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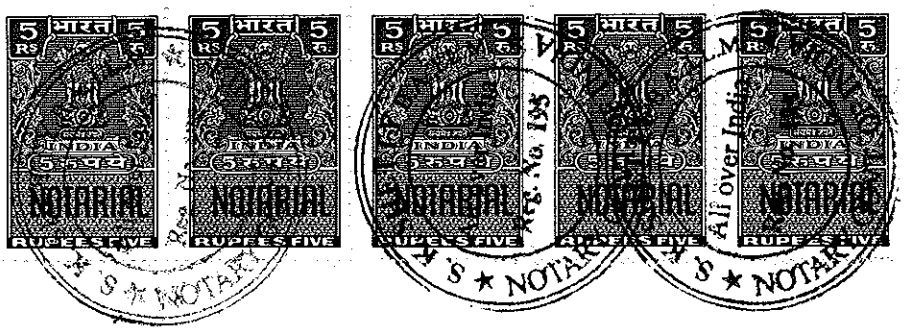

MINISTERIO DE RE... AUT... GUATE...

BIOEQUIVALENCE OF LEFLUNOMIDE

MAIN REPORT

ATTESTED BY ME
TRUE COPY
01/07/2004.
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THE INDIAN MERCHANTS' CHAMBER
30 JUN 2004
MUMBAI

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MUMBAI

For CIPLA LIMITED
P. VIRKAR
REGULATORY AFFAIRS

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STUDY TITLE

A randomized two way, two period, two treatment cross over bioequivalence study of LEFLUNOMIDE 100 mg., Tablet LEFLUNOMIDE manufactured by CIPLA LTD. (INDIA) in comparison with Tablet ARAVA 100 mg. manufactured by HMR (FRANCE) in 12 healthy male, adult, human volunteers under fasting condition.

STUDY NO. : 05/0/11

CHIEF INVESTIGATOR : Dr. K.A.PADGAONKAR M.D
Clinical Pharmacologist

HPLC ANALYST : Archana, Savita

DATE OF COMMENCEMENT : 28/11/2000

SUMMARY

On the basis of the pharmacokinetic parameters C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-inf.}$ $t_{1/2}$ and k_{el} studied, it can be concluded that the Test preparation LEFLUNOMIDE 100 mg., mfg. by CIPLA LTD. (INDIA) is bioequivalent with the Reference preparation, Tablet ARAVA, mfg. by HMR (FRANCE). The relative bioavailability of the Test preparation of LEFLUNOMIDE 100 mg., Tablet LEFLUNOMIDE was 102.91 % of that of the Reference preparation, Tablet ARAVA.

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S. K. S. ★ NOTARY

1. AIMS AND OBJECTIVES :

The aim and the objective of the present study was to evaluate the pharmacokinetic parameters and to compare the bioequivalence of two preparations of Tablet LEFLUNOMIDE 100 mg. in 12 healthy human volunteers in a randomized, two way complete crossover design.

2. SUBJECTS :

Subjects were adult, human, healthy male volunteers the mean age 25.75 ± 3.39 years and mean weight 59.08 ± 6.07 kgs. (Table 1), selected from the panel of volunteers. Volunteers were screened for inclusion in the study within 21 days before the commencement of the study.

They fulfilled the selection criteria as per the protocol submitted earlier. (Annexure I) Before admission to the study each subject was informed of the nature and the risks of the study and a written informed consent was obtained from the volunteers. They were allocated to the treatment A / B (Test or Reference preparation) in accordance with the randomization code.(Table 2)

3 MATERIALS AND METHODS

3.1 STUDY DESIGN :

This was a single dose, randomized, two treatment, two-way cross over study, with a washout period of 12 days between the two dosing sessions. In each dosing session, volunteers received either of the Test or the Reference preparation of LEFLUNOMIDE 100 mg. (3.1) only on the study day at a fixed time.

3.1.1 Dates of the Blood Collection for pharmacokinetic profiles (96 hrs.)

SESSION I	28/11/2000
SESSION II	13/12/2000

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3.2 PRODUCT INFORMATION :

Reference Preparation (A) :
Tablet ARAVA
containing LEFLUNOMIDE 100 mg.
Mfg. by HMR (FRANCE)
Batch No. : NPC 0088-2162-03
Exp. Date : 07/2001

Test Preparation (B) :
Tablet LEFLUNOMIDE
containing LEFLUNOMIDE 100 mg.
Mfg. by CIPLA LTD. (INDIA)
Batch No. : LMTI 00-060900
Mfg. Date : 09/2000

3.3 DOSE

With both the preparations the dose was one Tablet containing LEFLUNOMIDE 100 mg. (3.1)

3.4 BLOOD COLLECTION :

All the volunteers assembled in CPU ward at 6.00 a.m. on the study day of each session, after overnight fasting of 10 hrs. Their TPR, BP was recorded and an indwelling intravenous catheter was introduced with strict aseptic precautions in the suitable vein for blood collection. They received either of the study preparations (3.1) according to their code nos. (Table 2).

A total of 13 blood samples were collected at 0 hr. (before drug administration) and 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 48.0, 72.0, 96.0 (after drug administration) in the heparinised test tubes at each time point. Breakfast, lunch and dinner was provided after 3 hrs, 6 hrs., 13 hrs. respectively after drug ingestion. On the study days volunteers were permitted normal activities, excluding strenuous exercise. Collected blood samples were centrifuged immediately, plasma was separated and

RESULTS, DISCUSSION

AND

CONCLUSION

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stored frozen at - 20 °C with appropriate labeling of volunteer code no., study date and collection time.

symptoms were monitored, during the study period and for one week after the study period and if noticed, their details were entered in the case report sheets and tabulated at the end of the study.

4. HPLC Analysis

Samples were analyzed by HPLC after extracting the drug from plasma for the metabolite of the leflunomide and injecting it on the HPLC column for chromatographic analysis. The plasma values mentioned hereafter are of the metabolite of the leflunomide. (HPLC METHOD DETAILS : Annexure 3)

5. PHARMACOKINETIC VARIABLES STUDIED :

Plasma levels of LEFLUNOMIDE for every volunteer at each time point were plotted to obtain Time-Plasma concentration curves for the study preparations. The mean parameters of Bioavailability for this single dose study
were :-

C_{max} (Maximum Plasma Concentration)

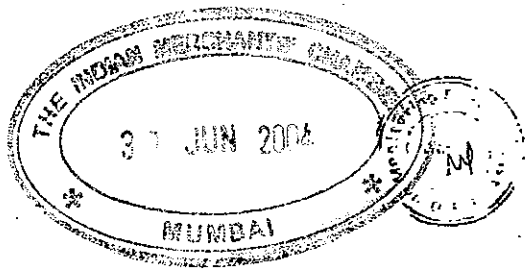
t_{max} (Time to Maximum Plasma Concentration)

$AUC_{(0-t)}$ (The area under plasma concentration time curve)

$AUC_{(0-inf)}$ (The area under plasma concentration time curve 0 to infinity)

$t_{1/2}$ (Elimination half life)

k_{el} (Elimination constant)



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6. RESULTS :

6.1 Pharmacokinetic parameters for Tablet LEFLUNOMIDE 100 mg. :

Administration of the Reference preparation, Tablet ARAVA, as a single dose in the fasting state produced the maximum plasma concentration of 17.27 ± 2.00 mcg/ ml (C_{max}) at the time 8.50 ± 2.28 hr.(t_{max}) whearas the Test preparation of Tablet LEFLUNOMIDE as a single dose in the fasting state produced the maximum plasma concentration 17.61 ± 2.00 mcg/ ml (C_{max}) at the time 8.33 ± 3.50 hr.(t_{max}) (Table 5 & 6).

Administration of the Reference preparation, Tablet ARAVA, produced the area under plasma concentration time curve (AUC_{0-t}) 349.70 ± 36.25 mcg.hr/ml, whereas administration of the Test preparation of Tablet LEFLUNOMIDE produced the area under plasma concentration time curve (AUC_{0-t}) 359.86 ± 56.56 mcg.hr/ml. (Table 7)

When administered as a single dose, in the fasting state, the Reference preparation, Tablet ARAVA, produced the area under plasma concentration time curve upto infinity (AUC_{0-inf}) 417.31 ± 61.17 mcg.hr /ml., whereas administration of the Test preparation of Tablet LEFLUNOMIDE produced the area under plasma concentration time curve upto infinity (AUC_{0-inf}) 428.08 ± 69.00 mcg.hr/ml. (Table 8)

Administration of the Reference preparation, Tablet ARAVA, produced the plasma elimination half life ($t_{1/2}$) 24.49 ± 7.10 hr. whereas administration of the Test preparation of Tablet LEFLUNOMIDE produced the plasma elimination half life ($t_{1/2}$) 26.61 ± 8.41 hr. (Table 8)

Administration of the Reference preparation, Tablet ARAVA, produced the plasma elimination constant (k_{el}) 0.031 ± 0.009 hr^{-1}, whereas administration of the Test preparation of Tablet LEFLUNOMIDE produced the plasma elimination constant (k_{el}) 0.029 ± 0.009 hr^{-1}. (Table 8)

On the basis of comparison of the AUC_{0-t} for LEFLUNOMIDE 100 mg., after single dose administration, the relative bioavailability of the Test preparation of Tablet LEFLUNOMIDE was 102.91 % of that of the Reference preparation, Tablet ARAVA.

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90 % confidence interval (conventional) for C_{max} values of Test preparation of Tablet LEFLUNOMIDE were 93.25 – 113.75 % of that of the Reference preparation.
90 % confidence interval (conventional) for AUC_{0-t} values of Test preparation of Tablet LEFLUNOMIDE were 92.61 – 115.76 % of that of the Reference preparation.
90 % confidence interval (conventional) for AUC_{0-inf} values of Test preparation of Tablet LEFLUNOMIDE were 90.89 – 119.09 % of that of the Reference preparation.

6.2 IN-VITRO DISSOLUTION AND IN-VIVO RATE OF ABSORPTION CORRELATION

In Vitro % dissolution of Test and the Reference preparation of LEFLUNOMIDE tablet were compared with in-vivo rate of absorption. The linear regression analysis was done to compare IN-VITRO DISSOLUTION AND IN-VIVO RATE OF ABSORPTION.

The correlation coefficient for the Reference and the Test preparation was 0.979 and 0.932 respectively.

This shows that there is a linear relation between the two preparations.

6.3 STATISTICAL INFERENCE

ANOVA (subject, period, treatment) was applied to the C_{max}, Ln C_{max}, AUC_{0-t} and Ln AUC_{0-t} values. ANOVA was found to be significant for the subject values of C_{max}, Ln C_{max}, AUC_{0-t} and Ln AUC_{0-t}. There was no statistically significant difference for the period and treatment values of C_{max}, Ln C_{max}, AUC_{0-t}, and Ln AUC_{0-t}.

90 % confidence interval (conventional) for C_{max}, Ln C_{max}, AUC_{0-t} and Ln AUC_{0-t} values of Test preparation of Tablet LEFLUNOMIDE were within the accepted limit of that of the Reference preparation (i.e. 80 % - 120 %).

Differences and ratios of C_{max}, Ln C_{max}, AUC_{0-t} and Ln AUC_{0-t} were within the normal limits for both the Test and the Reference preparation of LEFLUNOMIDE.

6.4 ADVERSE REACTIONS:

None of the volunteers complained of any adverse reaction on the pharmacokinetic profile days.

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7. DISCUSSION :

The single dose bioequivalence study of Tablet LEFLUNOMIDE 100 mg., was conducted in 12 adult healthy, human, male volunteers with two preparations of LEFLUNOMIDE . Values of C_{max}, t_{max} and AUC_{0-t} were comparable for the reference and the test preparation in the fasting state.

LEFLUNOMIDE was detected in plasma from 1 hour to about 72 hours in the Reference preparation as well as in the Test preparation. Peak plasma levels of LEFLUNOMIDE with the Reference preparation were achieved between 6 to 12 hours whereas with the Test preparation were achieved between 4 to 16 hours. The mean peak plasma levels of LEFLUNOMIDE with Reference preparation, Tablet ARAVA on the study day ranged between 14 – 20 mcg/ml. while with Test preparation of Tablet LEFLUNOMIDE ranged between 13 – 22 mcg/ml.

On the basis of comparison of the AUC_{0-t} for LEFLUNOMIDE after single dose administration, the relative bioavailability of the Test preparation of Tablet LEFLUNOMIDE 100 mg. was 102.91 % of that of the Reference preparation, Tablet ARAVA.

8. CONCLUSION :

On the basis of the pharmacokinetic parameters studied, it can be concluded that the Test preparation of Tablet LEFLUNOMIDE 100 mg. mfg. by CIPLA LTD. (INDIA) is bioequivalent with the Reference preparation, Tablet ARAVA, mfg. by HMR (FRANCE).

PRINCIPAL INVESTIGATOR

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