BIOEQUIVALENCE STUDIES OF TWO BRANDS OF MELOXICAM TABLETS IN HEALTHY PAKISTANI VOLUNTEERS

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ABSTRACT

The pharmacokinetic parameters of two oral formulations of meloxicam tablets were compared in a randomized, single oral dose; two treatments cross over design in 12 healthy male volunteers belonging to Pakistan under fasting conditions. After an overnight fast, the volunteers received 30 mg meloxicam and the blood samples were collected up to 96 hours and drug concentrations were determined by a validated HPLC method. Various pharmacokinetic parameters were determined from the plasma concentration-time curves of both formulations. The 90% confidence intervals obtained by analysis of variance were 87-94% for C_{max} and 88-97% for AUC_{0-t}, that fell well within the acceptance range of 80-125%. Also, no significant difference (α=0.05, Wilcoxon Signed rank test) were detected between T_{max} of both formulations. The two formulations were well tolerated and no adverse effect was reported during the study.

Keywords: Bioequivalence, meloxicam tablets, anti-inflammatory, analgesic, antipyretic drugs.

INTRODUCTION

Meloxicam (4–hydroxy–2–methyl–N-(5–methyl-1,3–thiazol–2-yl)-2H-1,2–benzothiazine–3- carboxamide 1,1-dioxide) is a NSAID belonging to the class of the enolic acids. It was chosen for pharmaceutical development because in animal tests, it showed a high potential for anti-arthritic activity, anti-inflammatory activity and at the same time less gastric and local tissue irritation as compared to NSAIDs available prior to its development (Stei and Püschner 1994 and Engelhardt *et al*., 1994). It is used in the management of rheumatoid arthritis, symptomatic treatment of osteoarthritis and ankylosing spondylitis (Sweetman 2005).

Meloxicam is almost completely absorbed when given parenterally, orally or rectally with an absolute bioavailability of 89% (Davies and Skjodt 1999 and Türck *et al*., 1997). It undergoes extensive metabolism, primarily by cytochrome P450, particularly by CYP2C9 and to a minor extent by CYP3A4 (Chesne *et al*., 1998), forming four major inactive metabolites (Schmid *et al*., 1995). The pharmacokinetics of meloxicam are linear over the entire dose range (7.5-30 mg) and remain unchanged from single to multiple dosing conditions, total meloxicam clearance found to be 7-8 ml/min with an elimination half-life around 20 hours (Türck *et al*., 1997).

Although several pharmacokinetic studies of meloxicam have been published, only few have been focused on bioequivalence (Türck *et al*., 1997, Dasandi *et al*., 2002, Marcelín-Jiménez *et al*., 2005, Rigato *et al*., 2006, Gschwend *et al*., 2007). The present study was carried out to determine the pharmacokinetic parameters of two brands of meloxicam tablets in fasting, healthy human volunteers belonging to Pakistan for the first time and to compare these parameters statistically to evaluate the bio-equivalence between the two brands.

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EXPERIMENTAL

Chemicals and reagents

Meloxicam reference standards was obtained through the courtesy of a local Pharmaceutical company. Acetonitrile, methanol, glacial acetic acid, perchloric acid (70-72%) and sodium acetate were purchased from Merck, Germany while heparin was obtained by BS M & B Co., Ltd., Shenzhen, China.

Instruments

The liquid chromatograph consisted of: an isocratic pump (LC-10A, Shimadzu, Japan), a spectrophotometric variable detector (SPD-10 AVP, UV-VIS detector, Shimadzu, Japan), a Rheodyne injector (Model-7725, USA equipped with 100 µl injector loop), a Communication Bus Module (CBM 102, Shimadzu, Japan), a reverse phase column (Lichrospher 5 µm RP-18 column (125x4.6 mm), Merck, Germany) and a computer (Pentium II 333 MHz) with software (LC–10 A for data handling). The other instruments used were: Centrifuge (Labofuge 200 Haraeus Septech, Kendro lab. Products, Germany), pH meter (Metler Toledo, Switzerland), ultrasonic bath (Clifton ultrasonic bath, Nickel electric Ltd., England), balance (Metler Toledo, Switzerland).

Study products

The study was conducted by using each of a test product which was a commercial formulation and a reference product which was the innovator product.

In vivo studies

Volunteers

Twelve (12) healthy male students of the Faculty of Pharmacy, University of Karachi volunteered in the present studies. The average age of the volunteers was 22.7±2.6 years, body weight was 68.8±10.2 kg and the body height was 171.7±3.7 cm. All participants were non-smokers and were selected on basis of negative past medical history. Normal physical examination was carried out by a registered medical practitioner and routine laboratory investigations (hematology, blood bio-chemistry, and urine analysis) were performed.

SELECTION OF VOLUNTEERS

Inclusion and exclusion criteria

The inclusion criteria for the volunteers were: male subjects belonging to Pakistan, age between 20-40 years, no history of allergic tendencies and reaction to NSAIDs, no history of alcohol abuse, with normal blood counts, normal liver and kidney function tests and without any abnormalities in physiology, urine and blood analysis, neither any treatment nor any drug taken for at least one month prior to the study and absence of any chronic disease or any pathological state.

The exclusion criteria were : a history of drug allergy, gastrointestinal disorders and cardiac, haematological, hepatic or renal diseases, concomitant medication on study days and repeated use of drugs that influence absorption and hepatic biotransformation of meloxicam during the 4 weeks prior to the study. This was done to ensure that the existing degree of variation was not influenced by illness or by other medications.

The study was approved by the Board of Advance Studies and Research (BASR), University of Karachi and was performed according to international guidelines and recommendations. Twenty volunteers were selected and out of which twelve volunteers, who met all of the inclusion criteria and met none of the exclusion criteria were enrolled in the study. A written informed consent was obtained from the enrolled volunteers before study initiation and after reception of written and oral information related to objectives, characteristics, procedures, risks and rights of participation in the study. The clinical phase of the study was performed at the Research Laboratory of the Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Pakistan.

Restrictions

The volunteers were not allowed to take any drug during the study. They were directed to report the investigator about any inter-current illness and the treatment taken. The purpose of this was to enable the investigator to make necessary adjustments in the procedure. No volunteer took any drug for at least one month prior to and during the study.

Design

This study was based on a single dose, randomized, two treatment, two periods crossover design.

Dosage

All participants received 30 mg meloxicam (4x7.5mg tablets) Test Formulation or Reference Formulation and a 2 week washout interval between formulation was established.

Study performance

All participants were required to refrain from caffeine, chocolate, tea or coke containing beverages at least 24 h before each dose. They were asked to fast from 10 h before until 5 h after drug administration. At 7:00 a.m. on the day of dosing, an indwelling cannula was applied in a suitable forearm vein of each volunteer and the zero hour blood sample was drawn. The first volunteer and then the remaining volunteers were asked to swallow one of the formulation with 240 ml of water at 8:00 a.m. The dietary regimen was similar for all subjects in both trial periods and consisted of two standard meals served 5 and 11 h after dosing. No other food was permitted during the study period. Liquid consumption was allowed ad libitum after lunch but xanthine-containing and acidic beverages were prohibited. The subjects were not allowed to remain in a supine position or to sleep after drug administration. Volunteers were ambulatory during the study but were prohibited from strenuous activity. During the two arms of the study, the subjects remained under constant medical surveillance by a physician and maintained daily contact with the clinical investigator and reported any adverse events, whether related or not to the ongoing drug treatment in his opinion. After each period of the study, the volunteers were re-examined by a physician.

Serial blood samples of 10 ml were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 24, 48, 72 and 96 h after drug administration by heparinized disposable syringes. All the volunteers were housed for 11 hours and up to 11 hours, samples were collected by indwelling cannula. Subjects were discharged from the research laboratory on the night of day 1 and they reported back to the laboratory at days 2, 3, 4 and 5 in ambulatory conditions for the last four blood samples (24, 48, 72 and 96 h postdose) and the samples were collected by vein puncture into heparinized disposable syringes. All blood samples were immediately transferred in centrifuge tubes and plasma was harvested by centrifugation at 3000 rpm for 10 minutes. The samples were stored at -20^{o}C until analyzed. After a period of 2 week, the study was repeated in the same manner to complete the crossover design.

Drug assay

A number of analytical techniques are available for the estimation of meloxicam in body fluids (Nageswara *et al*., 2005). The literature survey revealed that the high performance liquid chromatographic method reported by Dasandi *et al*., 2002 is simple and matches with the facilities available in the research laboratory of Pharmaceutics. This method was selected, modified and used successfully in the present study to extract and to analyze meloxicam in plasma samples using external standard method. The calibration curves were linear over the concentration range of 0.1 to 2.5 µg/ml using 100 µl plasma samples. Both the interday and intraday accuracy and precision were evaluated by replicate analysis of plasma samples at three different concentrations of meloxicam and were found well within the acceptable limits. Plasma samples were stable for three freeze thaw cycles at -20°C.

Sample preparation

To 1 ml of plasma sample, 0.1 ml of protein precipitating mixture (acetonitrile and perchloric acid; 1:1 v/v) was added and vortexed for 1 minute. After centrifugation to 4000 rpm for 20 minutes, a 0.1 ml supernatant was injected to the HPLC system.

Chromatographic conditions

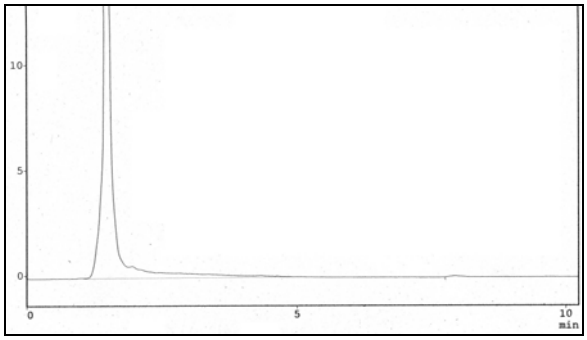
In HPLC system, mobile phase was composed of sodium acetate buffer (pH 3.3, 170 mmol) and acetonitrile (62:38 v/v). The flow rate was 1 ml/min., the detection wavelength was 355 nm. All assays were performed at ambient conditions. The retention time of meloxicam was approximately 8 minutes (Fig. 1).

Pharmacokinetics and statistical analysis

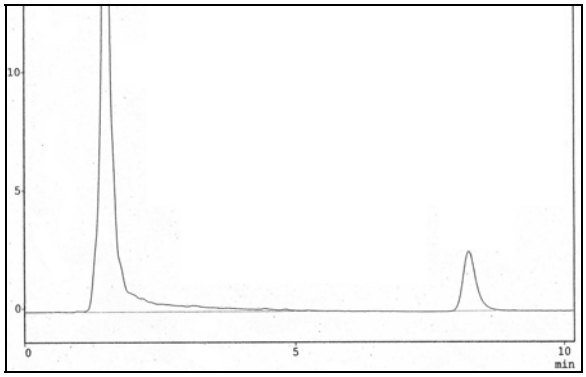
Pharmacokinetic parameters were determined using Kinetica® software, version 4.4.1. The C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were assessed by analysis of variance (ANOVA) while T_{max} were analyzed using Wilcoxon Signed rank test for paired samples at 0.05 level of significance. (Bolton 1997).

RESULTS AND DISCUSSION

The mean plasma concentrations of meloxicam in twelve healthy male volunteers after a single oral administration of 30 mg (4x7.5 mg tablets) reference and test formulations is depicted in Fig. 2. The calculated pharmacokinetic parameters of the two brands are shown in Table 1. Almost identical plasma meloxicam concentration profiles were obtained from both the formulations. The two brands of meloxicam were well tolerated by the volunteers in both phases of the study. Clinically relevant or drug related side-effects were not observed in any of the volunteers. There were no drop-outs and all the volunteers who had started the study continued to the end and were discharged in good health condition. The data from all volunteers was included in the pharmacokinetic analysis. Meloxicam was measurable at the post dose first sampling time in all the volunteers (1 h) and measurable titer was found in case of each brand even after 96 hours of administration.



(a)



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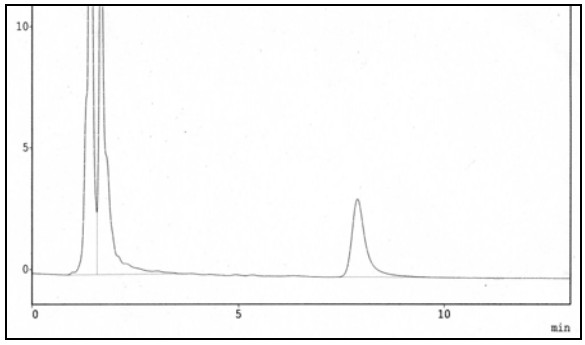

(c)

Fig. 1: Typical chromatograms of blank plasma (a), plasma spiked with meloxicam (0.5 µg/ml) (b) and plasma of a volunteer at 2 h after a single oral dose of 30 mg Reference formulation (c).

Following administration of meloxicam tablets, the mean ± standard deviation values for AUC_{0-t} were 47.12±3.53 µg.hr/ml (range: 43.90 to 54.28 µg.hr/ml) for the test formulation and 51.04±4.19 µg.hr/ml (range: 44.30 to 55.84 µg.hr/ml) for the reference formulation with a mean

Table 1: Pharmacokinetic parameters for the reference and test formulations of meloxicam after oral administration of 30 mg meloxicam to 12 healthy male volunteers.

Volunteer ID	Reference formulation				Test formulation			
	T_{max} hr	C_{max} µg/ml	AUC_{0-t} µg.hr/ml	$AUC_{0-\infty}$ µg.hr/ml	T_{max} hr	C_{max} µg/ml	AUC_{0-t} µg.hr/ml	$AUC_{0-\infty}$ µg.hr/ml
A	3.67	1.41	53.15	96.02	3.80	1.35	52.69	85.75
B	3.85	1.43	53.71	102.29	3.88	1.29	44.69	70.65
C	3.97	1.42	54.67	118.59	3.72	1.22	45.15	69.58
D	3.85	1.44	54.41	107.04	3.63	1.58	54.28	100.97
E	3.65	1.41	54.55	101.98	3.87	1.44	51.10	87.13
F	3.75	1.32	44.30	71.72	3.65	1.26	46.88	93.57
G	3.20	1.58	53.90	95.17	3.42	1.32	46.51	96.53
H	3.50	1.61	47.13	79.28	3.72	1.36	45.86	71.33
I	3.65	1.52	48.11	70.78	3.61	1.37	45.18	74.00
J	4.00	1.49	55.84	89.52	3.60	1.26	43.90	83.04
K	3.92	1.61	46.82	77.34	3.95	1.39	44.85	76.55
L	3.36	1.49	45.96	75.07	3.84	1.25	44.34	78.43
Mean	3.70	1.48	51.04	90.40	3.72	1.34	47.12	82.29
STD	0.25	0.09	4.19	15.57	0.15	0.10	3.53	10.65
% CV	6.76	6.08	8.21	17.22	4.03	7.46	7.49	12.94

T/R ratio of 0.93±0.08 (table 1). It is clear from the Table 1 that AUC_{0-t} obtained by reference formulation were slightly greater as compared to the test formulation. The ANOVA for ln transformed data for AUC_{0-t} detected a significant difference in treatments but no significant difference was detected in case of sequence, subjects within sequence and periods respectively. The 90% confidence interval for AUC_{0-t} ln transformed was 0.88-0.97 with mean ratio (T/R) of 0.98. Thus confidence intervals are within the bioequivalence limits of 0.80-1.25. Dasandi *et al*., 2002 had reported AUC_{0-72} value of 35.03 and 33.58 µg.hr/ml (test and reference formulations respectively) in Indian volunteers while AUC_{0-96} reported by Türck *et al*., 1997 in Germans was 62.3 µg.hr/ml following oral administration of 30 mg meloxicam. It is apparent that AUC_{0-t} of meloxicam in Pakistanis is greater than that reported in Indians while it is lower as reported in Germans. This indicates variation in absorption of meloxicam in various populations.

When the area under the plasma level time curve from time zero to time infinity ($AUC_{0-\infty}$) was compared, the test preparation gave (mean±SD) 82.29±10.65 µg.hr/ml (range: 69.58 to 100.97 µg.hr/ml) and the reference preparation 90.40±15.57 µg.hr/ml (range: 70.78 to 118.59 µg.hr/ml) with a mean T/R ratio of 0.93±0.18. The percentage relative bioavailability of test versus reference formulation of meloxicam was found to be 91.03 % which indicates almost complete absorption of meloxicam from both the formulation by GIT. It is apparent from Table 1 that in all cases, the difference between AUC_{0-t} and $AUC_{0-\infty}$ is greater than 10%. This indicates that the sampling period is not long enough to establish the AUC infinitive. The use of truncated (shortened) plasma drug concentration time curve may be more appropriate in the present case. It allows the measurement of peak absorption and decreases the time and cost for performing the bioequivalence study (Shargel *et al*., 2005). Therefore $AUC_{0-\infty}$ was not used for bioequivalence testing. The analysis of variance for ln transformed data for ($AUC_{0-\infty}$) detected a non-significant difference between the two formulations.

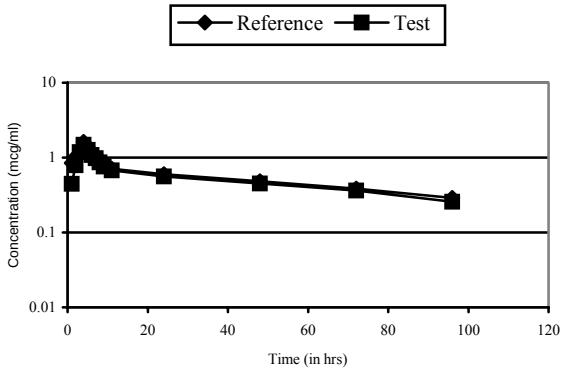

Fig. 2: Mean plasma concentration time profiles of the reference and the test formulations of meloxicam from 12 volunteers

When $AUC_{0-\infty}$ were examined in other studies at the same molar dose, the literature revealed values as 46.0 and 42.1 µg.hr/ml for test and reference formulations as reported by Dasandi *et al*., 2002, 65 µg.hr/ml as reported by Busch *et al*., 1991 and 67.5 µg.hr/ml as reported by Türck *et al*., 1997. In a study conducted by Xu *et al*., 2001 in healthy Chinese volunteers, the AUC reported for extensive metabolizers were 1.7 times than that reported in white

volunteers following same dose of meloxicam. Similar trend that is increase AUC as found in Chinese were obtained in our population. It appears that differences are there in the amounts of meloxicam absorbed in various populations.

The peak plasma drug concentration calculated (mean ± SD) found in the present studies was 1.34±0.10 µg/ml for the test preparation and 1.48±0.09 µg/ml for the reference preparation with a mean T/R ratio of 0.91±0.08 (Table 1). In case of test preparation, the highest C_{max} was obtained in volunteer 4 which was 1.58 µg/ml and the lowest in volunteer 3 which was 1.22 µg/ml while in case of reference preparation, the highest C_{max} was obtained in volunteer 8 and volunteer 11 which was 1.61 µg/ml and the lowest in volunteer 6 which was 1.32 µg/ml (Table 1). When ANOVA was computed for ln transformed data of $C_{max,}$ a non significant difference was found for subjects and subjects nested in sequence but a significant difference were observed for periods and treatments respectively. This may be due to variations in the GI tract of the volunteers caused by new food etc taken during the washout period. The 90% confidence interval was 0.87 to 0.94 with mean ratio (T/R) of 0.75. The confidence interval lies entirely within the bioequivalence limits. The C_{max} reported in other studies at the same molar dose of meloxicam ranged from 1.1 to 1.72 µg/ml (Dasandi *et al*., 2002, Türck *et al*., 1997). Thus our values of C_{max} are in close agreement with the previous findings.

When a comparison between the mean±SD values of time of the peak plasma concentration calculated (T_{max} cal) was made, the test preparation gave 3.72±0.15 hr (range: 3.42 to 3.95 hr) and the reference preparation gave 3.70±0.25 hr (range: 3.20 to 4.00 hr) with a mean T/R ratio of 1.01±0.07. From the results it is apparent that T_{max} of both reference and test formulations are almost same (Table 1). Non parametric analysis by the Wilcoxon Signed Rank test showed no statistically significant difference between the T_{max} values of both formulations. In a previous study conducted in Germans, T_{max} reported was 10.7 hr which were attained after administration of 30 mg meloxicam (Busch *et al*., 1991). Rani *et al*., 2004 had reported T_{max} of 2.91 hr after oral administration of 15 mg meloxicam in Indian volunteers. This was 2-3 times different as reported in the literature. In another study conducted in Mexican population, 65% faster T_{max} as compared to other population were reported (Marcelín-Jiménez *et al*., 2005). We also got an earlier T_{max} like Indians and Mexicans. It appears that an earlier T_{max} in various population might produce unwanted effects on prolonged use of meloxicam.

CONCLUSIONS

In conclusion, the results of our first *in-vivo* study in local population of Pakistan demonstrates that the formulations under study were bioequivalent and therefore likely to be exchangeable in clinical practice. The observed differences in extent of absorption may be attributed due to variations in the GI tract of the volunteers and also due to inter-individual variability in the volunteers of different populations. Thus it seems necessary to carry out further investigations in order to explore in detail the bioavailability and pharmacokinetic characteristics of this new molecule in our population.

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