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Comparative Bioavailability and Tolerability of Single and Multiple Doses of 2 Diclofenac Sodium Sustained-Release Tablet Formulations in Fasting, Healthy Chinese Male Volunteers☆

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ABSTRACT

Background: Diclofenac is a nonsteroidal anti-inflammatory drug used for the treatment of patients with osteoarthritis.

Objectives: Our primary objective was to compare bioavailability and tolerability of a generic sustained-release tablet with the established reference sustained-release tablet of diclofenac sodium in a fasting, healthy Chinese male population.

Methods: A randomized, open-label, single- and multiple-dose study design was used. After the single dose, volunteers received diclofenac sodium sustained-release tablet once daily for 5 days. In the single-dose phase, blood samples were collected from 0 to 36 hours after drug administration. In the multiple-dose phase, samples were obtained before drug administration at 8:00 AM on Days 3 and 4 to determine $C_{min,ss}$ of diclofenac sodium; on Day 5, samples were collected from 0 to 36 hours. Adverse events were monitored via subject interview, vital signs, and blood sampling.

Results: Twenty-four Chinese male volunteers were enrolled. The pharmacokinetic parameters (mean [SD]) for diclofenac after single dose of 75 and 100 mg were: C_{max} 473.5 [179.5] and 546.6 [154.9] ng/mL; $AUC_{0-\infty}$ 3841.2 [1402.3], and 5019.1 [2,314.0] ng · h/mL; T_{max} 4.9 [2.4], and 4.3 [2.2] hours; $t_{1/2}$ 5.9 [2.5], and 6.0 [2.2] hours. Mean [SD] values after multiple doses of 75 and 100 mg were: $C_{max,ss}$ 525.6 [127.4] and 650.5 [167.0] ng/mL, $C_{min,ss}$ 33.9 [20.9] and 62.9 [34.9] ng/mL, AUC_{ss} 4316.3 [633.0] and 5335.1 [1291.9] ng · h/mL, $C_{av,ss}$ 179.8 [26.4] and 222.3 [53.8] ng/mL, T_{max} 5.1 [1.8] and 4.5 [0.9] hours and $t_{1/2}$ 5.2 [2.9] and 5.5 [2.8] hours, respectively.

Conclusions: This diclofenac sodium 75 mg tablet has features compatible with the 100 mg sustained-release tablet and appeared to be well tolerated. ClinicalTrials.gov identifier: 2010L01969

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a preferred therapy for osteoarthritis because they effectively relieve pain and reduce inflammation with generally good tolerability.[1–3] Diclofenac is one of the most commonly used NSAIDs. It was approved in the United States in 1988 for the treatment of patients with osteoarthritis, rheumatoid arthritis, or ankylosing spondylitis.[4] It is an inhibitor of cyclooxygenase, and its potency is substantially greater than that of indomethacin, naproxen, or several other agents.[5–7] It is one of the most prescribed NSAIDs worldwide.

Diclofenac, like all NSAIDs, is highly bound to human serum proteins ($\geq$ 99.5%), specifically albumin.[8] It accumulates in synovial fluid after oral administration, which may explain the duration of therapeutic effect that is considerably longer that the plasma half-life.[9] The half-life of diclofenac sodium in plasma varies from 1 to 3 hours. Peak plasma concentrations occur in about 3 hours.[10] There are no data available on the distribution of diclofenac in organs or tissues of human beings. Animal studies have shown that the highest concentrations of diclofenac are found in bile, liver, and kidneys followed by blood, heart, and lung.[11,12] Diclofenac undergoes extensive hepatic metabolism by cytochrome P-450 (CYP) 2C9 and CYP3A.[13,14] CYP2C9 is polymorphic and is involved in the oxidation of a wide range of drugs, including NSAIDs such as naproxen, ibuprofen, and piroxicam.[15,16]

Diclofenac sodium has been marketed since 1973.[17] Experimental and clinical findings obtained to date have indicated that diclofenac sodium was synthesized on well-founded principles. Despite diclofenac sodium having been in widespread clinical use for > 20 years there is little published information on its pharmacokinetics of sustained-release tablet formulation in the Chinese

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population. A search of the Chinese- and English-language literature for reports on the pharmacokinetics of diclofenac identified only a small number of pharmacokinetic studies in white subjects.[10] No reports were identified concerning the pharmacokinetic properties of a sustained-release formulation of diclofenac sodium in a Chinese population.

Although the branded diclofenac sodium sustained-release tablet formulation was already marketed in China, information regarding the pharmacokinetics of diclofenac and the bioequivalence of these formulations in Chinese populations had seldom been reported to date. Before allowing the marketing of generic diclofenac sodium, the State Food and Drug Administration of China requires pharmacokinetic studies of the bioequivalence of generic and branded formulations. Therefore, the purpose of the present single- and multiple-dose study was to compare the bioavailability and tolerability of the proposed generic sustained-release formulation with the established reference sustained-release formulation of diclofenac sodium in a fasting, healthy Chinese male population.

Subjects and Methods

This was a single-center, randomized, open-label, 2-phase study conducted to determine the pharmacokinetics of a generic sustained-release formulation of diclofenac sodium after oral administration of single and multiple doses in healthy Chinese volunteers. This study was designed and conducted at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China, from May 2012 to June 2012. The study was conducted according to the principles of the Declaration of Helsinki and its amendments for biomedical research involving human subjects and the principles of the Good Clinical Practice guidelines. The clinical trial was approved by the State Food and Drug Administration of China (approval No. 2010L01969), and the clinical protocol and the informed consent form were approved by the local ethics committee at Tongji Medical College, Huazhong University of Science and Technology (approval No. [2012]046). All eligible subjects were informed of the aim and risks of the study by the clinical investigators and provided written informed consent before participation.

Inclusion and exclusion criteria

Healthy, nonsmoking, male Chinese volunteers aged 20 to 30 years with a body mass index from 19 to 24 were enrolled in the study. Before study entry, subjects were interviewed (regarding their occupation, smoking and drinking habits, and medical history) and underwent a routine physical examination, including vital sign monitoring (ie, blood pressure, heart rate, respiratory rate, and temperature), ECG, chest radiograph, and laboratory analysis (ie, hematology, blood biochemistry, hepatic and renal function, and urinalysis) to ensure that they were healthy enough to participate in the study.

Subjects were excluded if they had a history or evidence of a renal, gastrointestinal, hepatic, or hematologic abnormality; any acute or chronic disease; or an allergy to any chemicals. Subjects who had used drugs of any kind within the 2 weeks before the start of or during the study were excluded, as were those who consumed a moderate amount of ethanol daily (ie, >1 L beer or its equivalent [50 g/d ethanol]).

Single-dose phase

Subjects were hospitalized at 10:00 PM the night before the beginning of the study. They were randomly assigned, in a 1:1 ratio using a computer-generated table of random numbers, to receive a single dose of the test formulation (100 mg; lot No. 20110603, expiration date June 2013, Shenzhen Zhijun Pharmaceutical Co, Ltd, Shenzhen, China) or the reference formulation (75 mg; lot No. X0357, expiration date August 2013, Beijing Novartis Pharma Co Ltd, Beijing, China) of diclofenac sodium sustained-release tablets during Period 1 and the alternate formulation during Period 2. The 2 periods of treatment were separated by a 7-day washout period.

In each treatment period, at ~8:00 AM after the 12-hour (overnight) fast and before administration of the study drug, an indwelling venous catheter (Becton Dickinson Medical Devices Co, Ltd, Suzhou, China) was placed in a suitable forearm vein, and a 5-mL blood sample was drawn into a vacuum tube with heparin sodium (Tianjin Biochemical Pharmaceutical Factory Co, Ltd, Tianjin, China). Then subjects were administered, under the supervision of study investigators, a single diclofenac sodium sustained-release tablet orally with 250 mL water. Intake of food and beverages (other than water, which was allowed after 2 hours) was not permitted until 4 hours after drug administration; a standardized lunch and dinner (200 g cooked rice, 200 g vegetables, 50 g pork, and 50 mL tomato soup) were provided at 4 and 9 hours after administration, respectively.

Additional blood samples were drawn at 0.5, 1, 2, 3, 5, 6, 8, 12, 14, 24, and 36 hours after drug administration. Plasma was obtained by centrifugation at 1000 g for 10 minutes at 5°C (LDZ5-2 Auto-balance Table Centrifuge; Beijing Medical Centrifuges Ltd, Beijing, China) and stored at –80°C until analyzed using an LC-MS/MS method. Following the 7-day washout period after administration of the initial formulation, the participants returned to the study site, where the alternate formulation was administered and samples were drawn and analyzed as before.

Multiple-dose phase

Volunteers assigned to the diclofenac sodium in the single-dose phase continued into the multiple-dose phase, during which they were confined to the study center and received a diclofenac sodium sustained-release tablet (75 mg or 100 mg QD at 8:00 AM) for 5 consecutive days. This dose was selected for the multiple-dose phase because it is the dose likely to be used in clinical practice.

Samples of venous blood (5 mL each) were drawn from the indwelling catheter before drug administration on Days 3 and 4 to determine the $C_{min,ss}$. On Day 5, blood samples were also drawn at 0.5, 1, 2, 3, 5, 6, 8, 12, 14, 24, and 36 hours after drug administration. All other experimental conditions were the same as in the single-dose phase. Every period of treatment was separated by a 7-day washout period throughout the experiment.

Intense physical activity, smoking, and consumption of beverages containing xanthine derivatives or alcohol were not allowed over the course of the study. Subjects were under continuous medical supervision at the study site throughout the 5-week study period.

Determination of plasma concentrations

The analysis of the concentrations of diclofenac in plasma was conducted at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, after the completion of both periods. The concentration of diclofenac in plasma was measured using a validated LC-MS/MS method. The LC-MS/MS system was a Shimadzu LC-30AD pump (Shimadzu, Kyoto, Japan), and a SIL-30AC autosampler (Shimadzu, Kyoto, Japan) coupled to an API QTRAP 5500 triple quadrupole mass spectrometer with an electrospray ionization source (AB/MDSSciex, Concord, Ontario, Canada). The tandem mass spectrometer was operated

under the multiple reaction monitoring (MRM) using an electrospray ionization source in negative ion mode. The optimized condition consisted of a collision-activated dissociation gas of the medium, a curtain gas of 25 psi, a nebulizer gas of 50 psi, a TurboIonSpray gas of 30 psi, an ionspray voltage of –4500 V, a source temperature of 550°C, and an entrance potential of –4 V for diclofenac and –5 V for the internal standard (ie, indometacin). Quantification was operated in MRM of the transitions m/z 294.0 → m/z 250.0 for diclofenac, and m/z 356.1 → m/z 312.1 for indometacin. MRM data was acquired and the chromatograms were integrated with the software Analyst (version 1.6.1; AB Sciex, Concord, ON, Canada).

An aliquot of 20 μL internal standard solution (indometacin 4920 ng/mL in mobile phase) and 20 μL menthol were added to a 200-μL plasma sample in a screw-cap glass tube. After vortex mixing for 10 seconds, 500 μL acetonitrile was added to the mixture to precipitate protein and the sample was vortex mixed for 30 seconds and centrifuged at 13,000 revolutions/min at 4°C for 10 minutes; 150 μL of the upper layer was transferred to an injection bottle, which was loaded into an autosampler cabinet and 5 μL aliquot was injected into the LC-MS/MS system.

Chromatographic separation was performed on a Ultimate XB-CN column (150 mm × 2.1 mm, 5μm, Welch Materials, Potomac, MD). The mobile phase consisted of acetonitrile: 10 mM ammonium formate containing 0.05% formic acid (70:30 *v/v*) at an isocratic flow rate of 0.3 mL/minute, the injection volume was 5 μL, and the run time was 4 minutes. The temperatures of the analytical column and autosampler were set at 35°C and 4°C, respectively. Under these conditions, the retention times for diclofenac and indometacin were 2.5 minutes and 2.6 minutes, respectively.

The method was shown to be suitable for the determination of diclofenac in human plasma over the range of 8.3122 to 997.464 ng/mL ($r \geq 0.996$). Using weighted least-squares regression, the lower limit of quantitation was 8.3122 ng/mL plasma. Accuracy measured at three concentration levels was acceptable (varied from 95.23% to 99.97%) and the relative SD values were all $< 5.67\%$. Precision was likewise acceptable (between 3.58% and 7.09%). The values of intraday and interday precision were $< 7.08\%$ at 3 concentration levels. Diclofenac and the internal standard were stable in plasma at room temperature for at least 24 hours, as well as for 84 days, at –80°C after 3 freeze–thaw cycles.

The analytical method for diclofenac quantitation in plasma samples was validated and applied to the bioequivalence study according to international guidelines.[18,19]

Tolerability

Signs and symptoms of adverse effects of diclofenac sodium such as gastrointestinal bleeding, ulceration, nausea, vomiting, as well as any untoward effects, were collected using a daily written questionnaire and recorded by the study physicians. Tolerability was assessed using monitoring of vital signs (ie, blood pressure, body temperature, heart rate, and respiratory rate), physical examination, ECG, and routine blood and urine tests, along with blood biochemical tests (hepatic and renal function), at the start as well as at the end of the study. Blood pressure was measured using a standard mercury sphygmomanometer on the left arm after 5 minutes' rest, in the sitting position. In addition, vital signs were assessed at 2, 4, 8, 24, and 36 hours after drug administration. Adverse events were evaluated by the study physicians for intensity, seriousness, and relationship to the study medication. Adverse events were defined as mild (awareness of a sign or symptom but easily tolerated), moderate (discomfort sufficient to cause interference with normal activities), or severe (incapacitating, with an inability to perform normal activities). Causality between the study drug and an adverse event was described by the study physicians as "certainly," "probably," "possibly," "suspected," or "not related."

Pharmacokinetic and statistical analyses

Using a power analysis (expected value, $\geq 1\text{-}\beta = 0.8$), it was determined that the power of the ANOVA was > 0.8 at a 90% CI according to the US Food and Drug Administration guidelines on bioequivalence testing,[20] indicating that 24 subjects would be sufficient for the purposes of the study.

Single- and multiple-dose pharmacokinetic parameters were calculated from the plasma concentration-time data by noncompartmental methods. C_{max} and T_{max} were obtained directly from the observed data. The AUC_{0-t} was calculated using the linear trapezoidal rule.[19] The $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measurable concentration and λ_z is the slope of the log-linear regression of the terminal concentration data points.[21] The $t_{1/2}$ was calculated as $(\ln 2)/\lambda_z$. The mean concentration at steady state (C_{ss}) was calculated as $AUC_{ss\ (0-\tau)}/\tau$.

To test the bioequivalence of the test and reference formulations, ANOVA for the crossover design was conducted on ln-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Before any comparisons were performed, dose-dependent parameters (C_{max} and AUC) were normalized to the lower dose. The ratios of the ln-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were calculated using the *F* score. The probability of exceeding the limits of acceptance for bioequivalence established by the FDA (80%–125%) was obtained using two 1-sided *t* tests, as described by Schuirmann[21] and the US Food and Drug Administration.[22] The formulations were to be considered bioequivalent if the ln-transformed ratios (test/reference) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125% and if the *P* values were < 0.05 for the 90% CIs.[19,20]

All pharmacokinetic analyses were conducted using Drug and Statistics Software version 2.0 (Mathematical Pharmacology Professional Committee of China, Shanghai, China).[23] Individual pharmacokinetic values were calculated and then the means calculated. All statistical analyses were performed using SPSS version 11.5 (IBM-SPSS Inc, Armonk, NY).

Results

Subjects

A total of 24 subjects (mean [SD] age, 24.4 [2.3] years [range, 20–30 years]; weight, 63.6 [8.5] kg [range, 51.2–86.8 kg]; and height, 1.72 [0.07] m [range, 1.57–1.91 m]) were enrolled in the study. All subjects completed both treatment periods, with no protocol violations.

Single-dose phase

Table I summarizes the mean [SD] pharmacokinetic parameters of diclofenac sodium after single-dose administration of 2 sustained-release formulations in 24 healthy Chinese male volunteers. The mean C_{max} for diclofenac with the test formulation was 11.786 [3.459] ng/mL and T_{max} was 5.48 [2.06] hours. With the reference formulation, the corresponding values were 11.754 [3.292] ng/mL and 6.26 [5.77] hours, respectively. The $t_{1/2}$ values with the test and reference formulations were 30.86 [7.61] and 30.96 [6.91] hours, respectively. The mean plasma concentration-time profiles of the two sustained-release formulations (100 mg for the test formulation and 75 mg for the reference formulation)

Table I
Mean [SD] pharmacokinetic parameters of diclofenac after administration of 2 sustained-release formulations (100 mg for the test formulation and 75 mg for the reference formulation) of diclofenac sodium in 24 healthy Chinese male volunteers.

Parameter	Single-dose administration		Multiple-dose administration	
	Reference 75 mg (n = 24)	Test 100 mg (n = 24)	Reference 75 mg (n = 24)	Test 100 mg (n = 24)
			Mean [SD]	
Cmax, ng/mL	473.5 [179.5]	546.6 [154.9]	525.6 [127.4]	650.5 [167.0]
T_{max}, h	4.9 [2.4]	4.3 [2.2]	5.1 [1.8]	4.5 [0.9]
$t_{1/2}$, h	5.9 [2.5]	6.0 [2.2]	5.2 [2.9]	5.5 [2.8]
AUC_{0-36}, ng · h/mL	3624.3 [1335.2]	4565.5 [1985.6]	4316.3 [633.0]	5335.1 [1291.9]
$AUC_{0-\infty}$, ng · h/mL	3841.2 [1402.3]	5019.1 [2314.0]	179.8 [26.4]	222.3 [53.8]
$AUC_{0-36}/AUC_{0-\infty}$	0.942 [0.047]	0.923 [0.067]	33.9 [20.9]	62.9 [34.9]

after administration of a single oral dose of diclofenac sodium are shown in the **Figure**.

Multiple-dose phase

After administration of multiple oral doses of diclofenac sodium (100 mg for the test formulation and 75 mg for the reference formulation, QD for 5 days), there were no significant differences in C_{min} between days 3, 4, and 5 (**Table I**), indicating that steady-state conditions were achieved by Day 5 of multiple dosing. **Table II** shows the mean pharmacokinetic parameters for diclofenac after multiple-dose administration of 2 sustained-release formulations. Under steady-state conditions, the mean [SD] $t_{1/2}$ was 6.24 [2.52] hours. The pharmacokinetic parameters of diclofenac after multiple dosing were comparable to those after single dosing, indicating no significant accumulation of diclofenac with repeated dosing. The mean concentration–time profile after administration of multiple oral doses of diclofenac sodium is illustrated in the **Figure**.

Table II shows the 90% CIs of the ratios (test/reference) for the ln-transformed values of pharmacokinetic parameters, as well as the probability of exceeding the limits of acceptance for

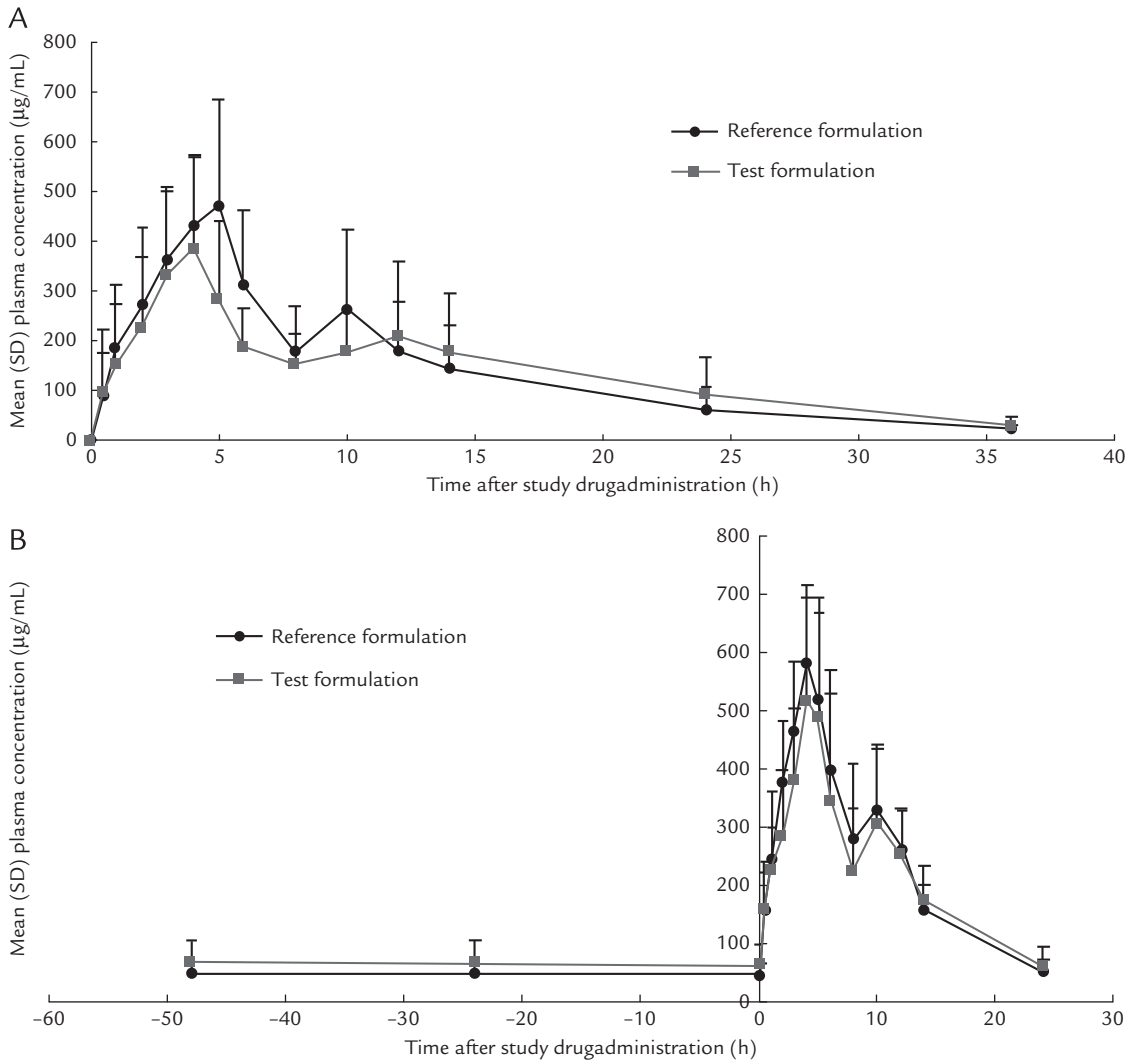

Figure. Mean [SD] plasma drug concentrations after administration of 2 sustained-release formulations (100 mg for the test formulation and 75 mg for the reference formulation, QD for 7 days) diclofenac sodium in 24 healthy Chinese male volunteers. (A) single-dose administration. (B) multiple-dose administration.

Table II
Comparison of 90% CIs for the ln-transformed ratios of pharmacokinetic parameters of 2 sustained-release formulations of diclofenac sodium after single-dose and multiple-dose administration in 24 healthy Chinese male volunteers; the probability of exceeding the limits of acceptance for bioavailability and power.

Parameter	Ratio, test/reference	90% CI	*P* for exceeding the limits of acceptance for bioavailability		Power
			< 80%	> 125%	
Single-dose administration					
C_{max}	89.6	82.1–97.8	< 0.001	< 0.001	0.99
AUC_{0-36}	93.8	81.9–107.4	< 0.001	< 0.001	0.99
$AUC_{0-\infty}$	95.9	83.2–110.6	< 0.001	< 0.001	0.99
Multiple-dose administration					
C_{max}	92.8	86.6–99.4	< 0.001	< 0.001	0.99
AUC_{ss}	91.3	84.2–99.1	< 0.001	< 0.001	0.99

bioavailability and the power of the test in the 24 healthy Chinese male volunteers. The results could meet the predetermined criteria for bioequivalence.

Tolerability

Diclofenac sodium appeared to be well tolerated by all volunteers. No adverse events were reported by subjects or found on analysis of vital signs or laboratory test results. No abnormalities were found in clinical or biochemical parameters when comparing baseline versus end-of-study assessments.

Discussion

Our study assessed the bioequivalence of single and multiple doses of 2 sustained-release tablet formulations of diclofenac sodium. There were no significant differences between formulations in pharmacokinetic properties in this small, selected, fasting, healthy Chinese male volunteer population.

The peak plasma concentrations were lower and occurred later after administration of either single or multiple doses of the sustained-release formulation of diclofenac compared with the normal tablet. Both formulations were apparently readily absorbed from the gastrointestinal tract, and diclofenac was measurable at the first sampling time (at 0.5 hour). The mean plasma profiles and bioavailability were similar both in single-dose and steady-state pharmacokinetic analyses.

In our study, no period or sequence effects for any pharmacokinetic property were found using ANOVA in the healthy Chinese male volunteers. The absence of a sequence effect in both pharmacokinetic parameters suggests that there was no carryover effect for these 24 subjects. These results indicate that a washout period of 7 days was adequate for total elimination of the drug between the 2 administration periods.

Our study found that the use of the sustained-release diclofenac sodium tablets under investigation might be suitable for therapeutic use, administered once a day. The adverse events reported in literatures were not related to higher peaks in plasma concentration of test formulation of diclofenac sodium in our study.

No subject withdrew from our study, and no adverse events were found on analysis of vital signs or laboratory test results. However, the study was limited by its short duration; its inclusion of only healthy male volunteers under fasted conditions; and by its single- and multiple-dose, open-label design. The mean age of these healthy subjects was 24.2 years (range, 20–30 years) and, therefore, the study results cannot be extrapolated to an older population. The results also cannot be used to predict performance in patients in clinical practice.

Conclusions

Our single- and multiple-dose study found that the test and reference formulations of diclofenac sodium met the regulatory criteria for bioequivalence in these fasting, healthy Chinese male volunteers. Both formulations appeared to be well tolerated.

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Conflicts of Interest

This research was sponsored by Shenzhen Zhijun Pharmaceutical Co, Ltd, Shenzhen, China. The sponsor ensured that the study was conducted according to clinical protocol but had no role in the design, conduct, analysis, or publication of the results. There were no benefits from commercial sources for the work reported in this article. The authors have indicated that they have no conflicts of interest regarding the content of this article.

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