Variation in pharmacokinetics of omeprazole and its metabolites by gender and CYP2C19 genotype in Pakistani male and female subjects

Shabnam Nazir[1], Zafar Iqbal*[2], Lateef Ahmad[3] and Sagheer Ahmad[4]
[1]Kohat University of Science and Technology, Department of Pharmacy, Kohat, Pakistan
[2]University of Peshawar, Peshawar, Department of Pharmacy, Peshawar, Pakistan
[3]University of Swabi, Department of Pharmacy, Swabi, Pakistan
[4]Kohat University of Science and Technology, Department of Pharmacy, Kohat, Pakistan

Abstract: Pharmacokinetics (PK) variation of drugs in males and females may affect therapeutic effectiveness and safety. In current study the PK differences for omeprazole and its metabolites5-hydroxy-omeprazole and omeprazole-sulphone were evaluated in males and females. The current study also considered PK comparison of Pakistani subjects using the CYP2C19 genotype as variable. A single oral dose (40mg omeprazole), open-labeland, non-controlled clinical trial was arranged. Samples were quantified using reversed phase HPLC-UV method. CYP2C19 genotype of subjects was determined by tetra primer polymerization chain reaction (PCR) assay. There was a significant increase in Cmax (from 2 to 2.9µg/ml, p=0.004**), (from 6.67 to 8.74µg-hr/ml, p=0.05*) and elimination half-life (from 1.05 to 2.1 hr, p=0.0001*) of omeprazole in females compared with males. Cmax and of 5-hydroxy-omeprazole (0.0248* and 0.0001***, respectively) and omeprazole-sulphone (0.0001*** and 0.001**, respectively) was significantly higher in females than males when compared at 95% confidence interval. The Cmax and AUC of omeprazole showed a significant raise (p=0.01* and 0.04*, respectively) in Homz PMs (Homozygous Poor Metabolizers) compared with Homz EMs (Homozygous Extensive Metabolizers) and Htrz PMs (Heterozygous Poor Metabolizers) while Cmax and AUC of 5-hydroxy-omeprazolewas significantly higher (p=0.01* and 0.04*, respectively) in Homz EMs compared with Homz PMs and HtrzPMs. AUC of omeprazole was significantly higher in females while its elimination also took longer compared with males. AUC of omeprazole was significantly higher in Homz PMs indicating that CYP2C19* displayed genetically deficient metabolism in its homozygous state.

Keyword: Pharmacokinetics, gender difference, CYP2C19, genetic polymorphism.

INTRODUCTION

Omeprazole is the most commonly used drug for hyperacidity, gastro esophageal reflux disease (Klinkenberg-Knol *et al.,* 1994) and Zollinger-Ellison syndrome (Frucht *et al.,* 1991). Omeprazole is mainly metabolized in the liver by CYP2C19 andCYP3A4 to 5-hydroxy-omeprazole (70%) (Cederberg *et al.,* 1989) and omeprazole-sulphone (30%). CYP2C19 exhibits genetic polymorphism and the individuals are classified as either extensive metabolizers (EMs) or poor metabolizers (PMs) based on CYP2C19 activity (Qiao *et al.,* 2006). Asians more frequently carry CYP2C19*3 allele (Jeong *et al.,* 2011). This finding may have clinical importance as cure rates for helicobacter pylori infection, duodenal and gastric ulcers are reportedly low in extensive metabolizers, high in intermediate metabolizers and very high in poor metabolizers based on CYP2C19 genotypic differences (Furuta *et al.,* 1998). Females are 1.5-1.7 fold more exposed to adverse drug reactions compared with the males (Yonkers *et al.,* 1992). PK of drugs is variable in males and females and genotypic groups based on CYP2C19 as well that may affect drug response (Risberg *et al.,* 2006, Shastry 2005).

PK studies of omeprazole have been conducted based on genetic polymorphism of CYP2C19 (Ieiri, *et al.,* 1996; Ramsjö, 2010; Sakai, *et al.,* 2001; T Andersson, 1990), racial or interethnic differences (Ieiri *et al.,* 1996, Kim *et al.,* 2004) and in various population groups(Chang *et al.,* 1995, Gonzalez *et al.,* 2003). Previously, metabolic activities of CYP2C19 and CYP3A4 have been investigated in males and females using omeprazole as a probe drug (Laine *et al.,* 2000). To the best of our knowledge, PK study of omeprazole in males and females has never been performed in Asian subjects.

The objective of the study was to evaluate PK of omeprazole, 5-hydroxy-omeprazole and omeprazole-sulphone as well as that of omeprazole and 5-hydroxy-omeprazole in various genotypic groups based on CYP2C19 activity in healthy male and female subjects

MATERIALS AND METHODS

Study design

A single dose (40mg omeprazole), open-label, non-controlled PK study was designed and performed in accordance with the principles of international conference on harmonization for conducting good clinical practice and declaration of Helsinki. The experimental procedure was approved by ethical committee of Department of Pharmacy, University of Peshawar.

*Corresponding author: e-mail: zafar_iqbal@upesh.edu.pk

(Application number 13/EC/Pharm. Reference number 12/Pharm).

Selection of volunteers

Sixteen male and sixteen female were age matched and included in the study protocol after normal findings from physical examination, previous medical histories and various laboratory tests such as hemoglobin, serum bilirubin and serum creatinine were included in the study protocol. Smokers and volunteers not qualifying healthy status per laboratory tests were excluded. All the volunteers gave written, informed consent prior to participation in the study protocol. The objective of the study and the procedure were carefully explained to the subjects. The demographic characters of volunteers are shown in tables 1 and 2.

Drug Administration and Blood Sampling

After overnight fast, at about 8.00 A.M, each volunteer received omeprazole capsules (40 mg) with full glass of water (ca ≈ 250ml). The blood samples (about 5ml) were collected at 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 hours following the drug administration into heparinized tubes. The median cubital vein was used for blood collection. A standard breakfast and lunch excluding all ingredients that may interact with drug under study were served after 3h and 5h, respectively, following the drug administration. The blood samples were centrifuged for 10 minutes at 10,000 rpm and 0◦C. The plasma obtained was separated and stored at -80◦C until assayed.

Chemicals and reagents

Omeprazole, 5-hydroxy-omeprazole and omeprazole-sulphone were gifts from Astra Zeneca (Sweden) while internal standard Pantoprazole was provided by Medicraft (Pvt.) Ltd. (Peshawar, Pakistan). HPLC grade methanol was purchased from Sigma-Aldrich (Oslo, Norway), potassium dihydrogen phosphate from Fischer Scientific (Leicestershire, UK), Sodium hydroxide from Merck (Darmstadt, Germany). Distilled water was prepared by Millipore (Milford, USA) distillation apparatus.

Chromatographic system and conditions

HPLC system of Perk in Elmer Series 200 (Norwalk, USA) equipped with online vacuum degasser, column oven and UV-VIS detector was used for quantitative analysis of omeprazole, 5-hydroxy-omeprazole and omeprazole-sulphone. The HPLC-UV method was developed and validated by the authors (Ahmad and Khan, 2011). The chromatographic responses were by processed by Total-chrome workstation 6.3.1(version), while network chromatography interface (NCI) 900 was used to connect it with the liquid chromatographic system.

Chromatographic separation was achieved at a column temperature of 45^{o}C on a Supelco/discovery C18 (150 x 4.6mm, 5mm) analytical column. The components of samples were eluted with the mobile phase consisting of potassium dihydrogen phosphate adjusted to pH 7.2 with NaOH and methanol (58: 42 v/v), pumped at a flow rate of 0.8ml/min. The effluent was monitored by ultra violet detection wavelength set at 302 nm. The temperature of column oven was set at 45°C.

Standard solutions

Stock solutions (1mg/ml) of omeprazole, 5-hydroxy-omeprazole and omeprazole-sulphone were prepared in methanol while serial dilutions were made with the mobile phase (Ahmad *et al.,* 2011)**.**

Sample preparation

To 150µL of plasma sample was added 50µL of internal standard, pantoprazole and 450µL methanol. The mixture was centrifuged (using Centurion® scientific UK) at 5000 rpm for 5 minutes. The upper clear layer of plasma was transferred to eppendroff tube (Ahmad *et al.,* 2011).

CYP2C19 genotyping

The CYP2C19 genetic polymorphism was investigated using two primers (1-GATCCAGAGCTTGGCATATT GTATC, 2-GGATCTAAACACACAACTAGTCAATGt, complementary to introns 4 and 5 which is unique intronic sequences of *CYP2C19*) and two primers (3-AAGATCTTTGTTATGGGTTCCC, 4-ATCGATCATTG ATTATTTCCCA) that are aimed for the allele-specific amplification (ASA) of the wild-type and the *Polymorphic* allele. In order to identify the *CYP2C19* polymorphism, wild-type and the *Polymorphic* allele were combined in one tetra-primer PCR assay. A 321-bp product is produced by Amplification of *CYP2C19* exon 5 with primers 1 and 2 that serves as internal control and as template, for the quality of the PCR amplification and for the ASA, respectively. The 229-bp PCR product specific for the *polymorphic* allele and the 127-bp PCR product specific for the wild-type allele are produced as result of ASA.

In order to genotype *CYP2C19 polymorphic allele* the tetra-primer PCR assay was used. The reaction mixture for this purpose was prepared by mixing: 16µL and 2.5 µL of water and of buffer (1.5mM MgCl2), respectively, 0.2µL and 0.5µL of Ampli Gold Taq (5U/µL) and of dNTP mixture (10mM), respectively, 0.4µL, 0.5µL, 0.75µL and 0.75µL of primer 1 (10mM), primer 2 (10mM), primer 3(10mM) and primer 4(10mM), respectively while 3.0µL of genomic DNA (50ng/µL). The cycling conditions used were: 10 min at 94°C; 44 cycles of 94°C for 30s, 50°C for 30s and 72°C for 60 s; and a final extension of 7 min at 72°C. In order to separate the PCR products, 2% agarose gel electrophoresis was used.

Determination of plasma drug concentrations

Following equation was used to determine plasma drug concentrations;

$$c = \frac{x}{y} \times \frac{a}{b} \times C_s \times F_D$$

Where x and y are peak areas for analyte, a and b are peak areas for internal standard in 1:1 mixture of (1µg/mL of each omeprazole, 5-hydroxy omeprazole, omeprazole sulphone and internal standard) and plasma samples respectively, Cs is the concentration of analyte in 1:1 mixture while F_D is the dilution factor.

Calibration curves were plotted as response ratio (ratio of peak areas of analyte and internal standard) versus relevant concentrations in a range of seven different levels for each omeprazole, 5-hydroxy omeprazole and omeprazole sulphone. The resulting plot was used to determine slope (m), intercept (b) and correlation coefficient (r).

Pharmacokinetic analysis

Plasma drug concentrations of omeprazole, 5-hydroxy-omeprazole and omeprazole- sulphone were plotted as a function of time on semilog and normal scale. PK-Solution®2.0 software was used for non-compartmental data analysis.

The non-compartmental analysis is based on assumption of linear kinetics being followed by drug. The Cmax and tmax were directly observed form plasma-concentration-time profile of omeprazole. The AUC was determined by trapezoid rule. By linear regression of the last points of the elimination phase, slope was determined. The elimination $t_{1/2}$ was calculated as:

$$t_{1/2} = \frac{0.693}{k} \quad 1.$$

The Vd/F was calculated as:

Apparent Volume of distribution, $Vd/F = \frac{Dose}{C}$ 2.

Where C□=the extrapolated serum concentration at time = 0, which for oral dose is calculated using a variation of the intravenous bolus equation ($C_o = C/e^{-ket}$).

$Cl/F = k\ Vd/F$ 3.

STATISTICAL ANALYSIS

The software used for statistical analysis was graph pad prism 5. PK parameters of omeprazole, 5-hydroxy-omeprazole and omeprazole-sulphone were compared in males and females and various CYP2C19 genotypic groups using t-test and one way Analysis of variance (ANOVA), respectively. The data are presented as mean ± SD.

RESULTS

The demographic characters of males (n=16) and female (n=16) volunteers showed significant (p<0.05) differences as shown in tables 1 and 2.

Gender based pharmacokinetic analysis of Omeprazole and its metabolites 5-OH-OMP and OMP-SUL

The plasma concentration-time plots and mean ± SDPK parameters ofomeprazole, 5-hydroxy-omeprazole and omeprazole-sulphoneinmales and females are shown in fig. 4.0, 5.0, 6.0 respectively while PK parameters are shown in table 3.0**.**

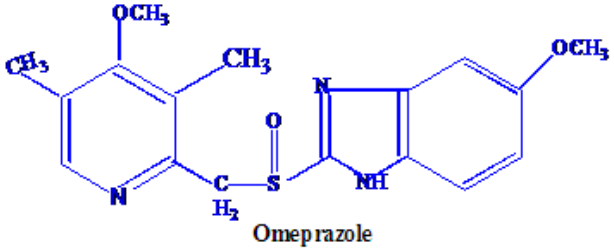

Fig. 1: Structure of omeprazole

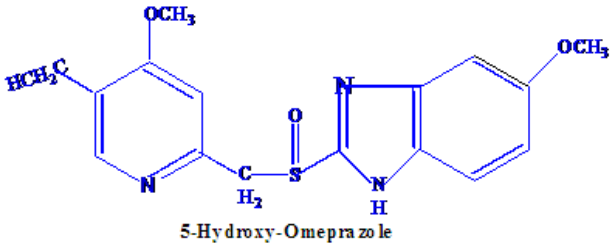

Fig. 2: Structure of 5-hydroxy-omeprazole

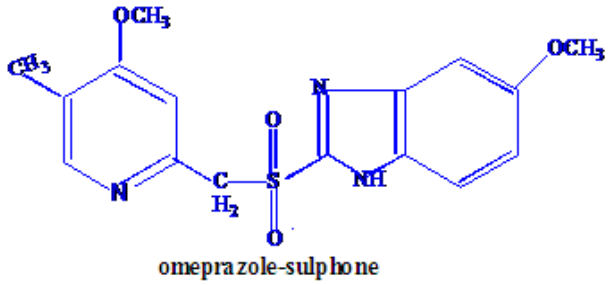

Fig. 3: Structure of omeprazole-sulphone

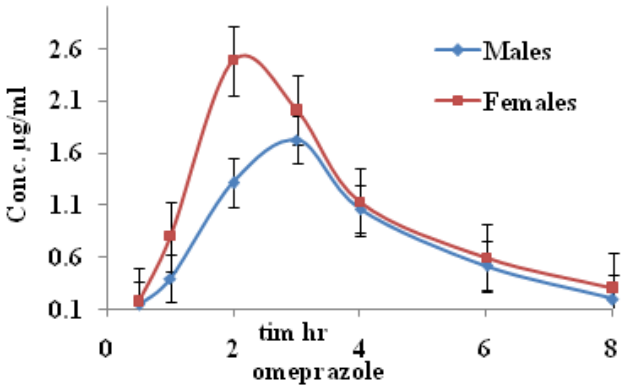

Fig. 4: Plasma Concentration Time Profile of Omeprazole in Males & Females on Linear scale.

The Cmax and of omeprazole was significantly higher (p <0.005) in females compared with males, while the elimination half-life of omeprazole was significantly

longer ($p<0.05$) in females compared with males. The Cmax of 5-hydroxy-omeprazole and omeprazole-sulphone were also significantly higher ($p<0.05$) in females compared with males while insignificant changes ($p>0.05$) were observed for the elimination half-life of 5-hydroxy omeprazole and omeprazole sulphone.

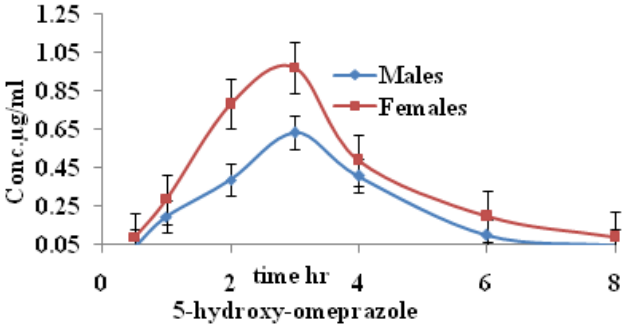

Fig. 5: Plasma Concentration Time Profile of 5-Hydroxy-Omeprazole in males & females on linear scale.

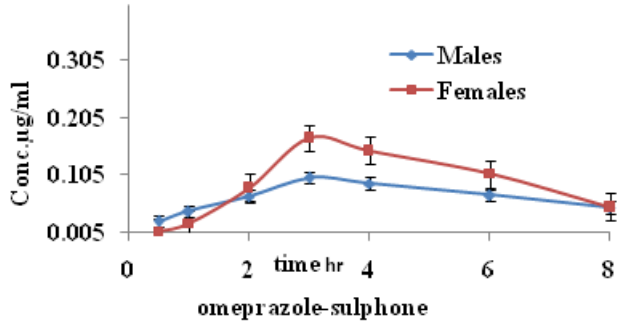

Fig. 6: Plasma Concentration Time Profile of Omeprazole-Sulphone in Males & Females Healthy Volunteers on Linear Scale

PK parameters of omeprazole and 5-hydroxy-omeparzole in genotypic groups of CYP2C19

Genotypic analysis was investigated in human volunteers (n=32), of these 15 subjects were homozygous m/m (mutant type) PMs, 5 were Homz w/w (wild type) EMs and 12 were Htrz m/w (mutant/wild) PMs. The Homz EMs were homozygous for CYP2C19 *1 and the Homz PMs were carrying CYP2C19*3 allele homozygously while Htrz PMs were containing both alleles *1 and * 3. The plasma concentration-time profile and mean ± SDPK parameters of omeprazole and 5-hydroxy-omeprazole for the three genotypic groups Homz PMs, Homz EMs and Htrz PMs are shown in fig. 7.0, 8.0 and table 4.

The Cmax of omeprazole was significantly higher ($p<0.05$) in Homz PMs compared with Homz EMs and Htrz PMs. The Cmax and of 5-hydroxy-omeprazole was significantly higher ($p<0.05$) in Homz EMs compared with Homz PMs and Htrz PMs.

The possible mechanism for gender difference in PK of omeprazole is shown in fig. 9.

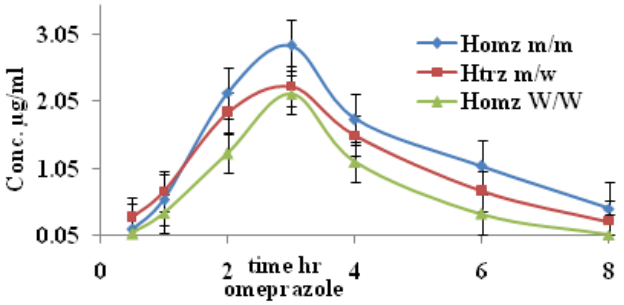

Fig. 7: Plasma Concentration Time Profile of Omeprazole in Different Genotypic Groups Based On CYP2C19 Activity on Linear Scale.

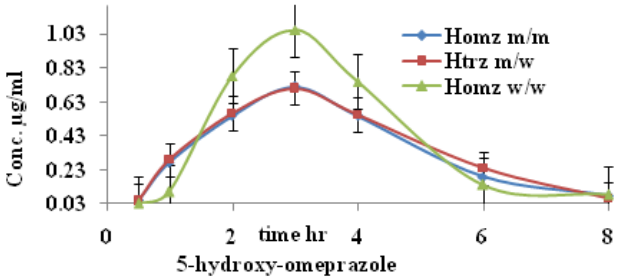

Fig. 8: Plasma Concentration Time Profile of 5-Hydroxy-Omeprazole in Different Genotypic Groups Based On CYP2C19 Activity on Linear Scale.

DISCUSSION

In current investigation, the Cmax and AUC of omeprazole were significantly higher in females compared with males. Omeprazole is a weak basic, lipophilic drug, formulated as an enteric-coated capsules or tablets in order to bypass the lower pH of the stomach and reach the alkaline environment of intestine, where absorption of the omeprazole is facilitated (Dressman *et al.*, 1990). Factors like pH of gastrointestinal tract (GIT), gastrointestinal motility and hepatic first-pass-effect control the bioavailability of orally administered drugs. The gastric and intestinal pH is higher in females compared with males (Dressman *et al.*, 1990) and slower gastrointestinal motility has also been reported in females (Booth *et al.*, 1957) that may be the possible reason for higher Cmax and AUC in females. The role of P-gp efflux is controversial (Yang *et al.*, 1989) as its expression is stimulated by estrogen (Bradley *et al.*, 1990) but progesterone inhibits the p-gp expression (Shapiro *et al.*, 1999).However, low P-gp activity and higher drug absorption in females has been reported (Fromm 2000).

The significantly longer elimination half-life in females shows slower clearance that may be an underlying cause for higher AUC observed in females as compared to males.

Table 1: Demographic Character of Male and Female Healthy Volunteers

Group	Males (n=6)	Females (n=6)	P-value
Age (years)	23.80±0.33	24.0±0.30	1.00
Weight (kg)	61.4±3.2	54.6±4.8***	0.0001***
Weight (cm)	158.2±4.7	137.9±18.2***	0.001***
BMI	24.6±1.6	23.2±1.3	0.7
S. Creatinine mg/dl	0.7	0.66	0.05*

BMI, body mass index. ****P*<0.0005, * *P*<0.05.

Table 2: Demographic Character of Healthy Volunteers in Various CYP2C19 Genotypic Groups

	Homz PMs (n=15)	Homz EMs (n=15)	Homz PMs (n=12)	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)	24.0±0.21	23.0±0.32	24.0±0.22	0.9
Weight (kg)	65.40±1.0	66.50±2.01	64.3±0.2	0.6
Weight (cm)	157.9±3.5	160±3.21	156±4.7	0.7
BMI	26.5±0.8	26±0.53	26.8±0.6	0.5
Plasma albumin mg/dl	4.3±0.4	2.6±0.6	3.4±0.5	0.04*
Serum Creatinine mg/It	0.7±0.1	0.6±0.1	0.4±0.2	0.03*

Homz EMs (homozygous extensive metabolizers), Htrz PMs (heterozygous poor metabolizers) and Homz PMs (homozygous poor metabolizers)

Table 3: PK Parameter of Omeprazole in Male (n=16) and Female (n=16) Human Volunteers Following Oral Administration of Omeprazole (40mg).

Omeprazole		Males	Females		95% CI Value
No	PK Parameter	Mean ±SD	Mean ±SD	P-Value	
1	Cmax (µg/ml)	2.006±0.98	2.913±0.61	0.004**	-1.495 to -0.3173
2	t_{max} (hr)	2.5±0.63	2.3±0.25	0.25	-0.11467 to 0.5467
3	AUC (µg-hr-ml)	6.67±4.32	8.74±2.23	0.05*	-4.121 to -0.02711
8	Elimination half-life (hr)	1.05±0.3	2.101±0.9	0.0001***	-4.476 to 0.6260
5-Hydroxy-omeprazole					
1	Cmax (µg/ml)	0.8±0.5	1.12±0.25	0.0248*	-0.5941 to -0.04338
2	t_{max} (hr)	2.8±0.7	2.62±0.27	0.5007	-0.2494 to 0.4994
3	AUC (µg-hr-ml)	2.1±1.1	3.12±0.56	0.0001***	-7.860 to -5.353
8	Elimination half-life (hr)	2.1±1.73	2.9±0.71	0.34	-0.507 to 1.407
Omeprazole-Sulphone					
1	Cmax (µg/ml)	0.15±0.1033	0.2813±0.0403	0.0001***	-0.1878 to -0.07465
2	t_{max} (hr)	2.875±1.5	3.5±0.4604	0.1216	-1.426 to 0.1760
3	AUC (µg-hr-ml)	0.55±0.50	0.8±0.54	0.001**	-2945 to -2.192
8	Elimination half-life (hr)	3.291±1.545	4.067±2.921	0.4	-2.463 to 0.9100

*p<0.05, **p<0.005, ***p<0.0005, Cmax = Peak plasma concentration, t_{max}= Time to achieve peak plasma concentration, =Area under the plasma concentration time curve (0 - t), = Area under the plasma concentration time curve (0-∞), Vd/F = Apparent volume of distribution, CL/F = Apparent clearance

The higher value for Cmax and AUC of 5-hydroxy-omeprazole in females shows more metabolites formation as compared to males. The possible reason may be higher activity of CYP2C19 in female (Hägg *et al.,* 2001) or prolonged exposure to omeprazole as is evident by longer elimination half life in females. According to present demographic data, females have lower plasma protein levels compared with males therefore, availability of free drug which could have resulted in the higher AUC as well as lead to an increase in metabolism.

The lower serum creatinine of male volunteersr effects the higher GFR in males that might be an indicative of faster renal clearance (Brøchner-Mortensen 1973) and shorter elimination half life. The Cmax and of omeprazole-sulphone was significantly higher in females suggesting higher CYP3A4 activity.

The Cmax and of omeprazole were significantly higher in Homz PMs compared with Homz EMs (with lowest value) and Htrz PMs. The higher level of omeprazole in

Table 4: PK parameter of Omeprazole in Homz PMs (n=12), Homz EMs (n=4) and Htrz PMs (n=10) (based on CYP2C19 Activity) Following Oral Administration of Omeprazole (40 mg).

Omeprazole		Homz PM	Homz FM	Htrs PM	P-Value
	PK-parameters	Mean ± SD	Mean ± SD	Mean ± SD	
1	Cmax (µg/ml)	3.5±1.4	2.5±1.1	2.6±0.7	0.01*
2	t_{max} (hr)	2.4±0.5	2.3±0.3	2.4±0.8	0.9
3	AUC (µg-hr-ml)	9.0±5.1	6.4±2.3	7.0±2.3	0.04*
8	Elimination half-life (hr)	2.2±1.7	1.7±0.7	1.06±0.2	0.06
5-Hydroxy-Omeprazole					
1	Cmax (µg/ml)	0.80±0.5	1.2±0.1	0.9±0.4	0.04*
2	t_{max} (hr)	2.9±0.6	2.1±0.5	2.5±0.5	0.03*
3	AUC (µg-hr-ml)	1.8±1.3	2.8±0.9	2.0±0.9	0.05*
8	Elimination half-life (hr)	2.5±1.9	1.7±0.4	3.9±2.4	0.09

*p<0.05, **p<0.005, ***p<0.0005

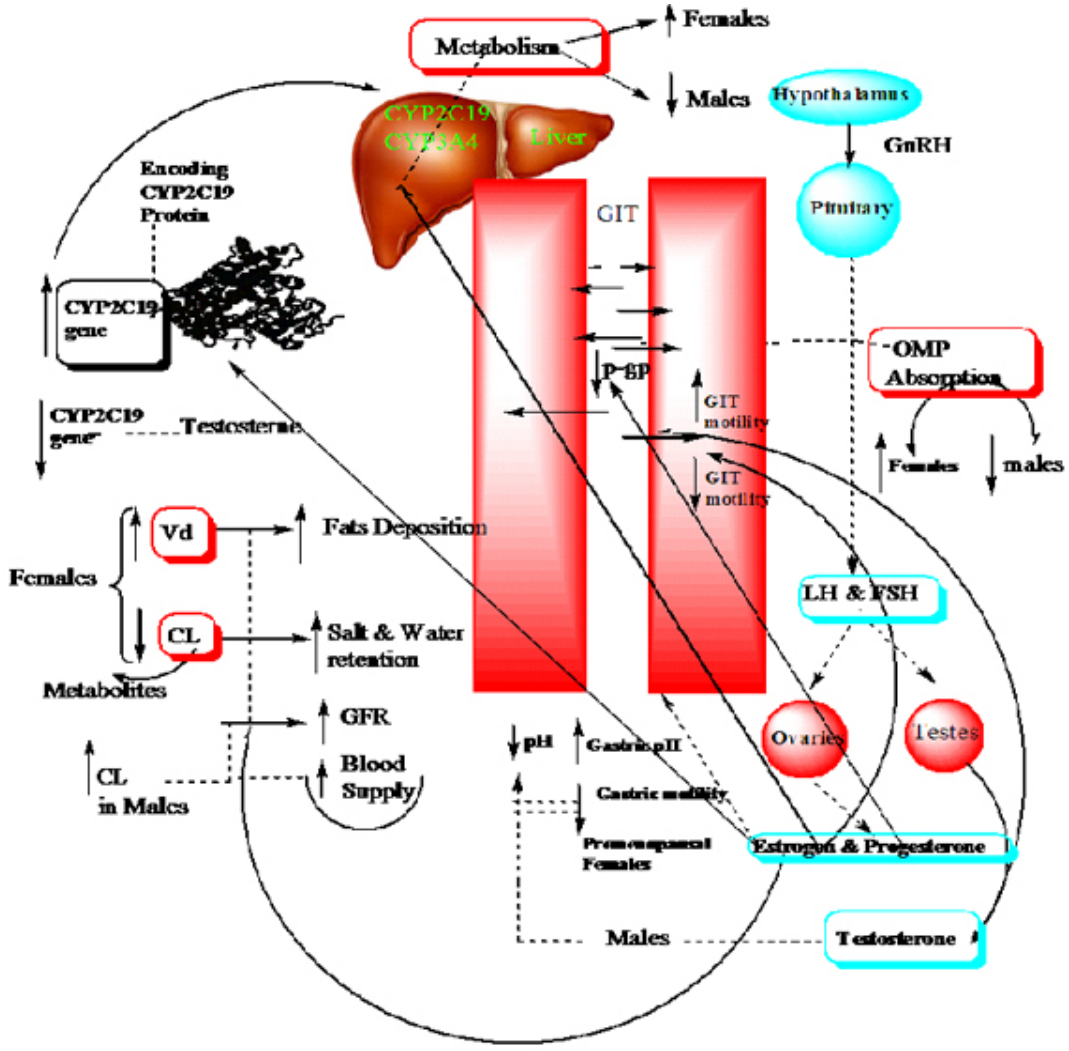

Fig. 9: Possible Mechanism for PK Differences between Males & Females.

Homz PMs may be attributed to the fact that mutant allele CYP2C19*3 in homozygous state slows down metabolism of omeprazole. CYP2C19*3 allele is responsible for poor metabolism and in Asians high frequencies of CYP2C19*2 and CYP2C19*3 have been observed (Mizutani 2003). In current investigation, highest AUC was observed for Homz PMs (CYP2C19*3 /*3 alleles) while lowest for Homz EMs (CYP2C19*1 /*1 alleles therefore, current finding supports the previous finding. The elimination half-life of omeprazole was

insignificantly longer in Homz PMs that may be due to slower CL.

The C_{max} for 5-hydroxy-omeprazole was insignificantly higher in Homz EMs while lowest values were observed for Homz PMs. In Homz EMs, the CYP2C19*1 allele being in homozygous state may cause extensive metabolism. This may be the quite possible reason for higher AUC values for 5-hydroxy-omeprazole in Homz EMs. Higher MR of 5-hydroxy-omeprazole was observed in Homz EMs and the lowest MR for Homz PMs.

CONCLUSION

The results show that poor metabolizers based on CYP2C19 genotype are more exposed to higher omeprazole levels. The wild type CYP2C19*1 allele completely expressed its metabolic activity in its homozygous state while mutant type CYP2C19*3 allele displayed genetically deficient metabolism of omeprazole. In current study, significant gender-based PK differences for omeprazole and its metabolites were found. The results showed that AUC of omeprazole was higher in females compared with males. An FDA drug release (May 25, 2010) regarding omeprazole safety clearly warns that bone fracture risk is associated with "High Dose, long-term "use of omeprazole and labeling of the drug will be changed for new safety information. There is growing awareness also that non-inclusion of females in clinical trials may lead to incorrect handling of drugs (Gleiter and Gundert-Remy 1996). The PK results obtained suggest higher plasma level of omeprazole in females that may cause higher incidence of side effects. Further clinical investigation is required in order to determine adjusted dosein females commensurate to the incidence and intensity of side effects in them.

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