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Research Article

Bioequivalence and Pharmacokinetics Evaluation of Two Formulation of Febuxostat 80 Mg Tablets In Healthy Indian Adult Subject

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ABSTRACT

In single center, open labeled, randomized, two-way, two-period, two-treatment, crossover, single dose bioequivalence study, test and reference formulation of Febuxostat were orally administered in twenty-six +2 (standby) healthy adult male human subjects under fasting condition, with five days washout period. Pharmacokinetic blood samples were collected periodically following drug administration. Febuxostat was measured in plasma samples by using validated high performance liquid chromatography method. The pharmacokinetics parameters C_{max}, T_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and $C_{max}/AUC_{(0-\infty)}$ were calculated applying non-compartmental analysis, followed by ANOVA of logarithmically transformed and untransformed values. The test versus reference ratio of geometric mean of C_{max}, $AUC_{(0-t)}$, and $AUC_{(0-\infty)}$ for febuxostat in both formulations were within the acceptance limit of 80-120%. No adverse event or clinical significant changes were observed in any of the subjects during the two runs of the study.

Keywords: Febuxostat, Bioequivalence, Pharmacokinetics

INTRODUCTION

Febuxostat, 2-[3-cyano-4- (2-methylpropoxy)phenyl)-4-methylthiazole-5-carboxylic acid (also known as TEI-6720 or TMX-67, Figure 1) is a non-purine, XO inhibitor (Hasegawa, 1998), clinical evaluated for the treatment of hyperuricemia and gout (Kamatani *et al*., 2003; Schumacher *et al*., 2002). It is previously reported that febuxostat is a potent, mixed-type

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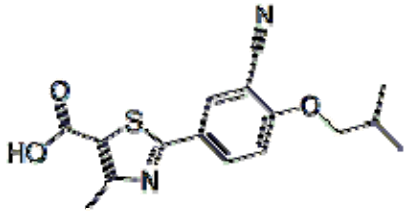

Figure 1: Chemical Structure of Febuxostat

inhibitor of bovine milk XO (Ki = 0.7 nM) in vitro and had a potent in vivo hypouricemic effect in rodents (Osada *et al*., 1993; Horiuchi *et al*., 1999) and chimpanzees (Komoriya *et al*., 1993).

Importantly, febuxostat does not inhibit other enzymes involved in purine and pyrimindine metabolism, such as purine nucleoside phosphorylase and orotidine-5-monophosphate decarboxylase, which are inhibited by allopurinol and/or its metabolites, therefore febuxostat is a potent non-purine, selective inhibitor of XO, and could be useful for the treatment of hyperuricemia and gout (T. Yasuhiro *et al*).

The clinical manifestations of gout, a spectrum of monoarthritic disorders characterized by crystallization of monosodium urate from supersaturated body fluids into tissues, have been well described for centuries. Although historically associated with royalty and affluent societies, increased longevity and shifts in patterns of diet and lifestyle have led to an increasing prevalence of gout worldwide, including in less-developed countries (Saag *et al*, 2006 and Annemans *et al*, 2008). Attacks of acute gouty arthritis are usually treated with NSAIDs, colchicine, or corticosteroids; however, because hyperuricemia is the primary antecedent biochemical abnormality observed in patients with acute gouty arthritis, urate-lowering agents are the foundation for prevention of further attacks.

In the absence of suitable alternatives, allopurinol has remained widely prescribed for the treatment of hyperuricemia. The availability of a new agent with improved tolerability and equivalent or greater efficacy would represent an important advance in the management of hyperuricemia in patients with gout. In February 2009, the US Food and Drug Administration (FDA) approved febuxostat, a nonpurine selective inhibitor of both the oxidized and reduced forms of xanthine oxidase, for the management of hyperuricemia in adults with gout. It is the first agent approved in the United States for the treatment of gout since allopurinol was first marketed in 1964 (T. Yasuhiro *et al*.).

As per the pharmacokinetic and pharmacodynamic study done by Mayer *et al*, plasma exposure to febuxostat and its metabolites were generally higher in subjects with increasing degrees of renal impairment, the percentages of decrease in serum uric acid were comparable

Table 1: Descriptive statistics of the pharmacokinetic parameters of Febuxostat

Pharmacokinetic Parameters	REFERENCE				TEST			
	Mean	**S.D.**	**S.E.**	**%CV**	**Mean**	**S.D.**	**S.E.**	**%CV**
C_{max} (µg/ml)	3.297	0.9284	0.1821	28.16	3.461	0.9367	0.1837	27.07
$AUC_{(0-t)}$ (µg/ml*hr.)	9.344	2.0437	0.4008	21.87	9.328	2.5538	0.5008	27.38
$AUC_{(0-\infty)}$ (µg/ml*hr.)	9.481	2.0597	0.4039	21.72	9.480	2.5394	0.4980	26.79
C_{max} / $AUC_{(0-\infty)}$ (hr^{-1})	0.3518	0.0872	0.0171	24.78	0.3811	0.1258	0.0247	33.01
T_{max} (hr)	1.818	1.2715	0.2494	69.93	1.843	1.1650	0.2285	63.20
K_{el} (hr^{-1})	0.329	0.121	0.024	36.68	0.322	0.0989	0.0194	30.67
$T_{½}$ (hr)	2.540	1.4646	0.2872	57.67	2.373	0.8147	0.1598	34.34
Ln C_{max} (µg/ml)	1.1514	0.3020	0.0592	26.23	1.2037	0.2863	0.0561	23.78
Ln $AUC_{(0-t)}$ (µg/ml*hr.)	2.2126	0.2136	0.0419	9.66	2.1983	0.2673	0.0524	12.16
Ln $AUC_{(0-\infty)}$ (µg/ml*hr.)	2.2276	0.2103	0.0412	9.44	2.2158	0.2620	0.0514	11.83
Ln (C_{max} / $AUC_{(0-\infty)}$)(hr^{-1})	-1.0763	0.2610	0.0512	-24.25	-1.0121	0.3093	0.0607	-30.56

regardless of the renal function group. A once-daily 80mg dose of febuxostat appears to be safe and well tolerated in different renal function groups and does not appear to require any dose adjustment based on differences in renal function. Effect of food or antacid, age and gender on pharmacokinetic and pharmacodynamics in healthy subject were reported by Khosravan *et al*. Although the pharmacokinetic and pharmacodynamics properties of the febuxostat have been published, no studies focused on these properties in the Indian

population. Therefore, the aim of the present study was to compare the bioequivalence and pharmacokinetic properties of both formulations of febuxostat 80 mg in healthy Indian volunteers.

Table 2: Geometric mean for Febuxostat (Test and Reference)

Pharmacokinetic parameters (Febuxostat)	**Geometric Mean**		**% Ratio of (Test / References)**
	Reference	**Test**	
$AUC_{(0-t)}$ (µg/ml*hr.)	9.139	9.010	98.59
$AUC_{(0-\infty)}$ (µg/ml*hr.)	9.278	9.169	98.82
C_{max} (µg/ml)	3.163	3.333	105.38

Relative Bioavailability for Febuxostat:

($AUC_{(0-t)}$ **Test** / $AUC_{(0-t)}$ **Reference**) X 100 = **98.59%**

MATERIAL AND METHODS

The study was conducted at Therapeutic Drug Monitoring Laboratory, Sion, Mumbai, India. The study participants were screened after obtaining signed informed consent form (ICF). The screened passed subject were enrolled and randomized to the one of the study treatments. The protocol, ICF and screening form were approved by the institutional ethical committee before conducting the study. The study was conducted in accordance with ICH guidelines on Good Clinical Practice, Indian Council of Medical Research, New Delhi, 2000, guidelines on biomedical research, CDSCO Bioavailablity and Bioequivalence guidelines and the provisions of Declaration of Helsinki (Seoul, October 2008).

Twenty six healthy, adult, male volunteers of Indian origin, with age of 30.4 ± 5.61 years and weight of 65.9 ± 8.01 kilograms completed the study. The subjects were confined at the clinical site at least 13 hours before dose administration. After overnight fasting for at least 10 hrs, one film coated 80 mg tablet of Febuxostat (Test Formulation, manufactured by Emcure Pharmaceuticals Ltd., Pune, India or Reference Formulation, manufactured by Takeda Pharmaceuticals America, Inc.) were administered orally to each subject in sitting posture, with 240 mL of water at ambient temperature, in each study period, as per the randomization code list. Dosing activity was followed by mouth check to assess the compliance to dosing. All subjects were restricted to intake of any fluid or water except water during dosing one hour pre and two hour post dose. Blood samples were obtained from an antecubital vein by an indwelling venous cannula using 5 ml sterile K_2-EDTA Vaccutainers.

On Study Day 1 of each study period, the cannula was placed into the subject's forearm about an hour before the scheduled time of dosing and it was retained for 24 hrs. Post dose sampling time points after formulation administration were 0.33, 0.66, 1.00, 1.33, 1.66, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00, 16.00, 18.00, 24.00 and 36.00 hours. Blood samples were collected after discarding the first 0.5 mL of blood from the cannula from 0.33 hrs till 24 hrs post-dose in each occasion. Blood samples were centrifuged to separate plasma within half hour of collection at 4000 rpm for 10 minutes at around 0-4ºC. Plasma was separated in duplicate and placed in polypropylene vials, stored frozen at -20°C ± 5°C. Standardized meals were provided to the subjects at 4.30, 7.30 and 12.80 hours after dosing. Respective meal contents were identical in both periods. Subject did not engaged in strenuous activity at any time during the study periods. Emergence of symptoms, if any was noted by asking subject during and at end of the study.

Table 3: 90% Confidence Interval for the pharmacokinetic parameters of Febuxostat (Test versus Reference)

Data	90% Confidence Interval		Accepted 90% Confidence Interval	
	Lower	Upper	Lower	Upper
Ln C_{max}	91.96	120.76	80.00	125.00
ln $AUC_{(0-t)}$	92.29	105.31	80.00	125.00
ln $AUC_{(0-\infty)}$	92.60	105.46	80.00	125.00

Plasma febuxostat was quantified according to high performance liquid chromatography system that was validated before the study. Protein precipitation extraction procedure was used to extract febuxostat from the plasma. 500.00 µl of subject plasma was taken in eppendorff tubes. The tubes were vortexed for 30 sec. 500 µl of acetonitrile was added to the tubes and vortexed for 60 sec. 600 µl of supernatant was pipette out in to another eppendorf tube. 50 µl of glacial acetic acid was added to the supernatant. The tubes were then centrifuged for 10 minutes at 15000 rpm in micro-centrifuge. 30 µl of the supernatant was injected onto the HPLC system. The linearity range for febuxostat was 0.10 µg/ml to 20.00 µg/ml. The validated method was found to be accurate and precise for intent use in subject sample analysis. Stability of febuxostat in plasma was evaluated as freeze-thaw cycle stability, bench top stability and long term stability in matrix. In addition, short and long term stock solution stability & HPLC autosampler stability were also evaluated. The acceptance criteria for the validated method were based on precision, accuracy, sensitivity, specificity

and stability. Standard and quality control samples were distributed throughout each batch of study sample analysis and demonstrated satisfactory performance of the method.

By the administration of the test and reference formulations, the plasma levels were produced in each subject establishing the pharmacokinetic profile. The area under curve (AUC) values for each participant and treatment were evaluated over the time intervals of 0 (time of dose) to infinity. The concentrations of febuxostat in plasma and derived pharmacokinetic parameters among treatments were compared by using an analysis of variance (ANOVA). The upper and lower limits of the 90% confidence interval (CI) were then antilogarithmically transformed to the linear scale. For logarithmically transformed data, this test procedure is equivalent to requiring the ordinary 90% CI of the geometric mean ratio to lie with 80 to 125%. Statistical significance was evaluated at 95% confidence level ($p > 0.05$). A certified validation WinNonlin version 3.0 program (Pharsight Corp., USA) was used for statistical evaluations of the pharmacokinetic parameters.

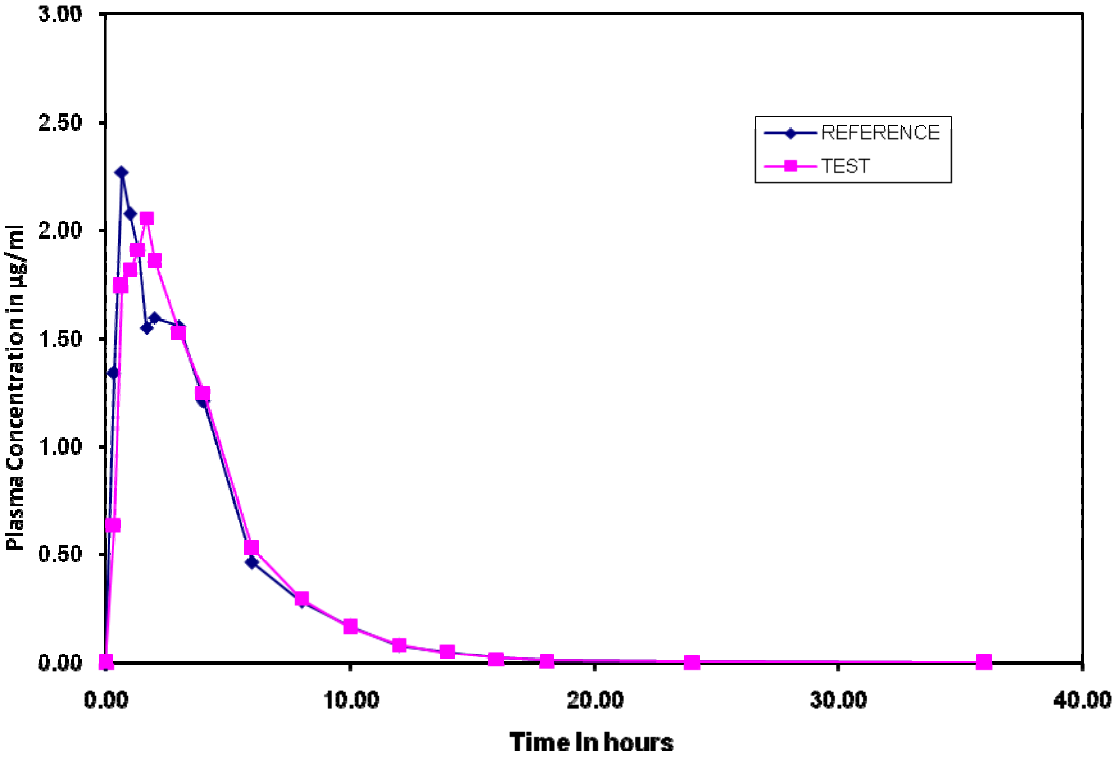

Figure 2: Mean Plasma Concentration of Febuxostat (Test and Reference)

RESULT AND DISCUSSIONS

Thirty eight healthy male Indian volunteers were enrolled in the study and all volunteers completed the study. Both the formulation of Febuxostat were endured by the volunteers in

both period of the study with no adverse effects were reported or observed. All volunteers continued to the end and were discharged in good health.

The HPLC analytical method for Febuxostat plasma sample showed good specificity, sensitivity, linearity, precision and accuracy. The linearity was observed within the range of 0.10 µg/ml to 20.00 µg/ml with the coefficient of correlation varied from 0.9912 to 0.9991. The between and within batch precision for all the low, middle and high quality control samples of Febuxostat were ranged from 94.74% to 102.02% and 91.16% to 99.91% respectively which is within the acceptance limit of 85% to 115%. Stock solution stability observed at room temperature was 6hrs and 12hrs. Long term stability at 2-8ºC was proved up-to 14 days. Plasma samples were stable for 30 days at -20 ± 5ºC. Consistent recoveries are observed for LQC and HQC with no carry-over in the method. All the stabilities performed were under acceptance criteria as per standard guidelines of FDA [13-14]. The HPLC method was found to be specific enough in the presence of different matrices collected from different sources.

The plasma levels produced by the administration of the test and reference formulations in each subject were used to establish the pharmacokinetic profile of all formulations (Figure 2). The pharmacokinetic parameters of Febuxostat are summarized in Table 1.

Febuxostat was detected from 0.33 hr in plasma in most of the subjects after ingestion of the formulations, reference and test. Febuxostat was detected up to 16.00 hr. in almost all of the subjects, post dose with both the formulations. C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $C_{max}/AUC_{0-\infty}$ values were comparable in both formulations. The T/R ratio of geometric mean of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for febuxostat in both formulations was 105.38%, 98.59% and 98.82% respectively (Table 2). These values were within the acceptance limit of 80-120%. ANOVA assessment found no significant sequence, period and treatment in the present study. The 90% confidence interval for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products fell within the established regulatory interval of 80%-125% (Table 3).

CONCLUSION

In this study, no statistically significant difference in C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were found between the Febuxostat film coated tablets containing Febuxostat 80 mg (Test Formulation, manufactured by Emcure Pharmaceuticals Ltd., Pune, India) and Uloric tablets containing Febuxostat 80 mg (Reference Formulation, manufactured by Takeda Pharmaceuticals America, Inc.). The present reported data were entirely within the bioequivalence acceptance

range proposed by the FDA of 80% to 125%. The test formulation can be considered a pharmaceutically and therapeutically equivalent alternative.

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