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Bioequivalence of Fenofibrate Tablet Formulations in Healthy Indian Male Subjects

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Fenofibrate is among the drugs of choice for treatment of hypertriglyceridemia and low levels of high-density lipoprotein. Its metabolite fenofibric acid is responsible for the pharmacodynamic effects of the drug. Two fenofibrate formulations were investigated in 18 healthy, Indian, male volunteers to demonstrate bioequivalence between these formulations. A single dose of 160 mg was given to volunteers in a two way randomized fashion with a wash out period of fourteen days. Blood samples were collected till 96 h following drug administration. Fenofibric acid concentrations were determined using a validated HPLC method using UV detector at wavelength of 287 nm. Additionally *in vitro* tests were performed using both the formulations to see the dissolution characteristics. The mean peak plasma concentration for the fenofibric acid (C_{max}) was 5.52 and 5.35 μg/ml for test and reference formulations. The C_{max} was achieved at 3.39 (test formulation) and 3.00 (reference formulation) h. The area under the plasma-concentration-time-curve AUC_{0-t} and $AUC_{0-\infty}$ had mean values of 135.1 and 156.4 μg.h/ml for the test formulation and 124.9 and 139.6 μg.h/ml for reference formulation. The resulting 90% confidence intervals of the parameter ratios were (85.0, 112.1) for C_{max}, (89.1, 117.5) for AUC_{0-t} and (90.3, 119.4) for $AUC_{0-\infty}$. Though the bioequivalence criteria were met, test formulation showed a little higher value of all the considered pharmacokinetic parameters. Interestingly, this difference correlated well with the observation of more dissolution from the test formulation.

Fenofibrate is a fibric acid derivative that has been marketed since the mid 1970's (1998 in the United States)[1]. Its metabolite fenofibric acid is responsible for the pharmacodynamic effects of the drug. Fenofibrate is a lipid-regulating drug that is structurally related to other fibric acid derivatives[2]. Fenofibrate reduces serum triglycerides, total cholesterol and LDL-cholesterol and raises HDL cholesterol to clinically relevant degrees. Amongst the fibrates, fenofibrate has the lowest and most variable bioavailability[3]. Reduction in particle size by micronisation of fenofibrate improves its solubility and substantially bioavailability[4]. Researchers have shown more favourable pharmacokinetics for micronized fenofibrate compared with immediate acting fenofibrate[5,6]. Micronized fenofibrate 67 to 201 mg/d is useful as monotherapy or as an adjunct to hyperlipidaemia. Fenofibrate appears to be highly effective in diabetic patients with hyperlipoproteinaemia. Fenofibrate is a well tolerated drug and during long term therapy it causes gastric disturbances, musculo-skeletal and dermatological reactions.

Present study was carried out to examine the relative bioavailability of the two formulations containing 160 mg fenofibrate. In addition, the dissolution rate for both formulations was determined *in vitro* using the classical paddle method.

MATERIALS AND METHODS

Fenocard tablet containing 160 mg fenofibrate (batch no. T-FEN-06; manufacturing date November, 2002) was the

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test formulation produced by Troikaa Pharmaceuticals Ltd., Ahmedabad. Tricor tablet containing 160 mg fenofibrate (batch no. 652572E21; manufacturing date November, 2002) was the reference formulation produced by Abbott Laboratories, North Chicago, USA.

In vitro dissolution studies:

The rate of fenofibrate release from Fenocard tablets produced by Troikaa Pharmaceuticals Ltd., and Tricor tablets by Abbott Laboratories, North Chicago, USA, was evaluated using USP type II dissolution testing apparatus (Paddle method). The dissolution tester was operated using 900 ml of dissolution medium maintained at 37±0.5° and a rotating speed of 75 rpm. The dissolution media used was 0.025 M sodium lauryl sulphate solution. Samples were drawn at 5, 10, 15 and 30 min and were analyzed by HPLC.

Bioequivalence study:

Eighteen (18) normal, healthy, male volunteers of age 19-38 were included in the study. Volunteers were recruited from our volunteer data bank, constituted after an extensive search and advertisement in local dailies. All the volunteers underwent a thorough physical examination, urinalysis and routine blood tests. The volunteers were also screened for the absence of Australia Antigen and HIV virus not more than seven days prior to the actual study date. They were instructed to refrain from all medications 7 d prior to the study, and until the study was completed. Alcohol was not permitted 24 h prior to and during the study. The study was conducted according to the principles outlined in the declaration of Helsinki. The study protocol was approved by the institutional ethics committee overseeing clinical studies in human. Volunteers gave a written consent before initiation of the study.

Study plan:

A two phase, double blind, randomized crossover study was conducted with a wash out period of 14 d. The clinical investigators as well as the volunteers were unaware of the allocation of the treatment. Allocation of treatments to volunteers was done using random numbers generated by computer. On the day of study, an intravenous cannula (20G) was inserted by the consulting physician at least one hour prior to the dosing time. The volunteers were administered one of the two treatments (after an overnight fast) with 240 ml of water. Five (5.0) ml blood samples was collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 16, 24, 36, 48, 72, and 96 h postdose. Up to 24 h, samples were collected by an indwelling venous cannula, which was kept patent with 0.5 ml saline and 0.2 ml of 1000 IU/ml heparin. From 48 to 96 h, the samples were collected by venipuncture in to heparanised disposable syringes. Blood was transferred into clean glass stoppered 15 ml tubes (as per blood collection schedule) through the indwelling venous cannula. Blood was transferred immediately to plasma separation room and plasma was separated by centrifugation at 4°, divided into two aliquots and stored in plastic vials at -20°, till assayed for fenofibric acid by HPLC. Standardized food was given after 4 h and 12 h of dosing. Standardized food was consisted of 10 small *chapatis*, 1 bowl vegetable, 1 bowl *dal* and 1 bowl of rice with 300 ml of drinking water.

Method of analysis:

Aliquots of 0.5 ml plasma were pippeted into the conical tubes. Five (5) micrograms of internal standard (mefenamic acid) was added into it and vortexed for 15 s. Twenty five (25) microlitres of 10% trichloro acetic acid (TCA) was added to this and vortexed for 15 s. In this mixture 5 ml of ethyl acetate was added and vortexed and then tubes were kept on extractor for 10 min. After extraction, the organic layer was centrifuged at 2500 rpm at 15° for 10 min. The organic supernatant was transferred in other tubes and kept for evaporation under N_2 gas till dryness. Then the sample was reconstituted in mobile phase and injected into the system.

Fenofibric acid was chromatographed on a reverse phase Flexit-Jour, Kromasil, RP-18 column (150 mmx4.00 mm 5 micron) and guard column C_{18} (Corasil) maintained at room temperature. The mobile phase consisting of a v/v mixture of 0.01mM KH_2PO_4 (pH 3.0): acetonitrile (45:55), pH adjusted to 3.0 with phosphoric acid, was pumped at a flow rate of 1.0 ml/min. Borwin software was used for the data analysis. The retention time for drug and internal standard were 4.0 and 6.8 min, respectively detected by UV detector at 287 nm. On each analysis day, a standard curve at the beginning and three quality control samples (high, mid and low) at the beginning, middle and end of sample assay were also analyzed.

Method validation:

The method was validated which included, Accuracy, Precision, Linearity, Percent recovery, Limit of Quantification, Limit of Detection, Inter- and Intra-day variations and Recovery. The plasma was spiked with fenofibric acid at concentration of 10 µg/0.5 ml, 3 µg/0.5 ml and 0.75 µg/0.5 ml for high, mid and low controls respectively. Repeatability of the assay showed mean (%CV, number of replicates) of

9.91 μg/0.5 ml, (0.95%, 10), 3.44 μg/0.5 ml (14.6%, 10) and 0.757 μg/0.5 ml (0.93%, 9) for high, mid and low controls respectively. The standard curves were linear in the range of 0.125 μg/0.5 ml to 16 μg/0.5ml for the drug with the correlation coefficient of >0.998 (extracted) and >0.999 (unextracted). The limit of detection (LOD) of the assay was 0.05 μg/0.5 ml. The percent recovery of drug (high, mid, low) and internal standard was 75.3%, 77.5%, 66.4% (high, mid, low) and 71.9%, respectively. Freeze thaw cycle showed that the drug was stable up to three cycles. Auto sampler stability showed that the drug was stable for 24 h. Plasma stability showed that the drug was stable for 1 m.

Data analysis:

The maximum plasma concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were directly determined from the plasma concentration versus time curves. The area under the curve from 0 h to t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve from 0 h to infinity ($AUC_{0-\infty}$) was estimated by summing the area from 0 to t (AUC_{0-t}) and t to infinity ($AUC_{t-\infty}$), where $AUC_{t-\infty}=C_t/k_{el}$, with '$C_t$' defined as the last measured plasma concentration at time t, and 'k_{el}' the slope of the terminal portion of the ln plasma concentration versus time curve, obtained by linear regression. The elimination half life ($t_{1/2}$) was calculated using the pharmacokinetic relationship $t_{1/2}=\ln(2)/k_{el}$[7].

Logarithmic transformation was done before data analysis for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. Analysis of variance (ANOVA) was used to assess sequence effect. The inclusion of the 90% confidence interval for the ratio of the product averages of test and reference formulations in bioequivalence range (80-125%) was used as bioequivalence criterion for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$[8]. Differences in T_{max} were assessed by the non parametric Wilcoxon matched pair signed rank test at 5% level of significance.

RESULTS

Dissolution study:

The results of the *in vitro* dissolution testing are depicted in fig. 1. The proportions of fenofibrate release from test and reference formulations were same for first 10 min. At 20 min, mean release values of fenofibrate were 89.2% for the test and 85.9% for the reference formulation. At the end of the dissolution profile, the mean of proportions of fenofibrate release from test and reference formulations were 95.1% and 88.3%. These results infer that the test formulation had more dissolution compared to the reference formulation after 15 min.

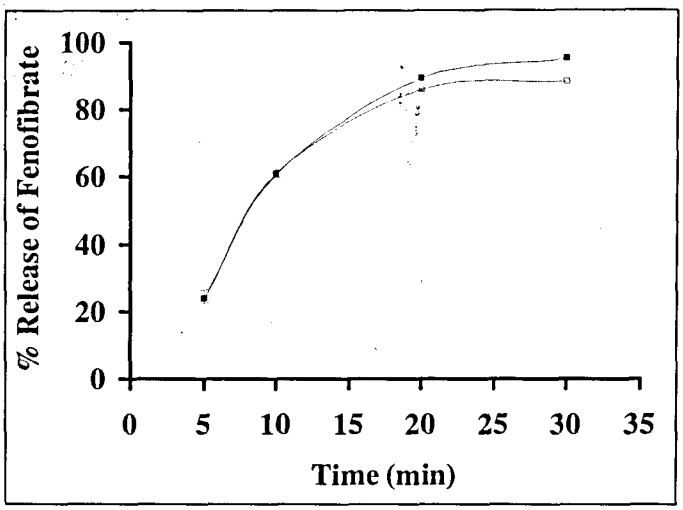

Fig. 1: Mean dissolution profile (mean±sd) of fenofibrate tablets (160 mg)

Mean dissolution profile for Tricor tablet (-□-) and Fenocard tablet (-¤-)

Bioequivalence study:

The mean plasma concentration-time profile of fenofibric acid following administration of single oral 160 mg dose to 18 healthy male volunteers is shown in fig. 2 and a summary of the pharmacokinetic parameters is presented in Table 1. The Test formulation exhibited a mean peak plasma concentration for the fenofibric acid, (C_{max}) of 5.52 μg/ml, and the time to reach C_{max} (T_{max}) was 3.39 h. The area under the plasma-concentration-time-curve AUC_{0-t} and

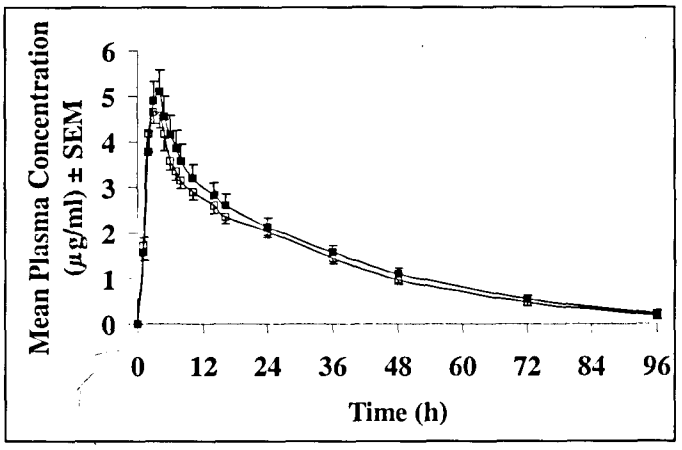

Fig. 2: Mean plasma concentration (mean±SEM) time profile of fenofibric acid

Mean concentration – time profile for Tricor tablet (-□-) and Fenocard tablet (-¤-)

TABLE 1: INDIVIDUAL PHARMACOKINETIC PARAMETERS OF FENOFIBRIC ACID IN PLASMA, FOLLOWING ADMINISTRATION OF THE REFERENCE AND TEST FORMULATIONS

Vol. No.	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-t} (μg.h/ml)	k_{el} (h^{-1})	$AUC_{0-\infty}$ (μg.h/ml)	$t_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-t} (μg.h/ml)	k_{el} (h^{-1})	$AUC_{0-\infty}$ (μg.h/ml)	$t_{1/2}$ (h)
	Reference Formulation						Test Formulation					
1	5.11	2.0	128.8	0.02	160.4	29.3	3.50	4.0	94.25	0.03	105.8	19.8
2	7.02	2.0	121.1	0.04	127.6	16.1	8.32	3.0	113.7	0.03	123.2	21.1
3	7.00	3.0	204.1	0.03	221.5	24.8	6.31	3.0	192.9	0.02	218.0	28.6
4	4.96	2.0	97.36	0.03	113.1	23.5	6.26	3.0	106.6	0.03	116.1	21.2
5	5.54	2.0	136.4	0.03	146.5	25.8	3.18	3.0	75.62	0.03	86.65	22.9
6	5.59	3.0	85.8	0.05	95.96	14.8	6.29	2.0	175.8	0.03	189.7	25.8
7	4.32	4.0	76.58	0.04	87.76	16.7	1.64	5.0	47.12	0.06	51.14	10.9
8	4.44	4.0	131.9	0.03	140.1	23.6	5.13	4.0	131.2	0.04	138.7	16.0
9	4.89	2.0	101.4	0.05	107.5	15.2	6.57	3.0	144.7	0.04	155.7	17.1
10	5.24	2.0	96.79	0.03	106.4	21.4	5.14	2.0	97.31	0.04	102.8	16.8
11	8.32	5.0	187.5	0.02	205.8	28.4	10.1	4.0	239.6	0.01	322.3	65.0
12	6.94	4.0	136.3	0.04	146.1	17.6	6.32	4.0	172.7	0.03	182.7	22.9
13	4.67	4.0	111.7	0.04	122.7	19.1	4.16	4.0	132.5	0.02	146.6	28.8
14	4.55	3.0	151.1	0.02	180.8	37.7	5.71	3.0	163.8	0.02	177.2	27.0
15	5.86	2.0	130.0	0.03	147.1	22.6	6.90	3.0	168.5	0.02	187.6	29.8
16	3.72	2.0	64.89	0.07	65.70	10.1	3.12	3.0	41.04	*	*	*
17	3.05	4.0	96.44	0.02	109.1	32.9	3.14	3.0	95.66	0.03	106.9	21.3
18	5.08	4.0	190.0	0.02	228.4	38.2	7.61	5.0	238.0	0.04	248.2	18.1
Mean	5.35	3.0	124.9	0.03	139.6	23.2	5.52	3.39	135.1	0.03	156.4	24.3
D	1.30	1.03	39.13	0.01	45.68	7.9	2.12	0.85	57.41	0.01	66.11	11.7
SEM	0.31	0.24	9.22	0.00	10.77	1.9	0.50	0.20	13.53	0.00	16.03	2.8
N	18	18	18	18	18	18	18	18	18	17	17	17

*Could not be calculated due to less number of data points in the elimination phase

$AUC_{0-\infty}$ had mean values of 135.1 μg.h/ml and 156.4 μg.h/ml. The corresponding C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the reference formulation were 5.35 μg/ml, 3.00 h, 124.9 μg.h/ml and 139.6 μg.h/ml. The resulting 90% confidence intervals of the parameter ratios for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$, are depicted in Table 2. These values were all within the range of 80-125% with (85.0, 112.1) for C_{max}, (89.1, 117.5) for AUC_{0-t} and (90.3, 119.4) for $AUC_{0-\infty}$ indicating that bioequivalence criteria was met. In addition, there was no statistically significant ($p>0.05$) difference between T_{max}. Both the formulations were well tolerated. The volunteers did not complain of any adverse reaction.

DISCUSSION

The present study showed that the test and reference formulations containing 160 mg fenofibrate are bioequivalent. No lag time in absorption phase was observed in the plasma profiles of the two formulations. The terminal half-life was approximately 24 h for both formulations, which is in accordance with the literature[2].

TABLE 2: 90% CONFIDENCE INTERVALS FOR THE RATIO OF THE TEST AND REFERENCE FORMULATIONS' AVERAGES OF VARIOUS PHARMACOKINETIC PARAMETERS FOR FENOFIBRIC ACID

Parameter	90% Confidence Interval	
	Lower Limit	Upper Limit
C_{max}	85.00	112.10
AUC_{0-t}	89.09	117.47
$AUC_{0-\infty}$	90.29	119.41

The two profiles appeared to be closely similar, although they exhibited a slight difference in the parameter values C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$. The parameters C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of the test formulations were comparatively higher than the reference formulations. Actually, the profiles were super-imposable in the first 2-3 h and then the concentrations continued to increase for the test formulation leading to a higher peak plasma concentration, time to peak plasma concentration and area under the curve compared to that of reference formulation. This was true even for most of the individual as well as for the mean plasma concentration-time profile. This was in accordance with the dissolution profile's pattern. Dissolution profiles were also identical until 10-15 min and then dissolution was more from the test formulation compared to the reference formulation.

The dissolution characteristic of a drug formulation plays a very important role in the rate of absorption of the active compound after oral intake. Much emphasis has been placed on the *in vitro* screening of a drug's dissolution properties as an index of *in vivo* performance of a given formulation. *In vitro* dissolution testing offers a simple and convenient method to evaluate the performance of pharmaceutical preparations during their developmental stage. Once correlation is established between the *in vitro* and *in vivo* results, the *in vitro* data can then be used reliably to monitor and validate the *in vivo* performance of different batches of preparations without performing human studies.

In conclusion, both the formulations were bioequivalent with respect to the parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. The variations seen in the bioavailability of fenofibrate from different formulations is small and beyond statistical significance based on the current regulatory criteria. However, the comparatively higher values of C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for the test formulation were reflected by the difference in the dissolution of the two formulations. Hence, we find it worthwhile to report very close *in vitro* and *in vivo* correlation observed here.

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