Randomized, Double-Blind, Crossover Comparison Between Two Levocetirizine Formulations on Histamine-Induced Cutaneous Response in Healthy Male Human Adult Volunteers

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ABSTRACT

Cetirizine is a highly efficacious and long acting second generation H1 receptor antagonist indicated for the treatment of allergic diseases. It is a racemate mixture composed of equal amounts of S- and R-enantiomers, and the R-enantioner, levocetirizine, carries the majority of the histamine H1-receptor-blocking activity. Recently, levocetirizine formulation has been introduced in India for the treatment of allergic rhinitis and urticaria.

Objective: The aim of this study was to compare the effect of levocetirizine (Indian formulation) versus an international brand of levocetirizine in 12 healthy male human volunteers under fasting conditions, using pharmacodynamic measure of inhibition of histamine induced wheal and flare response.

Methodology: Twelve healthy male volunteers were enrolled in this study. All volunteers gave written informed consent before entering in the study, which was approved by the Institutional Ethics Committee of Nizam's Institute of Medical Sciences. This was a balanced, randomized, double-blind, single oral dose, crossover study, where the subjects were randomized to receive either 5 mg levocetirizine reference or test formulation after overnight fast. A ten-day period was allowed between the 2 treatment schedules to eliminate the carry over effect of earlier treatment. Wheal and flare were induced on the forearm of the trial subjects by injecting freshly prepared histamine (0.1 mL containing 2 µg) intradermally while the

subject was lying comfortably with the arm resting on the bed. Ten minutes later, wheal and flare were visualized under a bright lamp. Histamine induced wheal and flare skin test was performed before and at 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, and 24 hours after drug administration.

Results: Ten minutes after intradermal injection, 2 µg of histamine produced significant wheal and flare cutaneous response in all subjects. Administration of reference and test formulations of levocetirizine significantly inhibited the histamine induced cutaneous response in all of the subjects. Maximum inhibition of histamine induced wheal response (I_w max %) with reference was 82.45% ± 8.8% and 77.9% ± 12.9% with test formulation. Maximum inhibition of histamine induced flare response (I_f max %) was 80% ± 4.4% and 81.58 ± 6.7% with reference and test formulations respectively. The area under the antihistaminic activity minus time profile curve for wheal was 2211 mm^2/hr ± 270 mm^2/hr and 2482 ± 368 mm^2/hr with reference and test formulations, respectively, and was found to be comparable. The least square mean ratio (%), T versus R for peak activity, Imax minus percent (maximum inhibition of histamine induced wheal and flare response), area under the activity time curve (AUC_{0-24} mm^2/hr and AUC_{0-24} %/hr) both for untransformed and log transformed data were found to be within 80% to 125% of 90% CI limits and both formulations were well tolerated.

Conclusion: It can thus be concluded that the test formulation of levocetirizine tablet is bioequivalent to reference levocetirizine tablet and both formulations are equally effective and well tolerated.

INTRODUCTION

Cetirizine is a highly efficacious and long acting second generation H1 receptor antagonist indicated in the treatment of allergic diseases. It is a racemate mixture composed of equal amounts of S- and R-enantiomers,[1] and the R-enantioner, levocetirizine, carries the majority of the histamine H1-receptor-blocking activity. Several studies that have suggested that histamine plays a pivotal role in pathophysiology of urticaria.[2,3] In addition, some studies have demonstrated that the responsiveness of the skin to histamine is slightly increased in patients with urticaria.[4] Histamine is stored primarily in the mast cells of skin, and when it is released leads to parasympathetic nerve stimulation and increased vascular permeability.[5,6] which in turn results in the formation of itchy, slowly expanding, erythematous wheals.[4] Leovcetirizine reduces histamine-induced wheal, flare, and itch significantly more than loratidine.[7] Inhibition of histamine induced wheal and flare response is apparent by one hour, reaches maximal inhibition by the sixth hour, and lasts for a mean duration of 30 hours.[1] Levocetirizine is rapidly and extensively absorbed and poorly metabolized in the body.[8] Recently, levocetirizine formulation has been introduced in India for the treatment of allergic rhinitis and urticaria. Consequently, the aim of this study was to compare the effect of levocetirizine (Indian formulation) versus the international brand of levocetirizine in 12 healthy male human volunteers under fasting conditions, using pharmacodynamic measures of inhibition of histamine induced wheal and flare response.

MATERIAL AND METHODS

Subjects

Twelve healthy male volunteers were enrolled in this study. All volunteers

gave written informed consent before entering in the study, which was approved by the Institutional Ethics Committee of Nizam's Institute of Medical Sciences. All volunteers were of average frame, height, and weight (within ± 15% of standard chart as per LIC of India). On entry, none of the volunteers showed any sign of illness, as indicated by medical history and a thorough clinical examination. All volunteers had normal electrocardiograms, clinically acceptable blood and urine biochemistry, hematology and serology lab investigations, including HIV and HbSAg tests. None of the volunteers were taking any prescribed or investigational medication during the 4 weeks preceding the enrollment. At screening, all volunteers demonstrated positive histamine-induced coetaneous response (wheal diameter more than 8 mm). None of the volunteers was a smoker or had a history of alcohol consumption. Volunteers with a history of skin disease or any allergic condition were excluded.

Study Design

This was a balanced, randomized, double-blind, single oral dose, crossover study. After the screening visit, each eligible volunteer entered into a randomized schedule to either receive 5 mg of reference formulation (UCB-Pharma AG, Zurich, Switzerland) or levocetirizine 5 mg tablet (Hetero Drugs, Hyderabad, India) after overnight fast.

All subjects were admitted in the clinical research ward in the evening prior to the dosing day and remained in the ward until 24 hours post dosing.

Treatment and Dosage

Each subject received a single dose of 5 mg levocetirizine tablet, either reference or test formulation, according to the prior randomization schedule. The products were administered orally with 240 mL of drinking water, to the test subject, at room temperature, after an overnight fast of 10 hours. The research nurse confirmed ingestion of the test drug by inspecting the oral cavity thoroughly, with a torch light. A ten-day period was allowed between the 2 treatment schedules to eliminate the carry over effect of earlier treatment.

The test drug was administered to subjects while seated and they remained seated comfortably for the first 2 hours following drug administration. Subjects were then allowed to do normal physical activity, but told to avoid any severe physical exertion during entire study period.

METHODS

Histamine Induced Wheal and Flare Cutaneous Response

Wheal and flare were induced on the forearm of the trial subjects, by injecting freshly prepared histamine (0.1 mL containing 2 μg) intradermally, while the subject was lying comfortably with arm resting on the bed.[1] Ten minutes later, wheal and flare were visualized under a bright lamp. The area of wheal and flare were outlined with thin tipped marker pen and were traced onto acetate sheet to make a permanent record, which was used subsequently for analysis of areas of wheal and flare. The area of wheal and flare will be expressed as square millimeter. In some subjects, wheal and flare cutaneous response induced with histamine were photographed, using a digital camera at baseline and peak effect (sixth hour post treatment), for documentation. Histamine induced wheal and flare skin test was performed before and at 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, and 24 hours after drug administration.

A standard breakfast, lunch and dinner was served to all subjects at 3, 6 and 12 hours, respectively, after drug administration. Subjects were not permitted to smoke, consume alcohol or caffeine con-

taining beverages 12 hours before dosing and throughout their stay in the unit. Vital signs like pulse rate, blood pressure, respiratory rate, and temperature were recorded before drug administration. Blood pressure and pulse rate were recorded before each histamine challenge. Intensity of itching sensation, drowsiness and dryness of mouth were also be recorded using 100 mm visual analogue scale during the study period.

Throughout the study, trial subjects were monitored for any adverse event. They were specifically asked about any adverse event, particularly drowsiness, during the entire study period.

Pharmacodynamic Parameters Considered for the Evaluation of Bioequivalence Between the Two Formulations

In individual subject, histamine induced cutaneous flare and wheal areas were determined by using graph paper and represented as mm squared, before and after 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, and 24 hours of test drug administration.

The following pharmacodynamic parameters calculated for the individual study subject:

I max: Maximum measured effect (inhibition of histamine induced wheal and flare response, as compared to baseline) over the specified time.

Tmax: Time of maximum measured effect, (inhibition of histamine induced wheal and flare response); if maximum value occurred at more than one point, tmax was defined as the first time point it appeared.

$AUC_{0\text{-}t}$*:* Area under the effect versus time curve from time zero to last measurable effect (24 hours), for histamine induced wheal and flare cutaneous responses, were calculated by linear trapezoidal method.

Statistical Analysis

The sample size was estimated by a power calculation done on basis of other studies. It was estimated that after treatment with two drugs, at least 12 volunteers were required to detect a significant difference of 20% between the two formulations at 90% power level with an alpha error of 5%.

Individual data of histamine induced cutaneous response of wheal and flare areas, percentage inhibition as compared to baseline, their mean and standard deviations are presented for all the subjects completing the study.

Analysis of variance: Analysis of variance was performed (0.05) on untransformed and log transformed parameters for I max, T max and $AUC_{0\text{-}24}$. The analysis of variance included, sequence, subject within sequence, period, and treatment as factors. Each analysis also included calculation of least square mean, difference between means and SD/SE.

Confidence intervals: These consisted of two one-sided tests for bioequivalence, 90% CI for difference between treatment; least square mean (LSM) was calculated for untransformed and log transformed I max, T max, and $AUC_{0\text{-}24}$. To be considered as bioequivalent, the 90% CI of these parameters should be within 80% to 125% of untransformed and 80% to 120% of log transformed data.

Power of test: The power (probability of detecting a 20% difference relative to the reference treatment, LSM at the 5% significance level using a *t* test under the null hypothesis of zero difference) was calculated for untransformed and log transformed I max, T max, and $AUC_{0\text{-}24.}$

RESULTS

A total of 12 healthy male volunteers

Table 1. Demographic Data of Volunteers

Volunteer Code	Age (years)	Height (cm)	Weight (kg)
1	31	164	60
2	32	169	64
3	32	172	82
4	26	168	66
5	26	163	61
6	23	165	65
7	27	162	62
8	32	157	58
9	30	164	63
10	28	168	71
11	27	168	62
12	29	160	64
Mean	28.58	165.00	64.83
SD	2.91	4.22	6.32

with a mean age of 29 years ± 3 years, mean height of 165 cm ± 4 cm and a mean weight of 65 kg ± 6 kg entered into the study (Table 1). At screening, each subject demonstrated a histamine induced flare and wheal cutaneous response. On entry, none of the volunteers had any sign of illness, as indicated by medical history and examination. They had normal electrocardiograms and clinically acceptable serum and urine biochemistry, hematology, and serology. None of the subjects were taking any prescribed or investigational drug during the study and 4 weeks preceding enrolment. All subjects gave written informed consent before entering into the study, which was approved by the institutional ethics committee.

Ten minutes after intradermal injection, 2 µg of histamine produced significant wheal and flare cutaneous response in all subjects. Administration of reference and test formulations of levocetirizine, significantly inhibited the histamine induced cutaneous response in all the subjects. The histamine induced wheal and flare areas was recorded before and after 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, and 24 hours of administration with both formulations. The mean area of histamine induced wheal and flare response with two formulations is shown in Figures 1 and 2. The mean percentage inhibition time curve of wheal and flare cutaneous response is shown in Figures 3 and 4, respectively. The maximum inhibition (I max), time to reach maximum inhibition (T max) and area under the curve for wheal and flare response-time curve (mm^2/hr and percentage inhibition-time curve (%/hr) is shown in Tables 2 and 3.

At the baseline, wheal and flare areas were not found to be significantly different before treatment with any formulation. The inhibition profile of histamine induced wheal and flare response for two levocetirizine tablet formulations demonstrated that they are comparable. Maximum inhibition of histamine induced wheal response (I_w max %) with reference was 82.45% ± 8.8% and 77.9% ± 12.9% with test formulation (Figure 3).

Maximum inhibition of histamine induced flare response (I_f max %) was 80% ± 4.4% and 81.58% ± 6.7% with

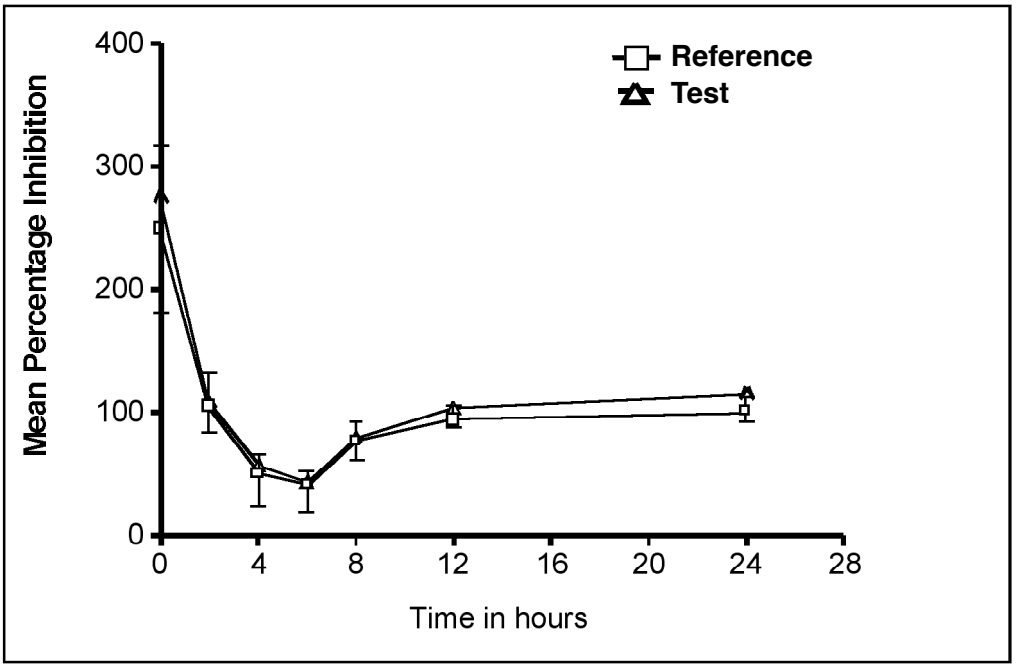

Figure 1. Effect of both treatment formulations of levocetirizine on histamine induced wheal response.

reference and test formulations, respectively (Figure 4).

Inhibition of wheal and flare areas was apparent and pronounced by two hours after dosage and was maximum at 5.27 hours ± 1.0 hours (wheal and flare response with reference formulation) and 5.0 hours ± 1.0 hours and 4.8 hours ± 1.0 hours (wheal and flare response with test formulation), respectively and persisted even at the end of 24 hours, both with reference and test formulations. There was no statistically significant difference in maximum inhibitory response and time to reach maximum inhibitory response between the two formulations.

The area under the antihistaminic activity-time profile curve for wheal was 2211 mm^2/hr ± 270 mm^2/hr and 2482 mm^2/hr ± 368 mm^2/hr with reference and test formulations, respectively and was found to be comparable. The AUC_{0-24} of percentage inhibition of histamine induced wheal response obtained with two formulations under the 24 hours study period was 1449% per hour ± 244.9% per hour (reference formulation) and 1425% per hour ± 277.5% per hour (test formulation) and was also found to be comparable.

Similar to wheal, the area under the antihistaminic activity-time profile curve for flare response was 14,450 mm^2/hr ± 2461 mm^2/hr and 16,160 mm^2/hr ± 2773 mm^2/hr with reference and test formulations, respectively and was found to be comparable.

The AUC_{0-24} of percentage inhibition of histamine induced flare response obtained with two formulations under the 24 hour study period was 1592% per hour ± 182.3% per hour (reference formulation) and 1619% per hour ± 158.8% per hour (test formulation) and did not differ between the two formulations.

The peak antihistaminic activity (I_w max and I_f max) was 82.45% ± 8.8% and 82.0% ± 4.4% with reference, while

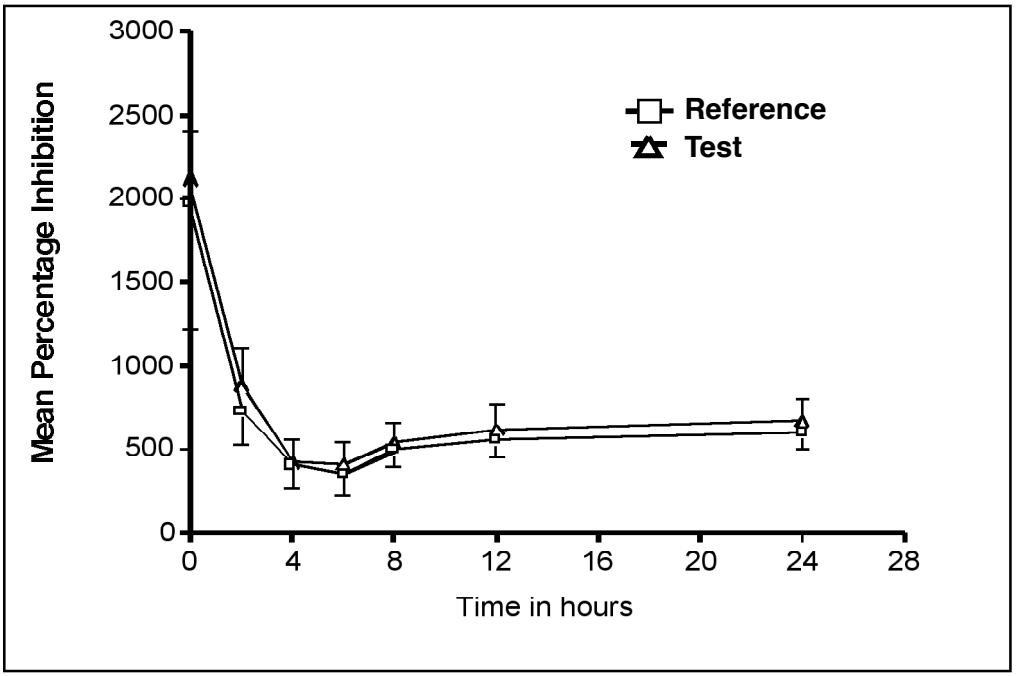

Figure 2. Effect of both treatment formulations of levocetirizine on histamine induced flare response.

it was 77.9% ± 12.9% and 81.58% ± 6.7% with test product. Least square mean (LSM) ratio of T/R for untransformed data of I max wheal was 94.5% (90% CI, 90.98-97.48); for log transformed data, LSM ratio was 93.78% (90% CI, 90.28-97.05). The least square mean ratio of T/R for untransformed data of I max flare was 99.48% (90% CI, 97.68-101.0), and LSM ratio of log transformed data was 99.3% (90% CI, 97.98-100.7).

The AUC-wheal $_{0\text{-}24}$ of untransformed data was 2211 mm^2/hr ± 270 mm^2/hr and 2482 mm^2/hr ± 368 mm^2/hr with reference and test respectively; with least square mean ratio of 112.2% (90% CI, 110-113.5), while with log transformed data the least square mean ratio was 111.94% (90% CI, 111.1-112.69).

The least square mean ratio of AUC-flare $_{0\text{-}24}$ of untransformed data was 111.8 % (90% CI, 112.3-111.4), while with the log transformed data LSM ratio was 111.6 % (90% CI, 111.9-111.4). Since the ratios lie between 80% and 125%, the test formulation of levocetirizine tablet is bioequivalent with reference levocetirizine tablet.

The AUC (%/hr)-wheal $_{0\text{-}24}$ of untransformed data was 1449% per hour ± 244.9% per hour and 1425% per hour ± 277.5% per hour with reference and test respectively; with least square mean ratio of 98.34% (90% CI, 97.2-99.3), while with log transformed data the least square mean ratio was 97.96% (90% CI, 97.3-98.8).

The least square mean ratio of AUC (%/hr)-flare $_{0\text{-}24}$ of untransformed data was 101.69% (90% CI, 103.01-100.59), while with the log transformed data it was 91.6% (90% CI, 90.84-92.1). As the ratio lies between 80% and 125%, the test formulation of levocetirizine tablet is bioequivalent with reference levocetirizine tablet.

Adverse Effects

Both treatments were well tolerated by

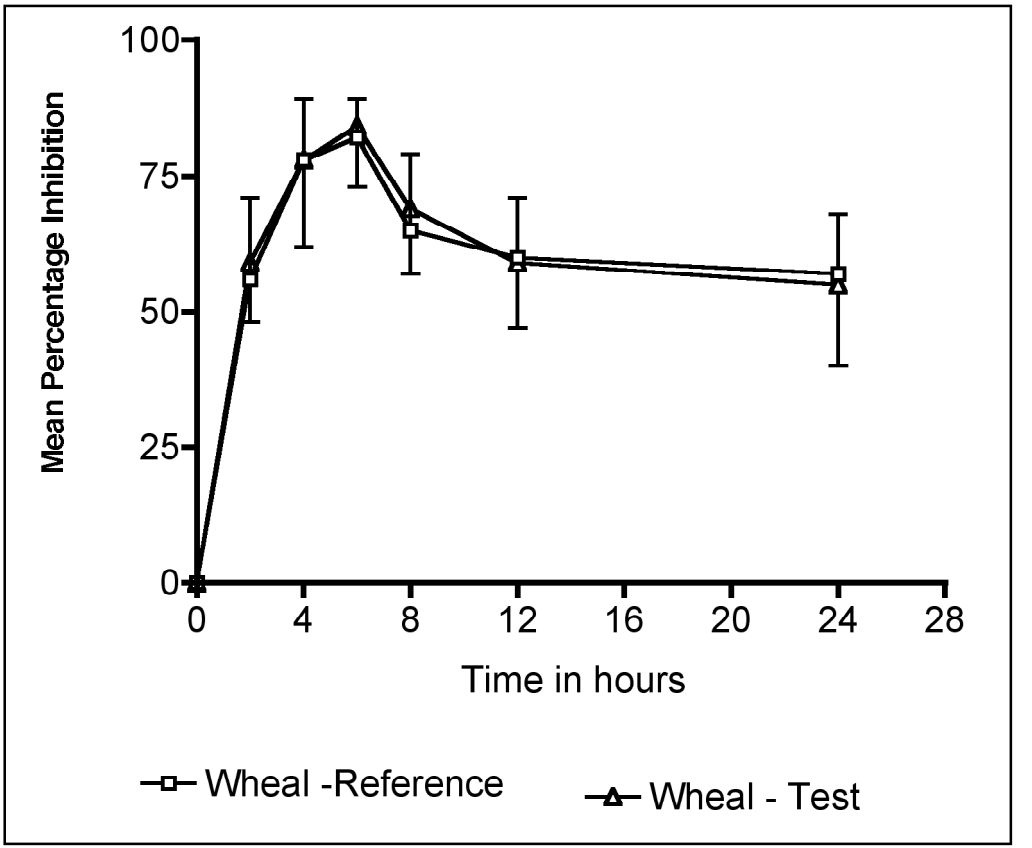

Figure 3. The percentage inhibition of cutaneous wheal response to histamine with both formulations.

the subjects and none of them complained of drowsiness or dryness of mouth. Itch sensation, produced by histamine injection at baseline, was significantly decreased after the administration of both reference and test levocetirizine formulations. None of the volunteers had any serious local or systemic adverse drug reactions.

DISCUSSION

In the present study, we could demonstrate that treatment with 5 mg test levocetirizine produced a marked inhibition of histamine-induced wheal and flare response in healthy human subjects, comparable to a 5 mg reference levocetirizine tablet formulation. Both formulations were comparable in activity and demonstrated apparent inhibition of wheal and flare response by 2 drug administrations; maximal activity was reached within 5 hours. Our study also demonstrated the time course of activity of the two formulations were comparable.

Comparison of pharmacodynamic effects has demonstrated equivalence for the levocetirizine tablet formulations with respect to the maximum level of inhibition that can be achieved. Further, the results of our study demonstrated that the degree and time course of histamine-induced wheal and flare inhibition were similar with both formulations. Our results are in accordance with the findings of Devalia et al[1], who demonstrated that the pharmacodynamic effects were similar when levocetirizine was administered either alone, as the single enan-

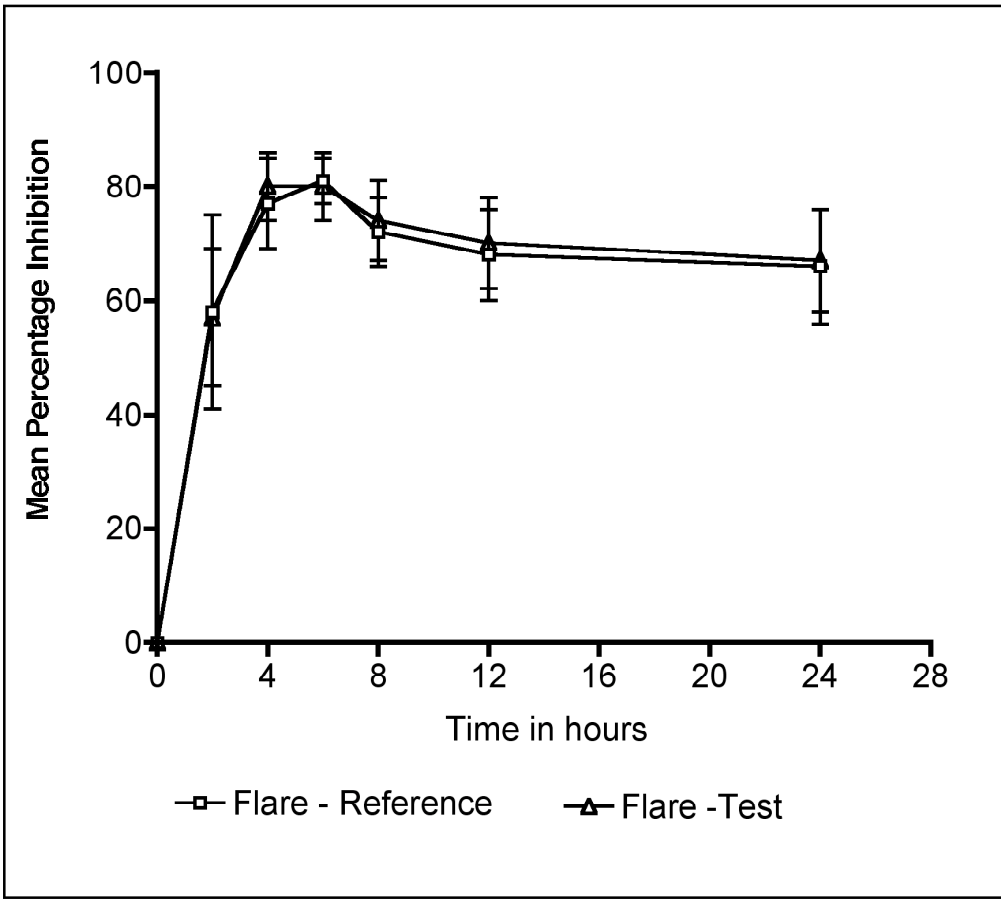

Figure 4. The percentage inhibition of cutaneous flare response to histamine with both formulations.

tiomer, or as a racemic mixture cetirizine.

Our results are in accordance with the findings of other researchers. Devalia et al[1] demonstrated marked inhibition of histamine-induced wheal and flare, which was apparent by one hour after dosage and reached maximum by 6 hours after treatment. In their study, they compared the activity of 2.5 mg levocetirizine versus 5.0 mg cetirizine and 2.5 mg ucb 28557 and the authors observed that treatment with levocetirizine was generally well tolerated in healthy volunteers and suggested the compound is safe.

In an earlier study, Grant et al[9], in a double-blind, randomized, placebo-controlled, crossover study, compared the inhibition profiles of various second-generation H1 antihistaminics including cetrizine. The authors demonstrated inhibition of histamine-induced wheal and flare response with administration of 10 mg cetirizine, evident by 1 hour after treatment and still apparent after 24 hours.

A similar study, conducted by Clough et al[7], compared the effects of levocetirizine and loratidine on histamine-induced wheal and flare response in human skin. The authors observed that the administration of 5 mg levocetirizine significantly reduced the flare,

Table 2. Levocetirizine Bioequivalence Study: Summary Statistics of Untransformed Data—Wheal Area

Product/Statistic	I Max (%)	AUC_{0-24} (mm^2/hr)	AUC_{0-24} (%/hr)	T max (hrs)
Reference				
Arithmetic Mean	82.45	2211.0	1449.0	5.27
SD	8.8	270.0	244.9	1.0
Test				
Arithmetic Mean	77.9	2482.0	1425.0	5.0
SD	12.9	368.0	277.5	1.0
90 % CI (T vs R)				
Lower limit	90.98	110.0	97.2	93.68
Upper limit	97.48	113.5	99.3	95.1
Geometrical Mean				
Reference	82.01	2195.0	1429.0	5.1
Test	76.89	2457.0	1400.0	4.89
Least square mean ratio (T/R) (%)	94.5	112.2	98.34	96.1
P value	ns	ns	ns	ns

Table 3. Summary Statistics of Untransformed Data—Wheal Area

Product/Statistics	Max (%) (mm^2/hr)	AUC_{0-24} (%/hr)	AUC_{0-24}	T max (hrs)
Reference				
Arithmetic Mean	82.0	14,450.0	1592.0	5.27
SD	4.4	2461.0	182.3	1.0
Test				
Arithmetic Mean	81.58	16,160.0	1619.0	4.8
SD	6.7	2773.0	158.8	1.0
90 % CI (T vs R)				
Lower limit	97.68	112.3	103.01	90.84
Upper limit	101.06	111.4	100.59	92.10
Geometrical Mean				
Reference	81.89	14,260.0	1582.0	5.17
Test	81.32	15,940.0	1612.0	4.73
Least square mean ratio (T/R) (%)	99.48	111.8	101.69	91.6
P value	ns	ns	ns	ns

wheal, and itch by 60%, 68%, and 91%, respectively, findings similar to our data, while the effects of loratidine were variable and not statistically significant. They concluded that levocetirizine (5 mg) is a potent inhibitor of the effects of histamine inhuman skin with efficacy that exceeded that of loratidine (10 mg) when single doses of the drugs were administered 4 hours before the test. Several double-blind, randomized, crossover studies are available comparing the inhibition profiles of cetirizine versus placebo or other antihistaminics but there are very few studies with levocetirizine. Simons et al[10] demonstrated that cetirizine was the most potent of all the antihistaminics investigated. In another study, it was suggested that the time of onset of action for cetirizine was 30 minutes, in attenuating histamine-induced wheal and flare response; this

correlates with the time at which the concentration of this drug peaks in plasma after its intake.[11]

CONCLUSION

In the present bioequivalence study, conducted on healthy subjects, levocetirizine 5 mg test formulation (Hetero Drugs Ltd, Hyderabad, India; batch number: 20032002; date of manufacture: March 2002; expiration date: February 2004) was found to be bioequivalent with reference levocetirizine 5 mg tablet formulation (Tab Xyzal, 5 mg levocetirizine dihydrochloride tablet formulation; UCB-Pharma AG, Zurich, Switzerland; lot number: 01 I 0 4; expiration date: September 2004). The least square mean ratio (%) T versus R for peak activity I max-percent (maximum inhibition of histamine induced wheal and flare response); area under the activity time curve (AUC_{0-24} mm^2/hr and AUC_{0-24} %/hr) both for untransformed and log transformed data were found to be within 80% and 125% of 90% CI limits. It can thus be concluded that the test formulation of levocetirizine tablet is bioequivalent to reference levocetirizine tablet.

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