Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of rheumatoid arthritis and osteoarthritis. However, gastrointestinal disturbances are the most frequently reported side effects of all NSAIDs, and include dyspepsia, gastric erosion, peptic ulcer formation and perforation, major upper gastrointestinal bleeding, inflammation and permeability changes of the intestine and lower bowel (1, 2). Consequently, there is a considerable need for a clinically effective NSAID with an improved safety profile, especially in terms of gastrointestinal side effects. Meloxicam is a cyclooxygenase-2, preferential inhibitor non-steroidal anti-inflammatory drug (NSAID) and belongs to an enolic acid (oxicam) class used for the treatment of osteoarthritis and rheumatoid arthritis. The purpose of this single dose randomized cross-over study was to assess bioequivalence of two brands of oral meloxicam tablets (Xobix® manufactured by Hilton Pharma (Pvt.) Ltd. as a reference and tablet Melfax® by AGP (Pvt.) Ltd. as a test) in 18 healthy male volunteers in local population of Pakistan. The data obtained were subjected to non-compartment model pharmacokinetic analysis. The value of $C_{\text{max}}$, calculated in present study was $1.051 \pm 3.762 \mu g/mL$ for reference formulation and $1.023 \pm 4.102 \mu g/mL$ (the mean ± SEM) for test sample. The value of $T_{\text{max}}$ was $3.125 \pm 1.004$ h for reference standard and $3.750 \pm 1.469$ h (the mean ± SEM) for test sample. The area under the curve from zero to infinity ($AUC_{0-\infty}$) was $28.667 \pm 0.414 \mu g\cdot h/mL$ for reference standard and $28.367 \pm 0.333 \mu g\cdot h/mL$ for test sample (the mean ± SEM). The $t_{1/2}$ values were $13.694 \pm 0.568$ h and $13.319 \pm 0.567$ h (the mean ± SEM) for reference formulation and test sample, respectively. The test formulation was found to be bioequivalent to reference formulation based on the pharmacokinetic parameters.

**Keywords:** meloxicam, bioequivalence, non-compartment model
Cam in domestic situation to ensure the rational usage and safety of this valuable medicinal agent.

EXPERIMENTAL

Chemicals and Reagents
Meloxicam was gifted by AGP, Pakistan. Methanol (HPLC grade), \( \alpha \)-phosphoric acid (85%) and hydrochloric acid were purchased from Merck, Germany. Di-sodium hydrogen phosphate was purchased from Riedel-de-Haen, England. All other chemicals and solvents were of analytical grade and used without any further purification.

Methods
The study was an open, single dose, cross-over complete two period of treatment dosing. Written informed consent was obtained from each subject before commencement of study. Eighteen healthy human male volunteers (age limit 20–26 years and body weight range 63–87 kg) were scheduled to participate in the study. Each volunteer received single dose of meloxicam (15 mg orally) separated by 2-week washout period. After dosing, serial blood samples were collected for a period of 72 h. Plasma was harvested and analyzed for meloxicam by a modified HPLC method.

Formulations
Test: Melfax® (meloxicam) 15 mg tablets, AGP, Karachi-Pakistan. Reference: Xobix® (meloxicam) 15 mg tablets, Hilton Pharma, Karachi-Pakistan.

Experimental design
The ethics of study was approved by the Board of Advanced Studies and Research, the Islamia University of Bahawalpur, Pakistan. Randomized two way cross over design was adopted for the study as given in Table 1. The volunteers were divided into two groups, 9 in each. The four volunteers of one group received single oral dose of 15 mg of meloxicam tablets (Xobix®, Hilton Pharma as a reference product designated as A), whereas the other four received 15 mg of meloxicam tablets (Melfax®, AGP Pvt. as a test product designated as B) in the treatment at first period. In the second period, reverse of the first period was administered. The single dose drug regimen was administered on an empty stomach and volunteers were housed at the study center from 1 h before to 72 h after the dosing. Each volunteer was instructed to fast overnight prior to the treatment visit. The volunteers were allowed to drink water at libitum. Each volunteer was provided with standardized breakfast two hours after the dosing. They were also provided the lunch and evening refreshment. A washout period of 14 days was allowed before the start of the second period.

Sample collection
A 20-gauge venous brannula was inserted into forearm for collection of blood samples. A blood sample was collected before drug was given (zero time) and then at 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0, 60.0 and 72.0 hours after dosing of meloxicam tablets (10). A 5 mL blood sample was collected each time. Blood samples were centrifuged at 3500 rpm for 10 min and plasma was collected and separated into labeled vials. The plasma samples were kept frozen at −20°C until analysis (11).

Extraction procedure
Extraction procedure was comprising of precipitation method. In extraction procedure, 1 mL of plasma sample was in glass centrifuge tube and 100 μL of 1 M HCl was added. Protein was precipitated out and vortex mixed for one minute then centrifuged at 4000 rpm for 20 min. Supernatant layer was taken and filtered through 0.45 μm Millipore filter, evaporated under the stream of nitrogen at ambient temperature, then reconstituted with 100 μL of the mobile phase and 20 μL were injected on the column for analysis (11).

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High performance liquid chromatography (HPLC)

Analysis was performed by using Perkin Elmer Liquid Chromatograph, with a pump series 200 and Perkin Elmer UV detector set at 346 nm. A reverse phase system was used consisting of C18 column (150 mm × 5 µm particle size × 4.6 mm I.D.). The mobile phase consisted of methanol : phosphate buffer (50 mM) (60:40, v/v). The pH of mobile phase was adjusted with ortho-phosphoric acid (85%) to 3.5 and the phase was degassed by passing nitrogen gas for about 2–3 min. The mobile phase was pumped at a rate of 1.0 mL/min. Injections of 20 µL volume were injected with a run time of 10 min (12).

Safety analysis

Health assessment including vital signs, physical examination and clinical laboratory testing was performed before and seven days after study. Subjects were interviewed at the beginning and end of each study period and were monitored throughout of the confinement period to determine any adverse events potentially related to study medication of procedures.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed by using non-compartmental method of analysis by using KineticaÆ 4.0 program.

Statistical analysis

Paired t-test was used to calculate the difference whether significant (p < 0.05) or insignificant (p > 0.05) between the values of the bioparameters of the two different brands of meloxicam, Xobix® and Melfax®. The confidence level was set at 95%.

RESULTS AND DISCUSSION

Maximum plasma concentration (C_{max})

The peak plasma drug concentration C_{max} represents maximum plasma drug concentration obtained after oral administration of drug. In the present study, the (the mean ± SEM) maximum plasma concentration (C_{max}) for reference and test product was found to be 1.051 ± 0.3762 µg/mL and 1.023 ± 0.4102 µg/mL, respectively. This difference was not significant (p > 0.05) and shows that both the brands were bioequivalent. The value for C_{max} in the present study has been found in good agreement with the previous studies conducted in healthy human volunteers. In these studies conducted in healthy male volunteers (8), maximum plasma concentration (C_{max}) was 1.72 mg/L with 18.8% coefficient of variation with a single oral dose of 30 mg of capsules. The higher value of C_{max} may be due to higher dose, i.e., 30 mg. The C_{max} reported in another study after a single oral dose of 15 mg in healthy subjects (the mean ± SD) was 1.20 ± 0.24 µg/mL. So by calculations of the dose, the value of C_{max} calculated in this study was found similar to that given in the literature. There was statistically non-significant difference (p > 0.05) between the values of C_{max} of both the brands.

Time of peak plasma concentration (T_{max})

The time of peak plasma concentration (T_{max}) corresponds to the time required to reach maximum drug concentration after drug administration. At T_{max} peak drug absorption occurs and the rate of drug absorption exactly equals to the rate of drug elimination (11). In the present study, T_{max} values of reference product and test product were 3.125 ± 1.004 h and 3.750 ± 1.469 h, respectively. Statistically non significant difference (p > 0.05) was found in the values of T_{max} of both the brands. These values for T_{max} (the mean ± SEM) are in good agreement with the previously reported values of T_{max} (after a single oral dose of 15 mg in healthy subjects) 4.365 ± 1.17 h (14), and 5.6 ± 3.2 h (the mean ± SD) (13). Another study (15), after a single oral dose of 15 mg in healthy subjects has reported a value of T_{max} as 6.3 ± 2.6 h and 5.3 ± 2.5 h (the mean ± SD) for test and reference product, respectively.

The absolute bioavailability of meloxicam tablets was 89% following a single oral dose of 30 mg. The mean maximum plasma concentration (C_{max}) was achieved within 4–5 h after a 7.5 mg meloxicam tablet taken under fasted conditions, indicating prolonged drug absorption.

Area under curve (AUC)

Area under the curve (AUC) reflects the total amount of drug that reaches the systemic circulation (11). AUC is directly proportional to the dose of the drug. As dose of the drug increases, AUC also increases. In the present study, the value of AUC_{0-72} (the mean ± SEM) for the reference product and test product were 28.667 ± 0.414 µg·h/mL and 28.367 ± 0.333 µg·h/mL, respectively. A statistically non significant (p > 0.05) difference was found between the values of AUC_{0-72} of both the brands.

In a previous study conducted on human volunteers (13), AUC_{0-8} (the mean ± SD) was found to be 29.17 ± 7.06 µg·h/mL which is in agreement with the values of AUC in the present study.
Half life (t1/2)

It is the time required by a drug to become one half of its original concentration in the plasma (11). In the present study, the plasma half life of reference and test products were 13.694 ± 0.568 h and 13.319 ± 0.567 h (the mean ± SEM), respectively. A statistically non significant (p > 0.05) difference was found between the values of AUC0-∞ of both the brands. These values are in agreement with the previous findings of 19.300 h of meloxicam in healthy subjects after oral dose of 15 mg capsule (16). Another study conducted on healthy volunteers using 30 mg tablet has reported t1/2 value of 17.5 h (1). A study was also conducted using two different tablets of 15 mg in healthy volunteers. The t1/2 (the mean ± SEM) reported was 19.953 ± 1.05 h and 19.050 ± 1.04 h for both the formulations, respectively (14). The half-lives of both the brands show no difference (p > 0.05) when compared statistically.

Comparison of the mean ± SEM of bioavailability and pharmacokinetic parameters of Xobix®-Hilton and Melfax®-AGP administered in the oral dose of 200 mg in normal subjects is in Table 2 and comparison of plasma concentration versus time profile of Xobix®-Hilton and Melfax®-AGP plotted on rectangular co-ordinate graph, administered in the oral dose of 15 mg to 8 subjects is presented in Figure 2.

The elimination half-life (t1/2) of meloxicam is approximately 20 h. This is reflected in a total plasma clearance (ClT) of 0.4–0.7 L/kg. Steady-state plasma concentrations are achieved within 3–5 days.

CONCLUSION

The results of reference and test brands showed that both these brands possess almost the same bioavailability and pharmacokinetic parameters. Therefore, on the basis of the values of pharmacokinetic and bioavailability parameters, it can be concluded that both the brands are bioequivalent and can be used as pharmaceutical substitute with each other.
REFERENCES


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