Original Article

Randomized, Open-Label, Two-Way Crossover Study To Compare The Bioequivalence Of Two Formulations Of Esomeprazole In Healthy Male Volunteers

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Abstract

A crossover-randomized bioequivalence study of two oral formulations of esomeprazole (40 mg) capsules were carried out in 16 healthy male Bangladeshi volunteers. The test and reference formulations were PRONEX™ (Drug International Ltd, Bangladesh) and NEXIUM™ (AstraZeneca AB, Sweden), respectively. Each tablet was administered with 150 mL of water to subjects after overnight fasting on 2 treatment days separated by 1 week washout period. After dosing, serial blood samples were collected for a period of 24 hours. The plasma concentrations of esomeprazole were estimated using a validated HPLC method. The pharmacokinetic parameters Cmax, Tmax, $AUC_{0\rightarrow 24h}$, t1/2, and Kel were determined. The mean (± SD) $AUC_{0\rightarrow 24h}$ for esomeprazole of test drug PRONEXTM for 16 volunteers was 1509 (± 546) ng.hr/mL whereas it was 1622 (± 589) ng.hr/mL for esomeprazole of NEXIUMTM. The relative bioavailability (PRONEXTM/NEXIUMTM ratio) was 93%. The Cmax, tmax, half-life of elimination (t1/2) and the rate of elimination (Kel) of esomeprazole of test drug were 1653 (± 706) ng/mL, 2.13 (± 0.81) hours, 2.00 (± 0.61) hour and 0.3465 respectively. The Cmax, tmax, half-life of elimination (t1/2) and the rate of elimination (Kel) of esomeprazole of reference drug were 1820 (± 877) ng/mL, 2.80 (± 0.67) hours, 2.14 (± 0.55) hour and 0.3238 respectively. The 90% CI for the test and reference drugs were found within the acceptance range of 80-125%. In conclusion, PRONEX™ is bioequivalent to NEXIUM ™ in terms of absorption.

***Key words**: Bioequivalence, esomeprazole, pharmacokinetics.*

Introduction

Esomeprazole is a proton pump inhibitor. The most potent suppressors of gastric acid secretion are inhibitors of the gastric H+, K+-ATP- ase (proton pump)[2]. Since their introduction in the late 1980s, these efficacious acid inhibitory agents have assumed the major role for the treatment of acid peptic disorders. PPIs are now among the most widely prescribed drugs world wide due to their outstanding efficacy and safety[1]. In typical doses these drugs diminish the daily production of acid (basal and stimulated) by 80% to 90%. Five proton pump inhibitors are available for clinical use[1]. All are substituted benzimidazole that resemble H2 antagonist in structure but have a completely different mechanism of action[2]. Omeprazole is a racemic mixture of R- and S-isomers; Esomeprazole, the S-isomer of omeprazole was developed with the aim of improving the pharmacokinetic and pharmacodynamic profiles of racemic omeprazole[3]. Esomeprazole is eliminated less rapidly than R-omeprazole, which theoretically provided a therapeutic advantage because of the increased half-life. Despite claims to the contrary, all PPIs have equivalent efficacy at comparable doses[1]. Esomeprazole and Pantoprazole are also available in intravenous formulations[2]. PPIs are administered as

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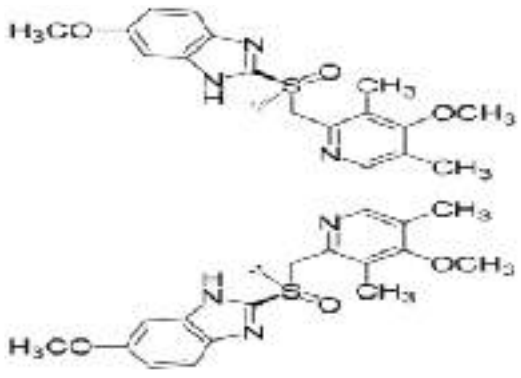
inactive prodrugs. To protect the acid labile prodrug from rapid destruction within the gastric lumen, oral products are formulated for delayed release as acid-resistant, enteric-coated capsules or tablet[2]. Among all proton pump inhibitors available, esomeprazole is the first to demonstrate significantly greater healing rates than omeprazole in the treatment of patients with erosive oesophagitis[4,5].

Esomeprazole is absorbed rapidly after oral administration[6]. PPIs and prodrug that require activation in an acid environment. After passing through the stomach into the alkaline intestinal lumen, the enteric coating dissolve and the prodrug is absorbed[2], the PPIs are lipophilic weak bases (pKa 4-5) and after intestinal absorption diffuse readily across lipid membranes into acidified compartment (eg. the parietal cell, canaliculus). The prodrug rapidly becomes protonated within the canaliculus and is concentrated more than 1000-fold by Henderson-Hesselbalch trapping. There, it rapidly undergoes a molecular conversion to the active form, a reactive thiophilic sulfonamide cation, which forms a covalent disulfide bond with the H+, K+ ATPase, irreversibly inactivating the enzyme[1]. The delayed-release and enteric coated tablets dissolve only at alkaline pH, while at mixture of omeprazole with sodium bicarbonate simply neutralizes stomach acid; both strategies substantially improve the oral bioavailability of these acid-labile drugs. Until recently the requirement for enteric coating posed a challenge to the administration of PPIs in patients for whom the oral route of administration is not available. An I/V formulation of esomeprazole is available in Europe but not in United states[2].

After absorption into the systemic circulation, the prodrug diffuses into the parietal cells of the stomach and accumulates in the acidic secretary canaliculi. Here it is activated by proton -catalyzed formation of a tetracyclic sulfonamide, trapping the drug so that it cannot diffuse back across the canalicular membrane. The activated form then binds covalently with sulfohydryl groups of cysteines in the H+, K+-ATPase, irreversibly inactivating the pump molecule. Acid secretion resumes only after new pump molecules are synthesized and inserted into the luminal membrane, providing a prolonged(up to 24-to 48 hour) suppression of acid secretion, despite the much shorter plasma half-lives(0.5to 2 hours) of the parent compounds. In contrast to H2 antagonist, PPIs inhibit both fasting and meal stimulated secretion because they block the final common pathway of acid secretion. In standard doses, PPIs inhibit 90-98% of 24-hours acid secretion. When administered at equivalent doses, the different agents show little difference in the clinical efficacy. In a crossover study of patients receiving long term therapy with all five PPIs, the mean 24-hour intragastric pH varied from 3.3(Pantoprazole, 40mg) to 4.0 (esomeprazole, 40mg) and the mean number of hours the pH was higher than 4 varied from 10.1 (Pantoprazole, 40mg) to 14.0 (esomeprazole, 40mg).

The bioavailability of all agents is decreased approximately 50% by food; hence, the drug should be administered on an empty stomach. In a fasting state, only 10% of proton pumps are actively secreting acid and susceptible to inhibition. PPIs should be administered approximately 1 hour before meal (usually breakfast) so that the peak serum concentration coincides with the maximal activity of proton-pump secretion. The drugs have a short serum half-life of about 1.5 hours, but acid inhibition lasts upto 24 hours owing to the irreversible inactivation of the proton pump. At least 18 hours are required for synthesis of new H+, K+ ATPase pump molecules[1].

Since an acidic pH in the parietal cell acid canaliculi is required for drug activation and since food stimulates acid production, these drugs ideally should be given about 30 minutes before meals. Concurrent administration of food may reduce somewhat the rate of absorption of PPIs but this affect is not thought to be clinically significant. The drug is primarily metabolized by CYP2C19 to hydroxyl and desmethyl metabolites and to a lesser degree by CYP3A4 to sulfone metabolites[7]. 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[8,9].



(S) and (R)-enantiomers of omeprazole

Materials and Methods

Chemicals: HPLC grade acetonitril was purchased from E. Merck (Germany). Sodium dihydrogen orthophosphate was purchased from Fisher Scientific UK limited.

Chromatographic condition: The HPLC-UV diode-array system consisted of Agilent model 1200 series solvent delivery pump, degasser, autosampler, column oven and photo diode array detector. Chromatographic data were collected and analyzed using Chemstation software.

A reverse-phase high performance liquid chromatography (HPLC) was used for the determination of esomeprazole. The chromatographic analyses were performed on an Agilent 5 µm C18 column (150 x 4.6 mm). The mobile phase used for analysis consisted of 35% acetonitril (HPLC grade; E. Merck, Germany) and 65% sodium di-hydrogen phosphate buffer (50mM, pH 4.5, adjusted with o-phosphoric acid) was delivered at a rate of 0.6 mL/min. The phosphate buffer was prepared freshly on each day of experiment and filtered using 0.45 µm nylon filter. The wavelength was set at 300 nm (bandwidth 2 nm). Separations were achieved at 40°C. Injection of sample (20 µL) was done using an autosampler. The peak with retention time and area were defined using software.

Stock solution preparation: One milligram of each esomeprazole (working standard) and Pantoprazole (internal standard) was dissolve into the mobile phase separately and then diluted into different concentrations.

Identification of esomeorazole and Pantoprazole: Twenty microgram of each solution (1µg/mL) concentration was injected into the HPLC system separately to identify the peak using retention time. Then standard and internal (1µg each) were mixed in mobile phase of which 20 µL was injected into the HPLC system

Sample preparation: One hundred microliter plasma was taken from each eppendrop tube stay few minutes for liquigification then 40µL (10µg/mL) Pantoprazole as internal standard were added vortex for few seconds in the meantime 400 µL phosphate buffer (50mM, pH 6.5) were added, mixed well staying for few minutes and subjected to liquid-liquid extraction using 2 ml dichloromethane as extracting solvent. After vortex mixing for 30 s and centrifugation (4 min at 10000 × g), the organic phase was removed and evaporated to dryness with nitrogen gas after evaporation the residue was reconstituted in 400µL mobile phase and injected 20µL to the HPLC system.

Purpose of the study: To assess the bioequivalence of a test product PRONEX™ (esomeprazole 40 mg per tablet; Drug International Ltd, Bangladesh) with a reference product NEXIUM™ (esomeprazole 40 mg per tablet; AstraZeneca AB, Sweden) by measurement of plasma concentrations by HPLC and calculation of bioequivalence parameters.

Study period:
Volunteers were collected from volunteer bank. Numbers of subjects were sixteen and average age of the volunteers with standard deviation was 27.37 ± 4.88 years, average height with standard deviation was163.56 ± 6.19 cm; average weight at screening examination with standard deviation was 55.37 ± 4.74 kg. Blood sample were collected after drug administration by two phases. Which was in phase I: 8/2/2011 to 9/2/2011 and 16/2/2011 to 17/2/2011 and in phase II: 23/2/2011 to 24/2/2011 and 2/3/2011 to 3/3/2011. Blood samples were estimated from 23/5/2011 to 21/6/2011.

Protocol/design: Randomized, open-label, two-way crossover bioequivalence study with a washout period of 7 days. There was no major deviation made from the approved protocol.

Study medication:
Treatment A (test formulation): PRONEXTM Batch No 07, Manufacturing date: November 2010, Expiry date: October 2012;

Manufacturer: Drug International Ltd, Bangladesh. Treatment B (reference formulation): NEXIUMTM Lot No. MB13257, Manufacturing date: February 2010, Expiry date: February 2012; Manufacturer: AstraZeneca AB, Sweden.

Dosage regimen: Each healthy volunteer received each of the treatments as a single dose in accordance with a randomization scheme (according to the protocol) with a washout period of 7 days.

Institutional review board: The protocol and the ethical aspect of this study were approved by the Institutional review board of Khwaja Yunus Ali Medical College Hospital. This comprises of 7- membered committee including a lawyer, local religious leader (Imam) and a woman representative. The protocol was approved with minor modifications. This study was conducted in accordance with International Conference

of Harmonization (ICH) Good Clinical Practice (CGP) guidelines adopted by the European Agency for the evaluation of Medicinal products (EMEA).

Informed consent: The purpose of the study was explained to each volunteer in local language (Bengali) before starting the study by medical officer. Written informed consent was only taken from a volunteer when he agreed to participate in the study after careful reading of the consent form. Any question raised by the volunteer was discussed with the medical officer in detail. A copy of written informed consent is attached in the protocol.

Hospital admission: After screening volunteers were admitted into the KYAMC hospital. Where especially designed 12 bedded ward for bioequivalence study could be done. Patients was admitted one day before starting the study. The ward is equipped with a 21 inch color TV for watching television programs and carom board for playing by the volunteers in their free time. In this study, at a time 8 volunteers were admitted into the ward. The blood samples were taken immediately before (2 mL in each time) and at 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours after administering esomeprazole. Blood pressure was measured both before and 6 hours after administering drug.

Drug analysis: Drug analysis of esomeprazole in plasma was performed by HPLC with a UV-Visible detector. The retention time of esomeprazole was 8.2 min. The plasma assay procedures were validated. The limit of detection (LOD) was 2ng/mL (3SD) whereas limit of quantification (LOQ) was 6.7 ng/mL (10SD). The extent of absorption was determined by $AUC0_{0\rightarrow 24h}$ of esomeprazole. The rate of absorption was determined by Cmax and tmax. The half-life of elimination (t1/2) and the rate of elimination (Kel) of esomeprazole was used to further characterize the pharmacokinetic outcome of this study.

Findings: The mean (± SD) $AUC_{0\rightarrow 24h}$ for esomeprazole of test drug PRONEXTM for 16 volunteers was 1509 (± 546) ng.hr/mL whereas it was 1622 (± 589) ng.hr/mL for esomeprazole of NEXIUMTM.The relative bioavailability (PRONEXTM/NEXIUMTM ratio) was 93%. The Cmax, tmax, half-life of elimination (t1/2) and the rate of elimination (Kel) of esomeprazole of test drug were 1653 (± 706) ng/mL, 2.13 (± 0.81) hours, 2.00 (± 0.61) hour and 0.3465 respectively. The Cmax, tmax, half-life of elimination (t1/2) and the rate of elimination (Kel) of esomeprazole of reference drug were 1820 (± 877) ng/mL, 2.80 (± 0.67) hours, 2.14 (± 0.55) hour and 0.3238 respectively.

Parameters	PRONEX™		NEXIUM™	
	Mean ± SD	90% CI	Mean ± SD	90% CI
Losartan				
C_{max}	1653 ± 706 ng/mL	82.43 to 117.56	1820 ± 877 ng/mL	80.18 to 119.81
T_{max}	2.13 ± 0.81 hours	84.36 to 115.63	2.80 ± 0.67 hours	90.16 to 109.83
$AUC_{0\rightarrow 24h}$	1509 ± 546 ng.hr/mL	84.12 to 114.87	1622 ± 589 ng.hr/mL	85.06 to 114.93
$t_{1/2}$	2.00 ± 0.61 hour	87.45 to 112.54	2.14 ± 0.55 hour	89.43 to 110.56
K_{el}	0.3465		0.3238	

Table I: Pharmacokinetic parameters following oral administration of PRONEXTM (test) and NEXIUMTM (reference)

$AUC_{0\rightarrow 24h}$ = Area under the plasma concentration-time curve from zero hours to 24 hours; Cmax = maximal plasma concentration; tmax = time for the maximal plasma concentration; t1/2 = half-life; Kel = elimination rate constant.

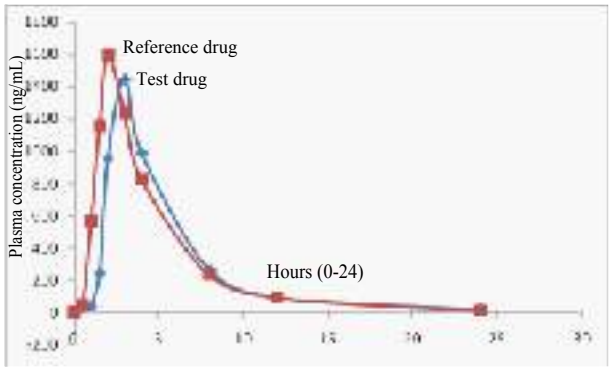


Figure 1: Mean plasma concentrations of esomeprazole in 16 human volunteers

Tolerance: Single dose of esomeprazole (40 mg) of both products was well tolerated by the volunteers.

Discussion

It is necessary to be bioequivalence for any local product to reference product to exclude any clinically important difference in the rate and extent at which the active entity of the drug becomes available at the site of action. The study was limited by of healthy male volunteers and each volunteer was administered a single dose in the fasted state. The current study had some limitations that should be considered.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[10]. Mean half-life of PRONEX (test) was found 2 hours, which was a bit lower than that of NEXIUM (reference) that was 2.14 hours. Omeprazole is a liver enzyme (cytochrome P450) inhibitor drug along with Disulfiram, Erythromycin, Valproate, Isoniazid, Cimetidine, Ciprofloxacin, Sulfonamide etc.This enzyme inhibition may be less in Asian people than Western, so increased enzyme activity may cause rapid inactivation of drug as well as lower half life. The study population might be fast acetylator than the Western people, so the half life was found lesser in PRONEX (test). Plasma protein level would be higher in Western population than the study population, which may be responsible for increased half-life of NEXIUM (reference). The acidity (pH) of stomach may be different in study population, which is required for activation of the drug. It might have influenced the half-life.

The 90% confidence intervals for the PRONEXTM (test) and NEXIUMTM (reference) were found within the acceptance range of 80-125%. So it can be concluded that PRONEX™ (test) is bioequivalent to NEXIUM™ (reference) in terms of absorption and can be used interchangeably in clinical setting.

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