Bioequivalence Evaluation of Two Esomeprazole 20 mg Capsule Formulations in Healthy Male Bangladeshi Volunteers

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Abstract

Bioequivalence study of two esomeprazole 20 mg capsule formulations namely Esolok®20 (Test product) and Nexium20 (Reference product) was carried out in the present study. 24 healthy male volunteers were enrolled into this randomized, single-dose, two-period, crossover, open-label pharmacokinetic study with one-week washout period. After administering a single dose of 20 mg of each formulation, blood samples were collected at different time intervals and analyzed for esomeprazole concentrations using a validated HPLC method. Non-compartmental method was used to determine different pharmacokinetic parameters. Obtained mean (SD) values for the test and reference products were 1.45 (0.53) and 1.53 (0.47) μg/ml for Cmax; 2.25 (0.57) and 2.21 (0.71) hr for Tmax; 4.36 (2.04) and 4.37 (2.35) hr-μg/ml for AUC0-12 and 4.59 (1.99) and 4.62 (2.39) hr-μg/ml for AUC0-∞, respectively. The 90% CIs of the test/reference mean ratios of the ln-transformed AUC0-12, AUC0-∞ and Cmax mean values were 102.51% (88.10% – 119.27%), 101.92% (87.32% – 118.96%) and 92.56% (85.73% – 99.93%) respectively, which were within the predetermined FDA bioequivalence range of 80% – 125%. In conclusion, the test and reference formulations of esomeprazole meet the regulatory criteria for bioequivalence both in terms of rate and extent of absorption.

Keywords: Esomeprazole; Proton pump inhibitors; Bioequivalence; Pharmacokinetics; Bangladeshi volunteers

Introduction

Esomeprazole, the S-isomer of omeprazole was developed with the aim of improving the pharmacokinetic and pharmacodynamic profiles of racemic omeprazole [1]. It suppresses the secretion of hydrochloric acid from gastric parietal cells via inhibition of the H+/K+ adenosine triphosphatase enzyme like other proton pump inhibitors [2]. Among all proton pump inhibitors available, esomeprazole is the first to demonstrate significantly greater healing rates than omeprazole in the treatment of patients with erosive oesophagitis [3,4].

Esomeprazole is absorbed rapidly after oral administration [5]. The peak serum concentration of esomeprazole (Cmax) was found to be within 0.5 hours of ingestion of an oral solution containing 20 mg and within 1 to 3.5 hours for encapsulated enteric-coated granules (40 mg) in two studies in a total of 32 healthy volunteers [5]. Increases in systemic exposure, as shown by areas under the plasma-concentration time curves (AUCs), are dose-related after single doses, increasing in a nonlinear fashion [6]. Esomeprazole is metabolized extensively in the liver by two cytochrome P450 isoenzymes to metabolites devoid of antisecretory activity [6,7]. The drug is primarily metabolized by CYP2C19 to hydroxyl and desmethyl metabolites and to a lesser degree by CYP3A4 to sulfone metabolites [8].

The use of generic drugs has increased due to their effectiveness and the increasing variety of drugs that are now available as generic formulations in the recent years. However, their use in clinical practice depends not only on their essential similarity (in formulation and composition, as determined by regulatory agencies), but also on their bioequivalence with their reference counterparts. Therefore, study of the comparative bioavailability of test and reference formulations is important for appropriate assessment by the scientific community [9]. Two drugs are considered to be bioequivalent if they are pharmaceutically equivalent and their bioavailability is so similar that they are unlikely to produce clinically relevant differences in regard to safety and efficacy [9,10].

The aim of this study was to investigate the pharmacokinetic profiles of two 20 mg esomeprazole formulations, namely Esolok®20 (Test product; Batch no: 38; Mfg. Date: Apr 2010; Exp. Date: Apr 2012) manufactured by The Ibn Sina Pharmaceutical Industry Ltd., Bangladesh and Nexium®20 (Reference product; Batch no: v1851; Exp. Date: 01–2012) manufactured by AstraZeneca, Wilmington, Delaware, US in healthy adult male Bangladeshi volunteers.

Materials and Method

Subjects

A total of 24 healthy subjects were enrolled into the study to achieve 80% power with 90% confidence interval [11] with mean (SD) age, 20.75 (0.965) years (range 19 – 22 years); mean (SD) body weight, 68.43 (9.7) kg (range 50 – 86 kg); mean (SD) height, 1.73 (0.07) m (range 1.60 – 1.81 m) and mean (SD) body mass index (BMI), 22.77 (2.22) kg/m2 (range 18.86 – 26.84 kg/m2).

All subjects were examined to verify their health status; these examinations included medical history, vital sign measurements, electrocardiography (ECG), blood sample analysis (basic profile, complete blood cell count, bleeding time, clotting time, prothrombin time, viral serology), and urinalysis (sediment, drugs). Subjects with...
relevant clinical, analytical, or ECG abnormalities were excluded from the trial. Additional exclusion criteria were as follows: smoking, history of alcohol or other drug abuse; consumption of any medication within one month prior to study commencement, participation in a clinical trial in the 4 months before enrollment; history of clinically important illness or major surgery in the 6 months before enrollment; inability to relate to and/or cooperate with the investigators; medication allergy; illnesses or disorders that could affect the absorption, distribution, metabolism, and/or excretion of drugs (e.g. malabsorption, edemas, renal and/or hepatic failure); a history of positive serology for hepatitis B or C (not due to immunization) or HIV; blood loss or donation in the 3 months before enrollment; blood or blood-derived transfusion in the 6 months before enrollment; and exhausting physical exercise in the 72 hours before enrollment.

Study design

Ethical permission and the protocol for the study was reviewed and approved by Bangladesh Medical Research Council (BMRC) (BMRC/ NREC/2010-2013/623). The study was conducted in the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Bangladesh in association with a well-equipped private clinic in Dhaka. The study was conducted in accordance with the International Conference of Harmonization (ICH) guideline for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki and its further amendments [12,13]. All eligible subjects provided written informed consent to participate and were free to withdraw from the study at any time without any obligation.

The study was a single-dose, randomized, open-label, two-period crossover design with a one week washout period. A single 20-mg capsule of either formulation (Esolok® 20 or Nexium® 20) was administered with 250 ml of water after an overnight fast. A standardized breakfast and lunch were given at 4 and 8 hours after drug administration. The consumption of alcohol, grapefruit juice, and beverages was not permitted for 72-hr prior to the study, or after drug administration, until final blood samples were collected. Food intake was strictly controlled and all volunteers received the same food to minimize the effects of food on the study outcomes. During the study period, the volunteers were under medical surveillance by two registered physicians to report any adverse events at all times.

Tolerability

Tolerability was determined by monitoring blood pressure, heart rate, body temperature at the start of the study, 4 hourly during the study, and at the end of each period. A full physical examination was also performed before and 24 hours after drug administration. Laboratory results (hematology, urinalysis, blood biochemistry) were collected before and after the study for all the subjects. The participants were interviewed by the physicians using a structured questionnaire and data collection system as well as nonspecific questioning. All the subjects were advised to report any adverse events at any time during the study period.

Blood sampling

A 20-G × 1.25-inch catheter (Vasofix® Braunüle®, B.Braun Melsungen AG, Melsungen, Germany) was inserted into a suitable forearm vein and 3 ml of blood was withdrawn during each time of collection. Venous blood samples were obtained prior to dosing 0 (baseline) and at 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 7.0, 9.0, and 12.0 h after dosing. The blood samples were kept for 30 minutes at ambient temperature in a dark place and then centrifuged at 3000 rpm for 15 minutes at 25°C. Collected serum was stored at −80°C until further analysis. Protective measures were taken against light during sample collection and analysis as esomeprazole is a light sensitive drug.

Pharmacokinetic and statistical analysis

Pharmacokinetic properties were calculated by a non-compartmental approach from serum concentrations of esomeprazole using software Kinetica (Version 4.4.1. Thermo Electron Corporation, UK). $C_{\text{max}}$ was estimated directly from observed concentrations, and $T_{\text{max}}$ as the corresponding time point at which $C_{\text{max}}$ occurred. $AUC_{\text{0-t}}$ was calculated by the linear trapezoidal method until the last measurable serum drug concentration, and $AUC_{\text{0-\infty}}$ was calculated as $AUC_{\text{0-\infty}} = AUC_{\text{0-t}} + C_{\text{t}}/k_{\text{e}}$. $k_{\text{e}}$ was the terminal elimination rate constant calculated by linear least square regression of the last three to four time points in the log concentration time profile and the terminal half-life was calculated by the following equation [14]: $t_{1/2} = 0.693/k_{\text{e}}$. The mean residence time (MRT) was calculated as:
MRT = $\frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$

The parameters were tested for difference by paired t-test at 5% level of significance. Analysis of variance (ANOVA) was carried out to evaluate the source of variations. The ANOVA model included sequence, subject nested within sequence, phase and treatment (test and reference) as factors [17]. Large sample based 90% Confidence Interval for pharmacokinetic parameters such as $C_{\text{max}}$, $AUC_{0-12}$ and $AUC_{0-\infty}$ were analyzed for the assessment of bioequivalence after logarithmic transformation according to the current FDA guidelines [10].

Results

Method validation

The analytical method was specific, sensitive, accurate and precise. The chromatograms showed that both the peaks were completely resolved from one another and also from serum components (Figure 1B). The peak area ratios were found to be constant across all samples.

Table 1: Serum pharmacokinetic parameters of all the volunteers for test and reference formulations.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test Formulation (n = 24)</th>
<th>Reference Formulation (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>1.45 ± 0.53</td>
<td>1.53 ± 0.47</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>2.25 ± 0.57</td>
<td>2.21 ± 0.71</td>
</tr>
<tr>
<td>$AUC_{0-12}$ (hr-μg/ml)</td>
<td>4.38 ± 2.04</td>
<td>4.37 ± 2.35</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (hr-μg/ml)</td>
<td>4.59 ± 3.96</td>
<td>4.62 ± 3.86</td>
</tr>
<tr>
<td>$k_{e}$ (hr$^{-1}$)</td>
<td>0.35 ± 0.11</td>
<td>0.39 ± 0.35</td>
</tr>
<tr>
<td>$AUMC_{0-12}$ (hr$^2$-μg/ml)</td>
<td>18.05 ± 11.34</td>
<td>18.10 ± 15.21</td>
</tr>
<tr>
<td>$AUMC_{0-\infty}$ (hr$^2$-μg/ml)</td>
<td>22.05 ± 12.91</td>
<td>22.16 ± 15.87</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>2.24 ± 0.57</td>
<td>2.21 ± 0.71</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>4.86 ± 1.22</td>
<td>4.69 ± 1.25</td>
</tr>
</tbody>
</table>

Table 2: P values of paired t-test.

Parameters | $p$-value |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (μg/ml)</td>
<td>0.198</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>0.878</td>
</tr>
<tr>
<td>$AUC_{0-12}$ (hr-μg/ml)</td>
<td>0.974</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (hr-μg/ml)</td>
<td>0.943</td>
</tr>
<tr>
<td>$k_{e}$ (hr$^{-1}$)</td>
<td>0.471</td>
</tr>
<tr>
<td>$AUMC_{0-12}$ (hr$^2$-μg/ml)</td>
<td>0.797</td>
</tr>
<tr>
<td>$AUMC_{0-\infty}$ (hr$^2$-μg/ml)</td>
<td>0.968</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>0.809</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>0.710</td>
</tr>
</tbody>
</table>

2) No interferences were seen in any individual study subject’s baseline of drug-free serum. The calibration curve was found to be linear over the concentration range of 0.02 to 2.0 μg/ml with regression coefficient of 0.9996. The limit of quantification was found as the...
lowest concentration on the calibration curve (0.02μg/ml) for which an acceptable accuracy of 105.3% and a precision of 5.97% were obtained, while the minimum detectable quantity of esomeprazole was found to be 0.01μg/ml. The accuracy was in the range of 95.63% to 105.34%. The intraday precision (expressed as the % CV for QC samples of 0.02, 0.2, 2.0μg/ml) was in the range of 2.48% to 8.26% and inter-day precision was 2.62% to 17.06%. The recovery of esomeprazole from serum was 96.5% while that for pantoprazole was 97.3% and the samples were stable after 6 hours at 25°C, after 1 day storage at – 80°C and after 3 freeze-thaw cycles.

**Tolerability**

Both the formulations were well-tolerated. All the 24 volunteers completed the study without any incidence of adverse effects. No clinically significant abnormalities on physical examination including vital signs measurement and ECG recordings and laboratory results were observed.

**Pharmacokinetic properties**

The pharmacokinetic parameters of esomeprazole are summarized in Table 1. The Mean (SD) Cmax for test and reference formulations are 1.45 (0.53) μg/mL and 1.53 (0.47) μg/mL, respectively, were attained at mean Tmax of 2.25 and 2.21 hours respectively. All subjects presented an AUC0−12/AUC∞ ratio was greater than 80%. The mean elimination half-life was 2.24 and 2.16 hours for test and reference formulations respectively. Mean serum drug concentrations of esomeprazole for both the test and reference formulations are presented in Figure 1.

**Statistical analysis**

The pharmacokinetic parameters were tested by paired t-test at 5% level of significance and the values are presented in Table 2. It can be observed that all the p-values are greater than 0.05 indicating no significant difference among the parameters obtained for test and reference formulations.

Analysis of variance (ANOVA) for crossover design was used to assess the effect of formulations, periods, sequences, and subjects on pharmacokinetic parameters [17]. Sequence effect was tested against the between subject mean squares. All other effects were tested against the within subject mean error. No significant formulation, period or sequence effect was observed (p > 0.05) for any of the pharmacokinetic parameters (Table 3). But in case of subject variation, significant (>0.05) for any of the pharmacokinetic parameters (Table 3).

<table>
<thead>
<tr>
<th>Sources of Variations</th>
<th>Cmax (μg/ml)</th>
<th>Tmax (hr)</th>
<th>AUC0−12 (hr−μg/ml)</th>
<th>AUC∞ (hr−μg/ml)</th>
<th>Kult (hr−1)</th>
<th>t1/2 (hr)</th>
<th>AUMC0−12 (hr2−μg/ml)</th>
<th>AUMC∞ (hr2−μg/ml)</th>
<th>MRT (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>0.111</td>
<td>0.629</td>
<td>0.781</td>
<td>0.829</td>
<td>0.644</td>
<td>0.660</td>
<td>0.506</td>
<td>0.721</td>
<td>0.674</td>
</tr>
<tr>
<td>Period</td>
<td>0.641</td>
<td>0.510</td>
<td>0.971</td>
<td>0.944</td>
<td>0.986</td>
<td>0.967</td>
<td>0.721</td>
<td>0.678</td>
<td>0.366</td>
</tr>
<tr>
<td>Sequence</td>
<td>0.704</td>
<td>0.968</td>
<td>0.758</td>
<td>0.831</td>
<td>0.407</td>
<td>0.431</td>
<td>0.994</td>
<td>0.876</td>
<td>0.620</td>
</tr>
<tr>
<td>Subjects</td>
<td>&lt;0.01</td>
<td>0.663</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.067</td>
<td>0.074</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.099</td>
</tr>
</tbody>
</table>

Table 3: p-values for sources of variations obtained from Analysis of Variance (ANOVA).

The pharmacokinetic properties of esomeprazole were stable after 6 hours at 25°C, after 1 day storage at – 80°C and after 3 freeze-thaw cycles. The 90% CI for esomeprazole Cmax, AUC0−12 and AUC∞ were 85.73% to 99.93%, 88.10% to 119.27% and 87.32% to 118.96%, respectively. All the values of the parameters were within the predetermined range of 80% to 125% according to the FDA requirement for bioequivalence (Table 4) [10].

**Discussions**

To exclude any clinically important differences in the rate and extent at which the active entity of the drugs becomes available at the time of action, assessment of bioequivalence of local product to reference product is necessary. The current study had some limitations that should be considered. This was an open-label study, so it might not address objectively the efficacy and safety profiles of the formulations tested. The study was also limited by inclusion of healthy male volunteers who were administered a single dose in the fasted state, the results cannot be extrapolated to a clinical setting. In the present study, food was strictly controlled and all subjects received the same standardized meal in two periods, thereby minimizing the effect of food consumption. These results remain to be tested for effect of food as well as in patients groups of various ages.

Our study examined the pharmacokinetic properties and bioequivalence of two formulations of esomeprazole capsules in healthy Bangladeshi male volunteers. The pharmacokinetic parameters calculated for both the test and reference formulations were not significantly different, which reflects the comparable pharmacokinetic characteristics of two formulations. All the 90% confidence intervals for important pharmacokinetics parameters were well within the FDA accepted limits for bioequivalent products (80 – 125%). Both the formulations were well-tolerated and all the 24 volunteers completed the study without any incidence of adverse effects. So it can be concluded that the two formulations are bioequivalent in terms of rate and extent of absorption and hence interchangeable.

**References**


