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Full Length Research Paper

Bioequivalence assessment of two formulations of celecoxib: Open label, single dose and two-way cross over study in healthy human male volunteers

Muhammad Akhtar[1]*, Mahmood Ahmad[1], Irshad Ahmad[1], Naveed Akhtar[1], Asad Ullah Madni[1], Muhammad Usman[1], Muhammad Naeem Aamir[1], Sonia Khiljee[1], Mohammad Sualeh[2], Shujaat Ali Khan[3] and Muhammad Aleem[4]

[1]Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan.
[2]Faculty of Pharmacy, Federal Urdu University, Karachi, Pakistan.
[3]Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad, Pakistan.
[4]Department of Statistics, the Islamia University of Bahawalpur, Pakistan.

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The purpose of this study was to assess bioequivalence of two marketed formulations of celecoxib capsules in healthy human male volunteers. The study was conducted according to a single dose, randomized sequence, open label, two-period and crossover design. Both test and reference formulations comprised labeled dose of 200 mg celecoxib and were administered to each subject after an overnight fasting on two treatment days separated by one week of washout period. After drug administration, blood samples were collected at predetermined time points for a period of 48 h. Plasma separated from blood was analyzed for celecoxib concentrations using validated reverse phase-high performance liquid chromatographic (RP-HPLC) method. Various pharmacokinetic parameters including C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$ and K_{el} were determined from the plasma concentration for both formulations. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, were evaluated for bioequivalence after log-transformation of data. The 90% confidence intervals for the ratio of C_{max} (93.26 to 100.70%), AUC_{0-t} (87.00 to 117.50%) and $AUC_{0-\infty}$ (86.49 to 118.56%), values for the test and reference products were within the acceptance range of 80 to 125%, proposed by Food and Drug Administration (FDA) and European Medicines Evaluation Agency (EMEA). Based on these statistical inferences, it was concluded that two formulations of celecoxib are bioequivalent in their rate and extent of absorption.

Key words: Celecoxib, pharmacokinetics, bioequivalence, healthy human male volunteers.

INTRODUCTION

Celecoxib (CEL) belongs to the group of non-steroidal anti-inflammatory drug (NSAID) approved for the treatment of rheumatoid arthritis and osteoarthritis that selectively inhibits cyclooxygenase-2 (COX-2) (Dutta et al., 2009). Drug dissolution in gastrointestinal tract is the first step in intestinal absorption process after an orally administered dosage form (Shono et al., 2009). It is also used in the treatment of orthopedics, familial adenomatous polyps (Devis et al., 2001) and in dental practice (Moore and Hersh, 2001) with comparable efficacy and better gastric tolerability (Tindall, 1999). Lack of carboxylic group and ability to orient into the COX-2 enzyme makes CEL different from other NSAIDs (Craig and Stitzel, 2004). Chemical structure of CEL is 4-[5-(4-methylphenyl)-3- (trifluoromethyl)- 1H-pyrazole-1-yl] benzenesulfonamide **(**Manzoori et al., 2005) as shown in Figure 1. Metabolism of CEL takes place in liver by the enzymes cytochrome P450 2C9 (Cyp 2C9) (Sandberg et al., 2002; Sweetman et al., 2007), a cytochrome P450 isoform that is known to exist as several genetic variants (Stormer et al., 2003). CEL eliminated primarily by metabolism and about 3% is recovered in urine and

*Corresponding author. E-mail: muhammad.akhtar@iub.edu.pk. Tel: +92-300-6720628. Fax: +92-62-9255565.

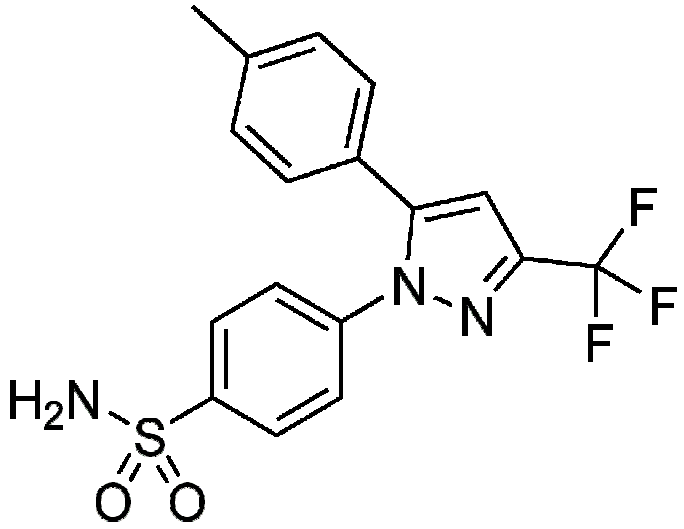

Figure 1. Chemical structure of celecoxib.

faeces as unchanged compound (Stempak et al., 2002). The European Medicines Evaluation Agency (EMEA) requires generic products that enter the marketplace to show bioequivalence to assess the possibility of alternative use between the reference product and an essentially similar medicinal product (CHMP, 2008). It was assumed that in the same subject an essentially similar plasma concentration time course will result in essentially similar concentrations at the site of action and thus, in an essentially similar effect. Medicinal products authorized and marketed on the basis of a full profile including chemical, biological, pharmaceutical, pharmacological, toxicological and clinical data are used as the reference product.

The aim of this study was to assess bioequivalence of two commercial formulations of CEL available as 200 mg capsules in Pakistani market.

MATERIALS AND METHODS

Product studied

CEL available as 200 mg capsule was purchased from the local market. Celbexx® (Getz Pharma) was used as the reference and Rheuoxib® (Highnoon Laboratories) as the test formulation.

Chemicals and reagents

HPLC grade acetonitrile, methanol and analytical grade chloroform were purchased from Merck-Germany. All other chemicals and solvents were of analytical grade and used as available.

Study design and performance

Only male subjects that meet the inclusion criteria of age from 22 to 36 years, body weight in the range of 56 to 70 kg, height in the range of 156 to 174 cm and was declared in good health based on medical history, physical examination and routine blood and urine analysis participated in this study, neither any treatment nor any drug was taken for at least one month prior to and during the study period, and have no drug allergy and hepatic or renal diseases. The study was an open label, single dose, randomized, two-period crossover design. Written informed consent was obtained from each subject before commencement of study. The study was performed in accordance with the revised 'Declaration of Helsinki' for biomedical research involving human subjects and the rules of 'Good Clinical Practice' (WHO, 1995; ICH, 1996). The clinical protocols of this study were approved by a local Pharmacy Ethical Committee (PEC) of The Islamia University of Bahawalpur. Eighteen healthy human male volunteers were scheduled to participate in this study and were divided into two groups. The study employed the subjects to receive single oral dose of CEL 200 mg on an empty stomach with about 250 ml of water. The subjects were instructed to fast over night prior to treatment. Both brands were administered randomly to two groups formulated for the study. Between two administrations there was a washout period of one week. The dietary schedule for all the subjects in both trial periods was same and consisted of two standard meals served after 6 and 12 h after dose. Liquid consumption was allowed but acidic beverages were prohibited. The subjects remained under constant surveillance by a physician throughout the study period.

HPLC analysis

A sensitive, accurate and validated reverse phase HPLC method was used (Ahmad et al., 2008; Itthipanichpong et al., 2005; Guirguis et al., 2001; Jalalizadeh et al., 2004). A brief description of method is given here. Blood samples were collected through 20 gauge venous cannula inserted into the forearm. Each time, 3 ml of blood was collected at predefined time points as before drug administration (zero time) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h after dosing. Blood samples were centrifuged at 3500 rpm and plasma was collected. The plasma samples were stored at -20°C until analyses. The mobile phase was composed of acetonitrile: water: triethylamine (50: 50: 0.05). The pH of mobile phase was adjusted to 5 with glacial acetic acid. Liquid-liquid extraction method was employed as the extraction procedure. Analysis was performed using HPLC (Perkin Elmer, USA pump series 200) with UV detector (Perkin Elmer, USA, series 200) set at 254 nm. The reverse phase system was consisted of base deactivated silica (BDS) Hypersil C_8 column (150 × 4.6 mm I.D. × 5 µ particle size).

Pharmacokinetic and statistical analysis

Non-compartmental analysis was performed to estimate pharmacokinetic parameters. Pharmacokinetic software Kinetica®, version 4.4.1 and SPSS version 12 were used for pharmacokinetic and statistical analysis. C_{max} (maximum plasma concentration) and t_{max} (time to reach C_{max}) were obtained directly from the data. AUC_{0-t} (area under plasma concentration time curve from time zero to time of last quantifiable concentration) was calculated using linear trapezoidal rule. K_{el} (terminal first order constant) was determined by a least square fit of terminal plasma concentrations. The constant K_{el} was used to extrapolate $AUC_{t-\infty}$ (area under plasma concentration time curve from time of last quantifiable concentration to infinite). $AUC_{0-\infty}$ (area under plasma concentration time curve from time zero to infinite time) was obtained as the sum of AUC_{0-t} and $AUC_{t-\infty}$. Bioequivalence between the products was determined by calculating 90% confidence intervals (CI) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products using logarithmic transformed data. Using the error variance (S^2) obtained from the ANOVA, the 90% confidence intervals (CIs) were

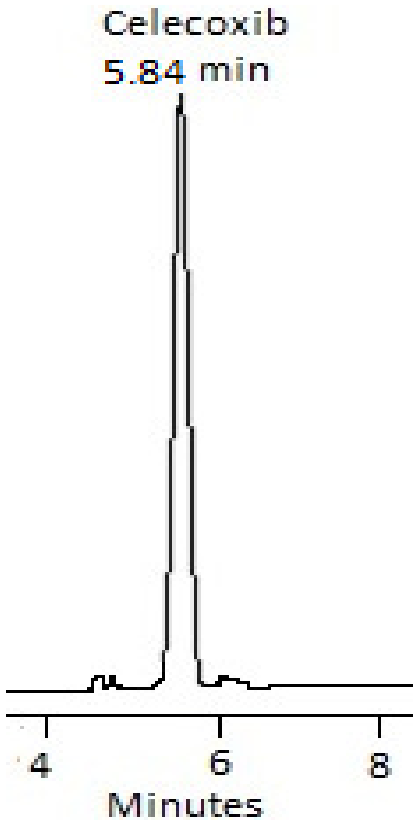

Figure 2. Representative HPLC chromatogram of celecoxib in volunteer 7 at 3 h.

calculated from the following equation:

$$90\%\ CI = \left(\bar{X}_T - \bar{X}_R\right) \pm t_{0.1(V)}\sqrt{s^2 \times \frac{2}{n}}$$

where $\bar{X}_T$ and $\bar{X}_R$ are the means of the ln transformed values for the test product (T) and reference product (R), S^2 is the error variance obtained from the ANOVA, n is the number of subjects, $t_{0.1}$ is the t value for 90% CI, v is the degree of freedom of the error variance from the ANOVA.

The anti ln of the aforementioned confidence intervals was the 90% Cls of the ratios of the test/the reference geometric means. The power of study would be 90% with 0.05 α. The acceptance criteria for bioequivalence were that the 90% Cls of the geometric mean ratios 0.80 to 1.25 for the AUC and C_{max}. The t_{max} difference was analyzed non-parametrically on the original data using Wilcoxon matched-pairs test (Setiawati et al., 2009).

RESULTS

The current HPLC method is suitable for CEL quantification in plasma samples. A representative HPLC chromatogram (Figure 2) of celecoxib was obtained in plasma sample of volunteer 7 at a time of 3 h after a single oral dose administration of 200 mg celecoxib. The representative plasma sample analyzed had a concentration of 0.937 µg/ml of celecoxib. Using an optimum flow rate of 1.0 ml/min of the mobile phase resulted in retention time of 5.84 min for celecoxib. The drug peak was well resolved with no tailing and no interfering peaks were observed. The tolerability for both the formulations under fasting conditions was good in all subjects. All volunteers who participated in this study continued to the end and were discharged in good health. The mean plasma concentration-time profile for the two formulations (test and reference) in healthy male volunteers after a single oral administration of 200 mg CEL capsules is shown in Figure 3. Both formulations appeared to be absorbed readily from gastrointestinal (GI) tract and measurable levels of CEL were found at the first sampling time (0.5 h) in all subjects. Almost identical plasma CEL concentration profiles were obtained from both formulations which are just about super imposable (Figure 3). All the calculated pharmacokinetic parameters for two brands of CEL are given in Table 1. The values of the analysis of variance (ANOVA) for the determination of product, group and period effects and the 90% confidence intervals (CI) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for both the reference and test products using logarithmic transformed data are shown in Table 2.

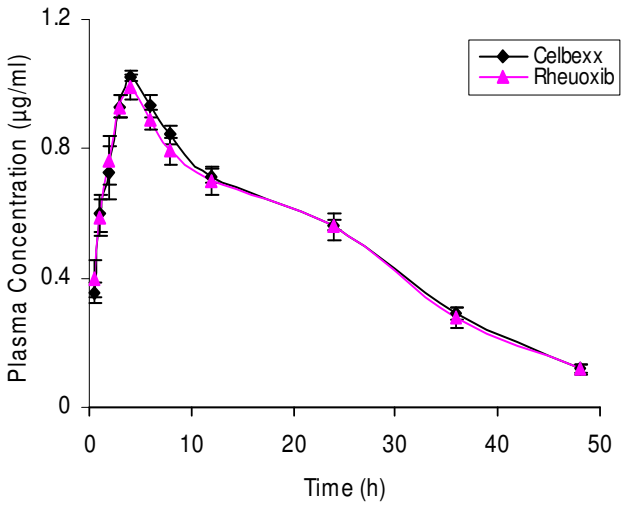

Figure 3. Mean (± SEM) of the plasma concentration of celecoxib for both reference and test products.

DISCUSSION

The current study has been conducted in eighteen healthy male volunteers to check the bioavailability of CEL as it is assumed that pharmacological effects of any drug substance are related to the available concentration

Table 1. Comparison of celecoxib pharmacokinetic parameters after single oral administration of 200 mg of the reference (R) and test (T) products.

Pharmacokinetic parameter	Celbexx® (R) (mean ± SEM)	Rheuoxib® (T) (mean ± SEM)
C_{max} (µg/ml)	1.03 ± 0.02	1.07 ± 0.01
t_{max} (h)	3.62 ± 0.26	3.5 ± 0.27
AUC_{0-t} (µg h/ml)	24.52 ± 0.61	23.96 ± 1.34
$AUC_{0-\infty}$ (µg h/ml)	26.63 ± 0.74	25.96 ± 1.56
$t_{1/2}$ (h)	11.80 ± 0.89	10.99 ± 0.97
K_{el} (h^{-1})	0.06 ± 0.01	0.07 ± 0.01

Table 2. ANOVA test (α = 0.05) for the assessment of treatment, group and period effects, and 90% CI for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the reference and test products.

Pharmacokinetic parameter	ANOVA (P-values)			90% CI
	Variation source			
	Product	Group	Period	
C_{max} (µg/ml)	0.067	0.101	0.09	93.26 - 100.70
AUC_{0-t} (µg h/ml)	0.181	0.689	0.728	87.00 - 117.50
$AUC_{0-\infty}$ (µg h/ml)	0.135	0.699	0.713	86.49 - 118.56

of drug in systemic circulation. According to FDA and EMEA regulation, the sampling schedule should be designed to provide a reliable estimate of the extent of absorption. As a general rule, this is achieved if AUC_{0-t} is at least 80% of $AUC_{0-\infty}$ (CHMP, 2008). Usually the sampling time should extend to at least three terminal elimination half-lives of the active drug ingredient, beyond t_{max}. The average $T_{1/2}$ of CEL was reported as 8.79 ± 5.49 h (mean ± SD) in the range of 3.51 to 19.91 h (Itthipanichpong et al., 2005) in one study and 12.76 ± 1.44 h (mean ± SEM) in another study (Emami et al., 2008). Thus, the total sampling time (48 h) was adequate to estimate the extent of absorption. The current study meets all the aforementioned criteria. The plasma decay half-life values in this study were 11.80 and 10.99 h for both the reference and test products, t_{max} values were 3.62 and 3.50 h for both reference and test products, respectively, and are comparable to the reported values (Sweetman et al., 2007; Paulson et al., 2001; Brunton et al., 2010). The low solubility of CEL will prolong absorption process and make elimination half-life more variable (Clemett and Goa, 2000). The C_{max} (mean ± SEM) values calculated in this study were 1.03 ± 0.02 and 1.07 ± 0.01 µg/ml for reference and test formulations, respectively and were comparable to similar previously conducted studies where C_{max} (mean ± SD) value was 806 ± 411 ng/ml (Paulson et al., 2001) and in another study C_{max} (mean ± SD) value was 686.83 ± 211.35 ng/ml (Itthipanichpong et al., 2005). The mean (± SEM) elimination half-life values for both reference and test products in current study were 11.80 ± 0.89 and 10.99 ± 0.97 h with the mean (± SEM) elimination rate constant of 0.06 ± 0.01 and 0.07 ± 0.01 h^{-1}, respectively. These values were in agreement to the reported values of a previously conducted study on CEL after 200 mg dose administration (Itthipanichpong et al., 2005). The area under the plasma concentration-time curve which reflects the extent of drug absorption from time zero to the last quantifiable concentration (AUC_{0-t}) and from time zero to infinity ($AUC_{0-\infty}$) for CEL in the current study were found to be 24.52 ± 0.61 and 26.63 ± 0.74 µg h/ml for reference formulation and 23.96 ± 1.34 and 25.96 ± 1.56 µg h/ml for test formulation, respectively. In a similar previous study the mean (± SD) values of AUC_{0-t} and $AUC_{0-\infty}$ were found to be 5157.12 ± 1499.46 and 5911.48 ± 1363.51 ng h/ml, respectively (Itthipanichpong et al., 2005). The absorption of CEL is minimally affected when administered with food in humans (Paulson et al., 2001). For CEL the racial differences in drug disposition and pharmacokinetic changes in elderly were reported (Davies et al., 2000). The differences in formulation and processing variables could also be the factors influencing the release of the drugs from the drug products, resulting in different pharmacokinetic values (Usman et al., 2009). The multivariate analysis, accomplished through analysis of variance (ANOVA) for assessment of product, group and period effects, revealed the absence of any of these effects in current study. For bioequivalence (BE) assessment, a standard BE range (80 to 125%) for basic pharmacokinetic parameters including C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ has been generally accepted. The 90% CI for the ratio of C_{max} (93.26 to 100.70%), AUC_{0-t} (87.00 to 117.50%) and $AUC_{0-\infty}$ (86.49 to 118.56%) values for the test and reference products are within the 80 to 125%

interval according to the criteria given by FDA and EMEA.

Conclusion

This single dose study found that the test and the reference products met the regulatory criteria for bioequivalence in healthy male volunteers. On the basis of statistical inferences, it is concluded that both the formulations of CEL are bioequivalent. The pharmacokinetic values determined in current study are also comparable to the previously reported studies.

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