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Comparative Bioavailability of Two Marketed Tenoxicam Formulations in Healthy Human Volunteers

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The objective of the study was to obtain the pharmacokinetic data of two marketed tablet formulations of tenoxicam and compare the relative bioavailability of the test formulation with standard formulation. A single dose 12x2 complete cross over study of the two marketed film coated tablet formulations of tenoxicam (20 mg) was carried out in twelve healthy male volunteers. Blood samples were collected at predetermined time intervals. The plasma concentrations of the drug were determined by RP HPLC. The plasma concentration of the tenoxicam and other pharmacokinetic parameters obtained were statistically analysed. The results of two way analysis of variance of plasma drug levels and pharmacokinetic parameters showed that there was no significant variation between the products and subjects at all the points of time. Products did not show significant difference between them with regard to the AUC_{0-72}, $AUC_{0-\infty}$, C_{max}, T_{max}, $T_{1/2}$ which are 153.53±54.54 μg h/ml, 278.92±78.98 μg h/ml, 4.45 μg/ml, 1.66±0.46 h, 65.59±28.19 h respectively for the product A and 157.25±55.02 μg h/ml, 283.84±85.03 μg h/ml, 4.80±1.13 μg/ml 2.04±0.96 h, 62.60±34.53 h for product B. It was therefore, concluded that the products A and B are bioequivalent.

Tenoxicam is a non steroidal antiinflammatory and analgesic agent belonging to the oxicam class of NSAIDs[1]. It is effective in the treatment of various rheumatic conditions. Tenoxicam is 99% bound to plasma proteins and has a half-life of 27±28 h. Tenoxicam allows for once daily dosage regimen. After the oral administration of a single dose of tenoxicam (20 mg) mean plasma concentrations ranging from 2-4 μg/ml are achieved[2]. Tenoxicam 20 mg film coated tablets, Tobitil (Ranbaxy Ltd) and Novotil (Recon Ltd) are available in the Indian market.

MATERIALS AND METHODS

Pure tenoxicam was obtained from Recon Ltd., Bangalore. Blood samples were centrifuged using Elteck Centrifuge, Ependroff micropipettes were used for spiking the plasma with internal standard. HPLC (Shimadzu Corp. Japan) was used to estimate the tenoxicam content in plasma. Disintegration test apparatus (Serwell) was used to determine disintegration time. Dissolution apparatus (Serwell, Bangalore) USP XXI and a UV spectrophotometer (Shimadzu), were used to determine the dissolution times of the tablet formulations and to estimate the drug content respectively.

Two marketed film coated tablet formulations, Tobitil (Ranbaxy Ltd) and Novotil (Recon Ltd) of tenoxicam (20 mg) were selected for the study. The products were coded as product A and B, respectively.

In vitro studies:

The assay was carried out with five tablets of each product. The powder equivalent to 10 mg of tenoxicam was extracted with methanol and was suitably diluted and the absorbance of the resulting solution was measured at 357 nm. The corresponding concentration was read from the calibration graph. The disintegration time of both the products was carried out using a disintegration test apparatus and distilled water was used as the medium maintained at 37±0.5°. The dissolution studies were carried out according to USP XXI using a single vessel rotating basket apparatus

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at 50 rpm, using 900 ml of distilled water maintained at 37±0.5°. Samples were collected at 15, 30, 45 and 60 min and were analysed spectrophotometrically.

***In vivo* studies:**

Study protocol and design have been approved by the J. S. S. Medical College ethical committee, Mysore and written and informed consent was obtained from all the volunteers prior to the study. Twelve healthy male volunteers in the age group of 20-25 y weighing 53-66 kg were selected for the study. All the volunteers were subjected for physical and biochemical and pathological examinations to ascertain that they are healthy. The concurrent use of other drugs, alcoholic beverages and smoking was restricted one week prior to the study and were prohibited during the study.

Study design:

A single dose 12x2 randomized complete cross over study was carried out on two different occasions separated by a washout period of 6 w. Volunteers were fasted overnight before the drug administration and for 2.5 h after drug administration. The products were administered with 200 ml of water. Blood sampling (5 ml) was done at 0.0, 0.5, 1.0, 1.5, 2, 3, 4, 8, 10, 12, 24, 48 and 72 h post dosing by using a 22 G" (0.9x25 mm) i.v. canula with injection port was positioned in the forearm vein and was kept with heparinised saline lock for the ensuring of 12 h blood sampling. Standard breakfast and lunch were served after 2.5 h and 6.5 h after drug administration. The blood samples were immediately centrifuged, plasma was separated and stored at -20° until analysis. A sensitive RP-HPLC method with UV detection at 361 nm was used for the quantification of tenoxicam in plasma samples[2]. Piroxicam was used as the internal standard 1 µg/ml.

Extraction:

One milliliter plasma samples were spiked with the 10 µl of internal standard piroxicam acidified with 0.3 ml of 10% perchloric acid and were mixed well. Ten milliliters of dichloromethane was added and the mixture was vortexed for 30 min. The mixture was then centrifuged at 2000 rpm for 5 min. The upper aqueous layer was discarded and the organic layer was evaporated to dryness at 50-60° on water bath. The residue was reconstituted in 200 µl of the mobile phase and 20 µl was injected into the C_{18}HPLC column.

Chromatographic conditions:

Chromatographic conditions were established using a HPLC Shimadzu LC 10AD recorder Wipro Acer PC, LC 10AD pump, SPD-10A variable wavelength detector and CBM integrator and a Shimpack 4.6x250 mm, 5 µm particle size RP column using mobile phase of acetonitrile:250 mM sodium acetate (30:70) at pH 6.0 with a flow rate of 1.0 ml/min using UV detection at 316 nm, 0.01 AUFS. The retention time for tenoxicam was 5.6 min and for the internal standard piroxicam was 5.2 min. The method was validated using spiked plasma samples and was found to be linear in the range of 1 to 5 µg of tenoxicam.

Data analysis:

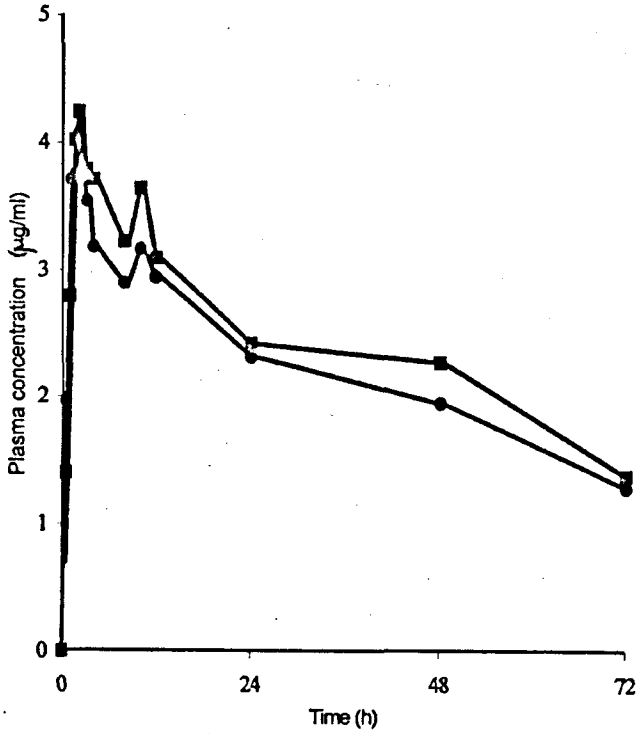

Fig. 1: Plasma concentration with time profile of tenoxican formulations

Mean plasma concentrations (µg/ml) of tenoxicam (n=12) at different time intervals following administration of two marketed products, product A (Tobitil, -●-) and product B (Novotil, -■-) each containing 20 mg of tenoxicam.

As a measure of the rate of drug absorption C_{max} and T_{max} were directly read from the plasma drug level profiles of each product. The rate of elimination (K) was calculated from the terminal elimination phase of the log plasma concentration versus time curve by the method of least square regression analysis[3]. The plasma drug concentrations and all the pharmacokinetic parameters were subjected to pooled t test and analysis of variance at 95% confidence interval.

TABLE 1: MEAN PHARMACOKINETIC PARAMETERS OF TENOXICAM FORMULATIONS.

Parameters	Products	
	A	B
C_{max} (µg/ml)	4.45±0.99	4.80±1.13
T_{max} (h)	1.66±0.46	2.04±0.96
$T_{1/2}$ (h)	65.59±28.19	62.60±34.53
AUC_{0-72} (µg h/ml)	153.53±54.54	157.25±55.02
$AUC_{0-\infty}$ (µg h/ml)	278.92±78.98	283.84±85.03

Pharmacokinetic parameters were determined in 12 healthy male volunteers after administering a single dose of either product A (Tobitil) or product B (Novotil) in a 12x2 randomized complete cross over study.

TABLE 2: POOLED T-TEST VALUES OF COMPARISON OF PHARMACOKINETIC PARAMETERS OF TENOXICAM FORMULATIONS.

Parameters	Between A and B
C_{max}	0.78 (NS)
T_{max}	1.80 (S)
$T_{1/2}$	0.21 (NS)
AUC_{0-72}	0.11 (NS)
$AUC_{0-\infty}$	0.14 (NS)

't' calculated values for the comparison of pharmacokinetic parameters of product A (Tobitil) and product B (Novotil). NS and S denote not significant and significant, respectively at $p<0.05$ ('t'-tabulated=1.717).

RESULTS AND DISCUSSION

The assay test results showed that product A and B have drug content within 98% of the label claim. The disintegration time for products A and B was observed to be 14.16 and 243.33 s respectively. The dissolution rate profiles showed that the products A and B showed more than 75% dissolution at 45 min and more than 90% at 60 min. The mean plasma drug levels obtained following the administration of product A and B are shown in fig. 1. The graphical concentration Vs time curve shows the C_{max} at 2 h for product A as well as product B. After C_{max}, there was a slight fall in plasma level followed by a raise in the concentration at 10 h of post dosing. Various pharmacokinetic parameters derived form the plasma levels are shown in the Table 1. The results of the pooled t-test for the comparison of pharmacokinetics parameters between product A and B is shown in the Table 2. There is a significant difference between the $t_{1/2}$ of the two products as determined by pooled t- test.

TABLE 3: TWO WAY ANOVA COMPARISON OF PLASMA LEVELS AND PHARMACOKINETIC PARAMETERS OF TENOXICAM FORMULATIONS.

Time in Hours	Source of Variation Subjects Inference Fs*		Source of Variation Products Inference Fp*	
0.5	1.98	NS	3.69	NS
1.0	0.79	NS	2.29	NS
1.5	0.29	NS	0.12	NS
2.0	0.39	NS	0.11	NS
3.0	0.38	NS	0.23	NS
4.0	2.12	NS	3.79	NS
8.0	1.76	NS	1.08	NS
10.0	4.19	S	2.81	NS
12.0	0.64	NS	0.86	NS
24.0	0.86	NS	0.22	Ns
48.0	0.49	NS	0.08	NS
72.0	0.89	NS	0.05	NS
C_{max}	0.66	NS	2.28	NS
T_{max}	0.61	NS	0.39	NS
$T_{1/2}$	0.04	NS	0.002	NS
AUC_{0-72}	0.42	NS	0.002	NS
$AUC_{0-\infty}$	0.74	NS	0.017	NS

Two way analysis of variance comparison of pharmacokinetic parameters of product A (Tobitil) and product B (Novotil). *Fs and Fp are F distribution values calculated between and within the variables for subjects and products at $p<0.05$ for different degrees of freedom of numerator and denominator. NS and S denote not significant and significant, respectively at $p<0.01$.

However, when the same data was subjected to ANOVA the products did not show any significant difference between them. The results of two way analysis of variance of plasma drug levels and pharmacokinetic parameters are shown in Table 3. It was observed that there was no significant variation between the products and subjects at all the points of time, except at 10 h when the inter-subject variation was significant. Pharmacokinetic parameters such as C_{max}, T_{max}, $T_{1/2}$ and AUC_{0-72} and $AUC_{0-\infty}$ for both products did not show any significant difference.

After oral administration of tenoxicam, the C_{max} of the products A and B were 4.55 and 4.8 µg/ml, respectively. These values of products A and B were attained at 1.66 and 2.04 h, respectively. The $t_{1/2}$ of the products A and B were 65.59 and 62.6 h, respectively. AUC_{0-72} for the products A and B was 153.53 and 157.25 µg h/ml, respectively. Products A and B complied with each other in all the *in vitro* studies. Both the products showed consistent pharmacokinetic parameters and the plasma drug levels did not show any significant difference between the products and individual volunteers. We therefore, would like to conclude that the products A and B are bioequivalent.

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REFERENCES

1. Guentert, T.W. and Heintz, R., **J. Pharm. Med.**, 1993, 3, 57.
2. Tamhankar, C.P., Patki, R.V., Hegde, D.A. and Tipnis, H.P., **Indian Drugs**, 1995, 32, 269.
3. Shah, Y.I., Paradkar, A.R. and Dhayagunde, M.G., In; Introduction to Biostatistics and Computer Science, 1st Edn., Nirali Prakashan, New Delhi, 1996, 97.