An Evaluation of Relative Bioavailability of Anastrozole Tablets 1 mg in Healthy, Adult, Postmenopausal/Surgically Sterile Indian Female Human Subjects under Fasting Conditions.

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Abstract
The oral bioavailability and pharmacokinetics of a locally manufactured test (Anastrozole) and reference (Arimidex®) formulations were compared in healthy postmenopausal/surgically sterile female volunteers. Volunteers were treated with 1 mg Anastrozole tablet, (test or reference) under fasting conditions with a washout period of 21 days. Blood samples were collected at scheduled time points and plasma concentrations of Anastrozole were analyzed by LC-MS/MS method.
Following oral administration of both formulations, there was rapid and extensive oral absorption from gastrointestinal tract achieving maximum serum concentration within 2 hrs. The concentration-time profile generated by the two products was found to be super imposable. Peak plasma concentration (Cmax), area under the serum concentration-time curve (AUC0-t), area under the serum concentration-time curve extrapolated to infinity (AUC0-∞) and elimination half-life (t1/2) were 16.24 ± 1.50 and 16.02 ± 1.59 ng/mL, 780.11 ± 1.61 and 800.57 ± 1.66 ng.hr/mL, 834.49 ± 1.64 and 866.11 ± 1.70 ng.hr/mL, 53.29 (31.55-79.13) and 57.06 (32.77-87.75) hr for Anastrozole and Arimidex® respectively.
The result indicates that these two formulations have similar rate as well as extent of absorption. Hence these two products can be said to have comparable bioavailability.

Key Words: Anastrozole, Arimidex, Postmenopausal woman, Bioavailability, Bioequivalence

INTRODUCTION:
Anastrozole is a potent and selective non-steroidal aromatase inhibitor which has been shown to possess superior efficacy and tolerability over established endocrine agents in advanced breast cancer. Inhibition of aromatase prevents the conversion of androgen substrates to oestrogen, its sole source in postmenopausal women, thereby leading to regression of hormone-sensitive breast carcinomas. Anastrozole may offer greater selectivity compared with other aromatase inhibitors, being without any intrinsic endocrine effects and with no apparent effect on the synthesis of adrenal steroids. It is well tolerated and has a convenient once-daily dosing regimen, ensuring maximum patient compliance.
Doses of 1 mg and higher resulted in suppression of mean serum concentrations of estradiol to the lower limit of detection (3.7pmol/L). The recommended daily dose, ARIMIDEX 1 mg, reduced estradiol by approximately 70 % within 24 hours and by approximately 80% after 14 days of daily dosing. Suppression of serum estradiol was maintained for up to 6 days after cessation of daily dosing with ARIMIDEX 1 mg.
This study was performed with an aim to evaluate the relative oral bioavailability of Anastrozole tablet 1mg, manufactured in India and Arimidex® (Anastrozole) tablet 1mg, manufactured in USA in healthy volunteers.

SUBJECTS AND METHODS:
Subjects
Fourteen healthy, nonsmoking adult postmenopausal / surgically sterile female volunteers (mean age ± SD, 53.14 ± 4.79; range 44-62 years; BMI 23.52 ± 1.67 kg/m 2) were enrolled in the study, of which only 12 subjects were dosed. All had normal renal and hepatic function. Subjects were enrolled in the study after normal findings from physical examination, laboratory investigations (including hematological, biochemical tests, serology and urine examination.) Exclusion criteria were any history of a significant gastrointestinal condition that could potentially impair the absorption or disposition of the study medicine, subjects suffering with Gastritis, peptic/duodenal ulcer and Zollinger–elision syndrome, use of any oral contraceptives within 28 days prior to period I dosing, use of female hormone replacement therapies, thyroid hormone replacement therapies, or antihypertensive therapies, pregnant females, breast feeding females, abuse of alcoholic beverages and history of allergy to the study drug. The study was approved by the Chennai Ethics Committee.
Study Drugs:
Anastrozole tablets 1 mg manufactured in India was used as test formulation. The pharmacokinetic parameters of Anastrozole were compared with that
Arimidex® which is manufactured by AstraZenica Pharmaceuticals LP, Wilmington, DE 19850, USA.

Study design:
This was an open labeled, randomized, two treatment, two period, single dose study. On the day of dosing in period I and period II, each volunteer was given one tablet of either test or reference formulation as per the randomization schedule. There was 21 days washout period between two dosing days. The drug was administered with about 240 ml of water. They received standard lunch after 4 hrs, snacks after 9 hrs and dinner after 13 hrs for drug administration. The volunteers were ambulatory throughout the study but were prohibited from strenuous physical activity, smoking, alcohol and stimulating beverages containing xanthine derivatives (tea, coffee and soft drinks containing caffeine). Volunteers were monitored constantly throughout the study period by a medical doctor.

Blood sampling:
Blood samples of 5 ml were collected in K3EDTA through an indwelling intravenous cannula placed in a forearm vein before drug administration and at 00.25, 00.50, 00.75, 01.00, 01.25, 01.75, 02.00, 02.25, 02.50, 03.00, 03.50, 04.00, 05.00, 06.00, 08.00, 12.00, 16.00, 24.00, 48.00, 72.00, 96.00, 144.0, 192.0 and 240.0 hours after dosing. The ambulatory samples were collected at 48.00, 72.00, 96.00, 144.0, 192.0 and 240.0 hours. Vaccutainers were placed upright in a rack kept in wet ice bath until centrifugation. Blood samples were centrifuged at 4000 RPM for 10 minutes at 04°C. The plasma samples were transferred into pre-labeled polypropylene tubes in double aliquots and stored at −50°C until analysis.

Analysis of Anastrozole concentration in human plasma:
The plasma samples were analyzed by Liquid Chromatography - Mass Spectrometry/Mass Spectrometry method. The lower limit of quantification was 0.2420 ng/mL during analysis. The samples (0.25 mL) were processed using liquid-liquid extraction technique. The analyte and internal standard were separated on Hypersil gold C18 100 x 4.6mm, 5um column.

Pharmacokinetic analysis:
The various pharmacokinetic parameters were calculated using WinNonlin software. The maximum plasma concentration (Cmax) and the time to reach Cmax (t max) were determined by visual inspection of the individual plasma-concentration time profiles. Terminal Elimination rate constant (Kel) was calculated by the regression coefficient of plasma-concentration vs. time. The area under the curve of concentration vs. time was calculated using one of the numerical integration methods called linear trapezoidal rule. AUC_%Extrap_obs has been derived using AUC0-t and AUC0-∞.

RESULTS:
Single oral dose of Anastrozole, manufactured in India or Arimidex® which is manufactured by AstraZenica Pharmaceuticals LP, Wilmington, DE 19850, USA were given to 12 healthy, adult, postmenopausal / surgically sterile volunteers (mean age ± SD, 53.08 ± 5.20; range 44-62; BMI 23.35 ± 1.75 kg/m²). The primary pharmacokinetic parameters Cmax, AUC0-t and AUC0-∞ and secondary pharmacokinetic parameters Kel, tmax, t1/2 and AUC_%Extrap_obs were calculated using WinNonlin® software.

The mean plasma concentration time profile of two formulations as shown in the graph was similar and superimposable (Figure 1)

The mean of Cmax was 16.24 ± 1.50 ng/mL (%CV 42.47) for test product and 16.02 ± 1.59 ng/mL (%CV 49.13) for reference product. For tmax the median values were found to be 1.75 (0.50-2.50) hr for test product and 1.75 (0.75 – 3.00) hr for reference product. For AUC0-t, the values obtained were 780.11 ± 1.61 ng* hr /mL (% CV 50.58) and 800.57 ± 1.66 ng* hr /mL (% CV 53.89) for for test and reference product respectively.

AUC is important in determining the BA and BE of a drug product. The values of AUC0-t for all volunteers were found to be greater than 80 % of AUC0-∞ which has been expressed as AUC_%Extrap_obs in which all the values are less than 20 %. The mean AUC0-∞ values were found to be 834.49 ± 1.64 ng* hr /mL (% CV 52.48) for test product and 866.11 ± 1.70 ng* hr /mL (% CV 57.13) for reference product.

Half life of test product was found to be 53.29 (31.55-79.13) hr and reference product was found to be 57.06 (32.77-87.75) hr. Kel was 0.0132 ± 0.0038 (hrs)-1 (% CV 28.40) for test and 0.0129 ± 0.0039 (hrs)-1 (% CV 30.42) for reference.

Table 2 shows the 90% confidence intervals of ratio of test and reference (T/R) between the two formulations regarding AUC0-t, AUC0-∞ and Cmax. Ratio of Least square Means (T/R) percent was found to be 101.39 % for Cmax, 97.44 % for AUC0-t and 96.35% for AUC0-∞. The study revealed that at a 90% confidence interval, Cmax, AUC0-t and AUC0-∞ were found to be within the range of 91.12% to 112.81%, 90.27% to 105.19% and 88.09% to
105.38% respectively. All of these values are within the bioequivalence accepted range of 80.00% - 125.00%.

**DISCUSSION:**

BA/BE studies provide important information which ensure safety and effectiveness of medicines to patients and practitioners.

The main objective of the study was to compare the pharmacokinetic profile and to evaluate the relative bioavailability of Anastrozole tablets 1 mg (Test product) and Arimidex® (Reference product). The study revealed that pharmacokinetic parameters of these two products are almost similar and Anastrozole concentration-time profiles generated by the two products were essentially super imposable.

![Mean linear plot of plasma concentration Vs. Time for Anastrozole](image)

Figure 1: Mean Plasma Concentrations of Test (Anastrozole tablets 1 mg) and Reference (Arimidex®) in 12 healthy postmenopausal / surgically sterile female volunteers.

**Table 1: Mean pharmacokinetic parameters of Anastrozole 1 mg tablets**

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Geometric mean</th>
<th>CV (%)</th>
</tr>
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<tbody>
<tr>
<td>Test product (N=12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>16.24 ± 1.50</td>
<td>42.47</td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng* hr /mL)</td>
<td>780.11 ± 1.61</td>
<td>50.58</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng* hr /mL)</td>
<td>834.49 ± 1.64</td>
<td>52.48</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (hr)</td>
<td>1.75 (0.50-2.50)</td>
<td>N/AP</td>
</tr>
<tr>
<td>( t_{\frac{1}{2}} ) (hr)</td>
<td>53.29 (31.55-79.13)</td>
<td>N/AP</td>
</tr>
<tr>
<td>( \text{Kel} ) (1 / hr)</td>
<td>0.0132 ± 0.0038</td>
<td>28.40</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{extra obs}} )</td>
<td>6.43 ± 4.15</td>
<td>64.60</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Reference product (N=12)</th>
<th>Geometric mean</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>16.02 ± 1.59</td>
<td>49.13</td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng* hr /mL)</td>
<td>800.57 ± 1.66</td>
<td>53.89</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng* hr /mL)</td>
<td>866.11 ± 1.70</td>
<td>57.13</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (hr)</td>
<td>1.75 (0.75-3.00)</td>
<td>N/AP</td>
</tr>
<tr>
<td>( t_{\frac{1}{2}} ) (hr)</td>
<td>57.06 (32.77-87.75)</td>
<td>N/AP</td>
</tr>
<tr>
<td>( \text{Kel} ) (1 / hr)</td>
<td>0.0129 ± 0.0039</td>
<td>30.42</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{extra obs}} )</td>
<td>7.45 ± 4.79</td>
<td>64.33</td>
</tr>
</tbody>
</table>

*Expressed in terms of median; N/AP – Not applicable

**Table 2: 90% confidence interval for different pharmacokinetic parameters from log data for assessment of bioequivalence.**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Ratio %</th>
<th>90% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>101.39 %</td>
<td>(91.12 % to 112.81 %)</td>
</tr>
<tr>
<td>( \text{AUC}_{0-t} ) (ng.hr/mL)</td>
<td>97.44 %</td>
<td>(90.27 % to 105.19 %)</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng.hr/mL)</td>
<td>96.35 %</td>
<td>(88.09 % to 105.38 %)</td>
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</table>
Analysis of variance for Ln-transformed pharmacokinetic parameters revealed as follows: C_{\text{max}} formulation sequence and period effect for Ln-transformed pharmacokinetic parameters were statistically insignificant at 5% level of significance. AUC_{0-t} period effect for Ln-transformed pharmacokinetic parameters was statistically significant whereas formulation and sequence effect is statistically insignificant at 5% level of significance. AUC_{0-\infty} formulation period and sequence effect for Ln-transformed pharmacokinetic parameters were statistically insignificant.

Based on the results obtained in this study, it can be concluded that Anastrozole tablets 1 mg (Test) and Arimidex® (containing Anastrozole) tablets 1 mg (Reference) are bioequivalent in 12 healthy, adult, postmenopausal/surgically sterile female human subjects under fasting conditions.

**ACKNOWLEDGEMENT:**
The authors wish to express their hearty gratitude to Micro Therapeutics Research Labs Pvt. Ltd for overall support.

**REFERENCE**
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