered dose being excreted unchanged in the urine (Bort *et al.*, 1996). It is metabolized via the CYP2C9 enzyme to 4-hydroxyaceclofenac, diclofenac, and 4'-hydroxydiclofenac, which are active metabolites that are further conjugated to inactive metabolites (Yamazaki *et al.*, 1999). Reduced daily dosing is generally not needed in elderly patients or in those with mild renal impairment, but one study suggests dose reduction to half the daily dose (100 mg daily) in patients with hepatic impairment (Dooley *et al.*, 2001).

Genetic variations of the CYP2C9 enzyme can lead to interpatient variability in pharmacokinetics (PKs) and efficacy (Lee *et al.*, 2007). Several CYP2C9 single nucleotide polymorphisms (SNPs) such as *CYP2C9*2* (rs1799853) or *CYP2C9*3* (rs1057910) were associated with decreased CYP2C9 enzyme activity (Agundez *et al.*, 2009; Chaudhry *et al.*, 2014). Those genetic variations can alter the PK characteristics of NSAIDs (Lopez-Rodriguez *et al.*, 2008), increasing the risk of developing acute gastrointestinal bleeding (Ma *et al.*, 2008).

A Korean domestic pharmaceutical company has developed a new formulation of aceclofenac 100 mg. The aim of this study was to compare the PK properties of the two aceclofenac 100 mg tablet formulations and evaluate their bioequivalence.

Methods

Study design and subject selection

A randomized, single-dose, 2 × 2 crossover study was conducted on 26 healthy Korean male volunteers. Subjects randomly received either the test formulation of aceclofenac 100 mg (Afenac®, Korea Arlico pharm. Co., Ltd., Seoul, Korea) or 100 mg of the reference formulation (Airtal®, Dae Woong Pharmaceutical Co., Ltd., Seoul, Korea). After the initial dose, they underwent a 1 week washout and then crossed over. Subjects were hospitalized at the Clinical Trials Center of Kyung Hee University Hospital, Seoul, Korea, the day before drug administration. After an overnight fast, a single dose of the test or reference formulation of aceclofenac 100 mg was administered to the subjects with 240 ml of water. The subjects were under continuous medical supervision at the study site for the duration of the study, during which adverse events and vital signs were monitored to assess tolerability. This study was approved by the Institutional Review Boards of Kyung Hee University Hospital and was conducted in accordance with the Declaration of Helsinki (2014) and the International Conference on Harmonization Good Clinical Practice guidelines. All of the subjects who participated were informed of the aim and risks of the study by the clinical investigators, and all subjects provided written informed consent before participating.

Blood sample collection and determination of aceclofenac concentration

To analyze the plasma concentration of aceclofenac, serial blood samples were obtained using a lithium heparin-coated tube before dosing (0) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12 hours after drug administration. All plasma samples were analyzed using validated high-performance liquid chromatography (HPLC) coupled with triple quadrupole tandem mass spectrometry (Agilent Technologies, Inc., Santa Clara, California, USA) (Srinivas, 2009). To prepare the samples for analysis, 100 μL of plasma specimen was mixed with 0.1% formic acid in acetonitrile (35:65, v/v) in the presence of an internal standard (ramipril). The mixture was vortexed for 1 min and then centrifuged for 8 min at 12 000 rpm. An aliquot of the supernatant was transferred to an autosampler vial and 1 μL was injected onto the Gemini C18 110A column (50 x 2.0 mm, 3 μm, Phenomena, Inc., Torrance, California, USA) at a flow rate of 0.2 mL/min using gradient elution. Detection of precursor-to-product ion transition was achieved by employing electrospray ionization in the positive ion

mode along with multiple reactions monitoring (MRM). The precursor/product ion pair mass-to-charge ratio (*m/z*) for aceclofenac was 351.86/79.40. The calibration curves were linear over the range of 0.5-50 μg/mL. Interday precision and accuracy were determined by repeated analyses on 5 consecutive days using 0.5, 1, 20, and 50 μg/mL of aceclofenac. Intraday percent coefficients of variation (% CV) and accuracy were 1.95-6.58% and 96.84-104.92%, respectively. Interday % CV and accuracy were 4.00-7.03% and 98.97-101.22%, respectively.

PK analysis

The plasma concentration of aceclofenac was analyzed by noncompartmental analysis using Phoenix® WinNonlin® software version 6.3 (Certara, St. Louis, MO, USA). Area under the plasma concentration versus time curve from 0 h to the last measurable concentration (AUC_{last}) was calculated using the linear trapezoidal method. The observed concentrations and times were used to estimate the maximum plasma concentration (C_{max}), and time required to reach the maximum plasma concentration (T_{max}). The apparent terminal elimination rate constant (λ_z) was estimated from regression of log-transformed plasma concentration of aceclofenac versus time over the terminal log-linear disposition portion of the concentration-time profiles, and the elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = \ln 2 / \lambda_z$. Total apparent clearance (CL/F, where CL is clearance and F is the bioavailability) of aceclofenac was calculated as the administered dose (100 mg) divided by the AUC_{last}, and the apparent volume of distribution (V_z/F, where V_z is the volume of distribution and F is the bioavailability) was calculated as CL/F divided by λ_z.

Statistical analysis

We calculated the arithmetic mean and standard deviation (SD) for every demographic characteristic and PK parameter. To compare the bioavailability of the test and reference formulations, a generalized linear mixed effects model was developed for $\log(AUC_{last})$ and $\log(C_{max})$, with period, sequence, and treatment as fixed effects and subjects nested in sequence as random effects. Using this model, the geometric mean ratios (test/reference) with a 90% confidence interval for AUC_{last} and C_{max} were calculated. Bioequivalence was concluded if the GMRs and 90% CIs for AUC_{last} and C_{max} fell within range of 0.80 to 1.25, as proposed by the

Ministry of Food and Drug Safety of Korea. Statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Subjects

A total of 26 healthy male subjects were enrolled in this study. Three of them withdrew consent before drug administration in the first period. This left a total of 23 subjects to complete the study as planned. Mean subject age, height, and weight (with standard deviations) were 24.83 (3.83) years, 174.07 (5.60) cm, and 71.45 (12.80) kg, respectively. Age, height, and weight were not statistically different between sequence groups.

Pharmacokinetics

Systemic exposure to aceclofenac after a single oral administration of the test formulation was similar to that of the reference formulation. The mean plasma concentration versus time curves for the test and reference drugs were similar (Figure 1). The GMRs with 90% CIs for AUC_{last} and C_{max} were within the range of 0.8 to 1.25 (Table 1), which satisfies the conventional regulatory criteria for bioequivalence. No trend was found in individual comparisons between the test and reference formulations for AUC_{last} and C_{max} (Figure 2). Other PK parameters, including T_{max}, $t_{1/2}$, CL/F, and V_z/F, were also comparable between the test and reference formulations (Table 1).

Tolerability

Aceclofenac was well tolerated by all of the volunteers, with no clinically significant adverse events related to aceclofenac administration reported. All vital signs and physical examinations assessed during the study period did not reveal anything clinically significant.

Discussion

The results indicate that the newly developed aceclofenac 100 mg tablet formulation has a PK and tolerability profile that is comparable to the reference formulation. The GMRs with a 90% CI for AUC_{last} and C_{max} were entirely within the conventionally accepted range for bioequivalence (0.8 to 1.25). The mean plasma concentration versus time curves for the two formulations were superimposable (Figure 1), and no systemic deviations were found for individual comparisons of AUC_{last} and C_{max} (Figure 2). Both the formulations were well tolerated by healthy volunteers after a single oral administration.

This study's design was adequate for comparing the PK characteristics of the test and reference formulations of aceclofenac. The blood sampling time points were adequate for observing the C_{max} of aceclofenac (0 to 2 hours). The 12-hour sampling from each dosing (more than 10-fold longer than the $t_{1/2}$) was long enough to assess the elimination phase of aceclofenac. To avoid a carry-over effect, the washout period lasted 10-fold longer than the $t_{1/2}$, so that no measurable plasma concentration was detected in any of the subjects before dosing in the second period. The number of subjects was adequate to show the bioequivalence of the test and reference aceclofenac formulations, with a post hoc power analysis revealing that the actual statistical power of our study was greater than 99%.

The PK parameters observed in this study differed from those reported in an earlier study performed on Indian subjects, but genetic variation of the *CYP2C9* might explain this difference. After Indian subjects ingested a single oral dose of aceclofenac 100 mg, the mean AUC_{last} and $t_{1/2}$ were calculated to be 27.52 to 28.46 $\mu g \cdot h \cdot mL^{-1}$ and 2.83 to 3.06 hours, respectively; whereas the corresponding parameters in this study were 22.26 to 23.96 $\mu g \cdot h \cdot mL^{-1}$ and 1.1 to 1.2 hours, respectively (Gowda *et al.*, 2006). The minor allele frequencies of *CYP2C9*2* and *CYP2C9*3* SNPs in Indians were 7% and 9%, respectively (Bhatt *et al.*, 2014), and approximately 0% and 3.2%, respectively, in Koreans. Therefore, a proportionately greater number of Indians would have decreased CYP2C9 enzymatic activity, with a corresponding decreased ability to metabolize aceclofenac compared to Koreans (Martinez *et al.*, 2006; Myrand *et al.*, 2008). We believe the decreased enzymatic activity of CYP2C9 SNP (Falzoi *et al.*, 2010) in Indian subjects resulted in higher AUC_{last} and $t_{1/2}$ values than that observed in the Korean subjects of this study. The PK parameters we calculated were comparable to those of a previous study performed in healthy Korean subjects, and our data supports the observation of the interethnic variability of aceclofenac PK (Llerena *et al.*, 2014).

168 We conclude that the test formulation of aceclofenac 100 mg tablet is bioequivalent to the reference
169 formulation.

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263 Table 1. Comparison of pharmacokinetic parameters between the two aceclofenac 100 mg formulations (test and
264 reference) after single oral administration

PK parameters	Test (Afenac®, N=23)	Reference (Airtal®, N=23)	GMR[2] (Test/Reference, 90% CI)	Intrasubject CV (%)	Intersubject CV (%)
C_{max} (µg/mL)	11.02 ± 3.23	10.95 ± 2.86	0.994 (0.917-1.077)	15.95	24.55
AUC_{last} (µg·h·mL^{-1})	22.26 ± 8.48	23.96 ± 7.71	0.895 (0.859-0.932)	9.06	35.51
T_{max}[1] (h)	2.2 [1.0-4.0]	2.1 [1.0-4.0]	-	-	-
CL/F (ml/h)	4626.21 ± 1867.89	4341.85 ± 1472.94	-	-	-
Vz/F (ml)	6527.86 ± 777.88	6800.73 ± 1528.54	-	-	-
$t_{1/2}$ (h)	1.1 ± 0.4	1.2 ± 0.4	-	-	-

T_{max}, time to reach the maximum blood concentration after administration of drug; C_{max}, maximum plasma concentration of drug; AUC_{last}, area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration; $t_{1/2}$, terminal elimination half-life; CL/F, apparent clearance; Vz/F, Apparent volume of distribution during terminal phase after non-intravenous administration.

C_{max}, AUC_{last}, CL/F, Vz/F and $t_{1/2}$ are presented as mean ± standard deviation

[1]T_{max} is presented as median [minimum-maximum]

[2]Geometric mean ratio

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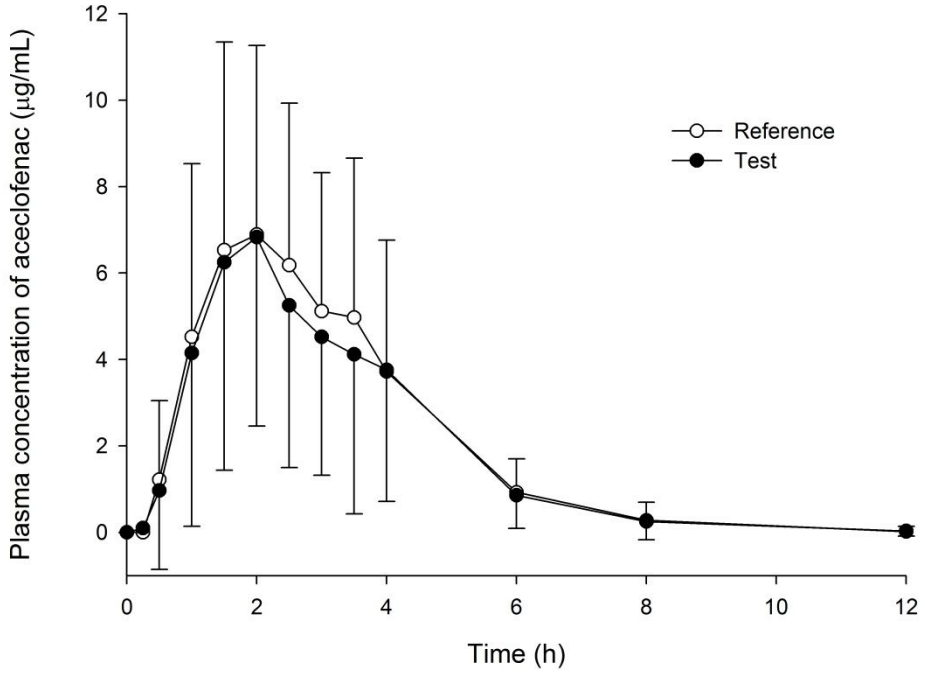
267 FIGURE LEGENDS

268 Figure 1. Mean plasma concentration-time profile of aceclofenac after a single oral administration of test (filled
269 circle) and reference (open circle) formulation of aceclofenac 100 mg. The error bars denote the standard
270 deviations.

271 Figure 2. Comparison of C_{max} and AUC_{last} between the two aceclofenac formulations (test and reference) in indi
272 vidual subjects.

273 Figure 1



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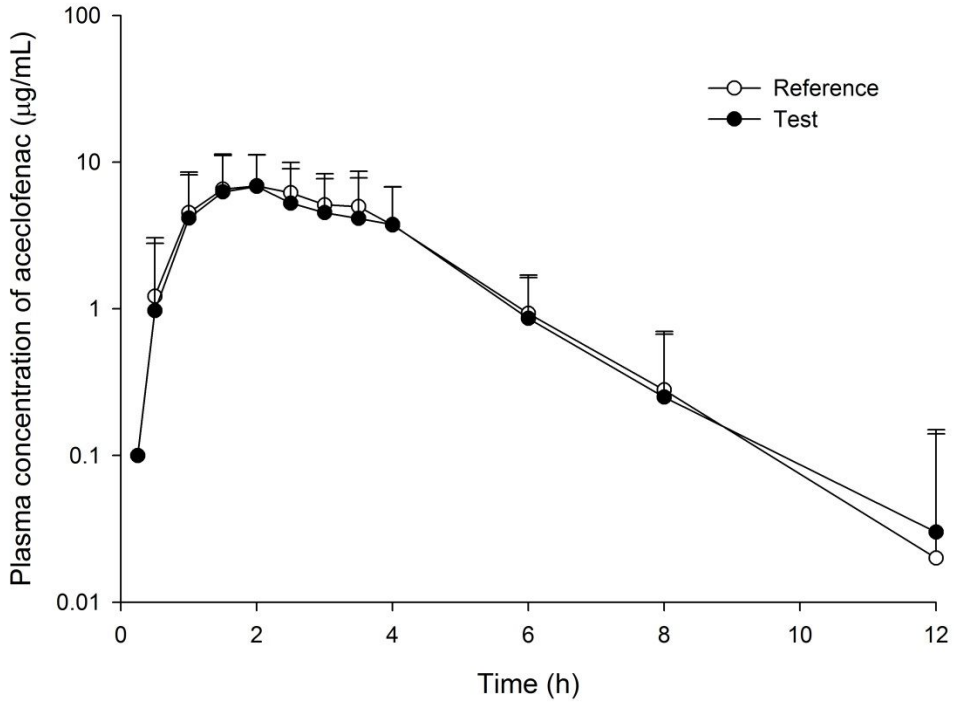

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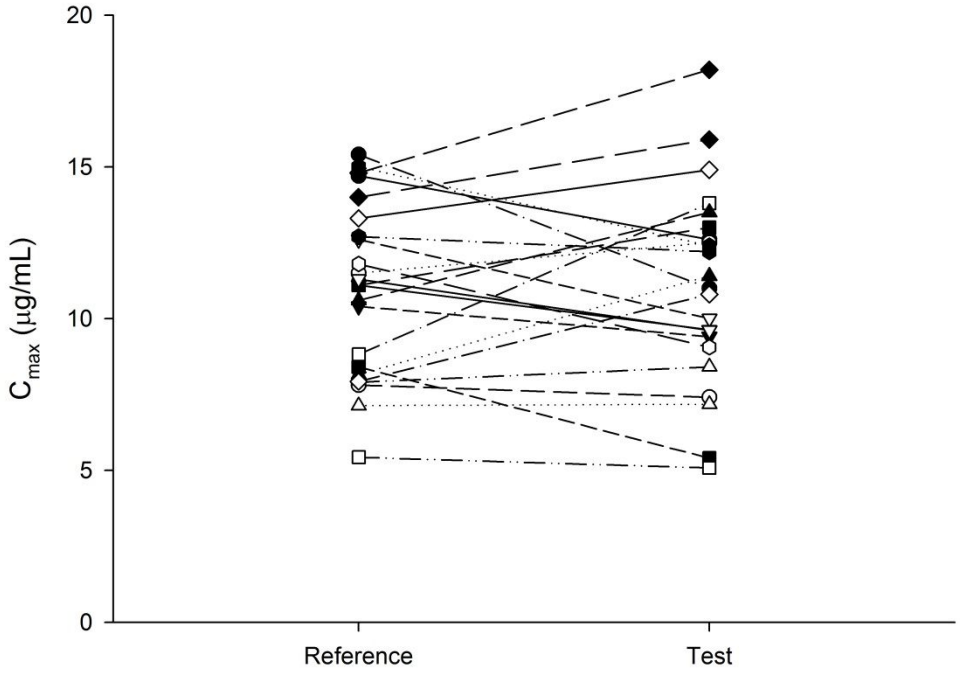
276 Figure 2



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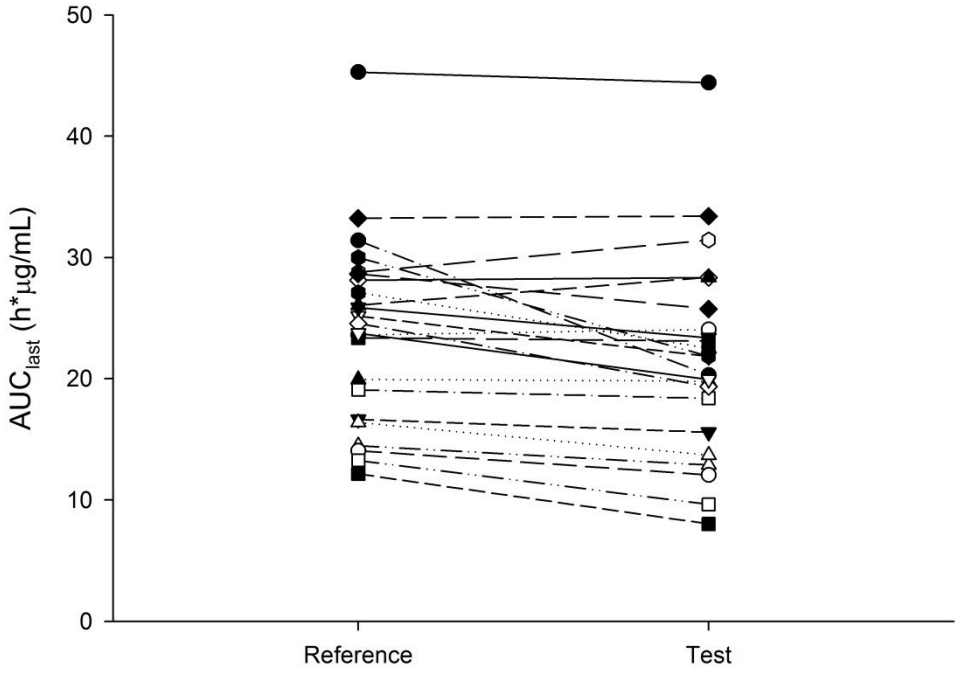

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