Assessment of potential interaction between simvastatin and clarithromycin in healthy adult male subjects

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Abstract: Cardiac patients with weak immune system are susceptible to bacterial infections. Their prescriptions frequently contain simvastatin and clarithromycin together. The objective of present project was to assess the potential interaction between simvastatin and clarithromycin by evaluating the clarithromycin effects on the pharmacokinetics of simvastatin in healthy adult male subjects. The study design comprised of two phases, used at interval of one week. In first phase simvastatin 20 mg alone was administered to each volunteer. In second phase, co-administration of simvastatin 20 mg with clarithromycin 250 mg was made under similar specified conditions. Blood samples were collected at specified time intervals. Simvastatin plasma concentrations were analyzed through High Performance Liquid Chromatography with UV detector at 238 nm wavelength. Using one compartment open model, MW/PHARM version 3.02 software program was used by F. Rombut for pharmacokinetic parameters calculation. Clarithromycin co-treatment resulted in 2.3 fold increase in maximum plasma concentration C_{max} (from 2.47±0.34 ng.mL^{-1} to 5.66±1.18 ng.mL^{-1}; $p<0.05$) and 3.9 fold increase in area under time versus concentration curve from 0 to 10 hours AUC_{0-10} (from 15.10±3.73 ng.hr.mL^{-1} to 58.49±15.73 ng.hr.mL^{-1}; $p<0.05$) of simvastatin. These results suggest that co-prescription of simvastatin and clarithromycin should be avoided to minimize the adverse events resulting from high simvastatin concentration, without sacrificing therapeutic worth of simvastatin.

Keywords: Simvastatin, plasma, clarithromycin, HPLC, pharmacokinetic interaction.

INTRODUCTION

Poly-pharmacy in old age, multi-morbid conditions and day by day progression in drug development are substantially involved in drug-drug interactions (Corsonello *et al*., 2007; Köberlein *et al*., 2013). Cardiovascular patients are on complex therapeutic regimen therefore, expected to be potentially at higher rate of drug-drug interactions (Sharma *et al*., 2014). Familiarity with the drug-drug interaction is necessary in health care set up because of prevalence of hazardous outcomes of these adverse drug reactions, which may be aggressive enough to subsume hospitalization or even death (Savova *et al*., 2013).

Simvastatin is a hypolipidemic agent that inhibits HMG-CoA reductase to reduce plasma cholesterol level therefore lowers the threats of cardiovascular adverse events (Stein *et al*., 2000; Alakhali *et al*., 2013). It is a prodrug in the form of inactive closed lactone ring which is absorbed in the gastrointestinal tract and generate an active open ring hydroxyl acid after hydrolysis in liver and intestine because of the presence of cytochrome P450 3A4 (Erturk *et al*., 2003). Simvastatin shows only 5 % systemic availability and the phenomena behind such a low bioavailability is involvement of extensive first pass metabolism (Tornio *et al*., 2005; Jin *et al*., 2014). Monotherapy with simvastatin has less adverse consequences but chances of muscular toxicity intensify at high doses (Bellosta *et al*., 2004; Bottorff, 2006). These adverse consequences only become the case of risk with concomitant prescription of CYP3A4 inhibitors such as grape fruit juice, calcium channel blockers, antiviral and antifungal agents. Elderly patients were considered to be at high risk of developing severe rhabdomyolysis with warning signs of muscle weakness, pain and dark urine with acute renal failure (Lilja *et al*., 2004; Fallah *et al*., 2013).

Clarithromycin is a semi synthetic macrolide agent with an advantage of acid stability. But being a CYP3A4 down regulator at duodenal epithelium, it interferes with metabolism of drugs that are substrate for CYP3A. Clarithromycin involved in increasing in the steady state concentration of these drugs by disrupting their metabolism. A significant proportion of prescriptions comprised of both statins and CYP3A4 inhibitors on similar days have been investigated in Norway, Switzerland, UK and USA (Ming *et al*., 2008; Devold *et al*., 2009; Bakhai *et al*., 2012). The purpose of this pharmacokinetic interaction study was to determine the effect of CYP3A4 inhibitor, clarithromycin, on the bioavailability of simvastatin within local healthy male subjects under indigenous environmental conditions.

MATERIALS AND METHODS

Subjects

Eight healthy adult volunteers participated in the study. Each volunteer was ascertained to be in good health by a

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medical history, clinical examination and routine laboratory testing. Inclusion criteria required that volunteers be healthy males between 22 and 28 years of age, have body weight between 60 and 70 kg and be able to understand and read the Urdu language. Candidates were excluded if they were smokers, consumed alcohol regularly or used any drug or food such as grapefruit products, caffeinated beverages and chocolates (from 2 weeks before the first study day) that can either affect or be metabolized by CYP3A4. The study protocol was approved by the Ethics Committee of Institute Physiology and Pharmacology, University of Agriculture Faisalabad for studies in Healthy Subjects of local population. The subjects were provided with written informed consent before entering the study.

Study design

The study design comprised of two phases, used at an interval of one week. The volunteers fasted overnight before oral administration of simvastatin tablets 20 mg with 250 mL of water and standardized meal was served during the sampling schedule. Subjects were not allowed to take any medication from 2 weeks before the study. In second phase, concomitant administration of simvastatin 20 mg along with clarithromycin tablets 250 mg was made after seven days wash out period of first phase.

Blood sampling

For the collection of blood, a plastic cannula/branulla was inserted in one of the brachial veins under strict aseptic conditions to each subject. Blank samples and timed post drug administered samples were collected in EDTA (Ethylenediamine Tetraacetic Acid) tubes. The pH of each sample was measured by pH meter (Beckman HS, Germany) with glass electrode at 37^{o}C. Blood samples were centrifuged at 4000 rpm for 30 minutes; plasma was separated and stored at -20^{o}C until further analysis.

Simvastatin analysis chemicals

Simvastatin was supplied by Merck Sharp & Dohme Italia (Rome, Italy)**.** HPLC-grade acetonitrile was obtained from Farmitalia Carlo Erba (Milan, Italy). Water was purified and deionized using a Mini-Q ion-exchange filtration system (Millipore, Bedford, MA, USA). Water was filtered through WCN 0.45 µm filters, while acetonitrile was filtered through WTP 0.5 µm filters (Whatman Ltd, Maidstone, UK). Sodium dihydrogen phosphate analytical grade was obtained from J.T. Baker (B.H. Schilling, Milan, Italy).

Calibration and extraction procedure

Mobile phase consisted of a mixture of 0.025 M sodium dihydrogenphosphate (pH 4.5)-acetonitrile (35:65, v/v). After mixing both the solvents the mobile phase was passed through filtration assembly, having the filter paper size 0.45 µm white nylon HNWP 47 mm. Then, the filtered mobile phase was sonicated to remove any air bubbles for 15 minutes. Working standards having simvastatin concentrations 1, 10, 100, 250 and 500 ng.mL^{-1} was prepared. Concentration versus peak area data was plotted on a graph to construct the calibration curve. The curve was linear over the range of 1 to 500 ng mL^{-1} (R^2 =0.998; y = 31.394 x + 244.38).

HPLC analysis

The reversed phase, isocratic high performance liquid chromatography system equipped with liquid chromatographic pump Sykam S1122, sample injector Sykam S5111, C18 column (Thermo, BDS Hypersil. 5 µm; 4.6 mm × 250 mm) and UV visible detector Sykam S3210 was used to analyze the simvastatin plasma concentrations at 238 nm, using a flow rate of 1.5 mL/min at room temperature with 20 µL sample using (Carlucci *et al*., 1992; Novakova *et al*., 2008). The limit of quantification for simvastatin was 0.10 ng mL^{-1}. The separation was achieved at the retention time of 8 minutes and compared with standard. The data of plasma concentration versus time of simvastatin was semilogarithmically prepared.

Pharmacokinetic analysis

The pharmacokinetic parameters of simvastatin alone and combine with clarithromycin in the present project were described best by one compartment open model, using a computer program, MW/PHARM version 3.02 by F. Rombut, in cooperation with University Center for Pharmacy, Department of Pharmacology and Therapeutics, University of Gronigen and Merdi/Ware, copy right 1987-1991. Maximum plasma concentration (C_{max}) and time to attained maximum concentration (T_{max}) were determined directly from the individual plasma concentration versus time curves.

STATISTICAL ANALYSIS

The data was analysed statistically through Student *t*-test and values were expressed as mean values ± SD. For all variables, 95% confidence intervals (CI) were calculated. The statistical program SAS software, version 9.2 was used for the analysis. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Clarithromycin considerably increased plasma concentrations of simvastatin in each subject (table 1 and fig.1). On the basis of comparison of pharmacokinetic parameters it became sure that drug-drug interaction existed among simvastatin and clarithromycin (table 2).

The mean area under the curve of simvastatin concentration versus time $AUC_{(0–10)}$ was increased 3.9 folds (from 15.10±3.73 ng.hr.mL^{-1} to 58.49±15.73 ng.hr.mL^{-1}; $p<0.05$) and the mean peak plasma

concentration C_{max} was increased 2.3 folds (from 2.47± 0.34 ng.mL^{-1} to 5.66±1.17 ng.mL^{-1}; $p<0.05$) due to clarithromycin concomitant administration. There were no statistically significant changes in the T_{max} and $t_{1/2\beta}$ of simvastatin.

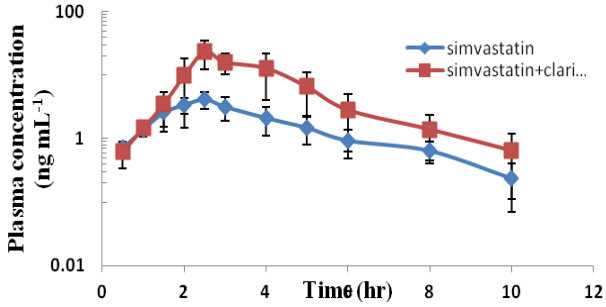

Fig. 1: Plasma concentration of simvastatin alone (blue curve) and with clarithromycin (red curve) on a semilogrithmic scale versus time in eight healthy adult male subjects.

DISCUSSION

The present study illustrates that clarithromycin 250 mg considerably increased plasma concentration of simvastatin 20 mg. Even lowest clinically recommended dose of clarithromycin 250 mg increased the peak plasma concentration up to 2.3 folds of simvastatin 20 mg and that in area under the time versus concentration curve (AUC_{0-10}) up to 3.9 folds increased. Area under the curve shows linear behaviour with plasma concentration of drug (Mauro, 1993). The area under the curve (AUC) after single oral intake of simvastatin 20 mg in present project obtained 15.10±3.73 ng.hr.mL^{-1}. The AUC for 40 mg Simvastatin literature cited values are 44.3±41.7 ng.hr.mL^{-1} (O'Brien *et al*., 2003), 31.6±17.2 ng.hr.mL^{-1} (Lilja *et al.,* 2004), for 10 mg dose of simvastatin AUC was 6.82 ng.hr.mL^{-1} (Kosoglou *et al*., 2002). It is assumed that as dose increases plasma concentration also increases and peak plasma concentration correlates well with area under the curve, therefore AUC with respect to concentration increases as well. This indicates the potential of interaction between simvastatin and clarithromycin. The variation pattern in peak plasma concentration has also been studied due to environmental variations (Dresser *et al*., 2000), human race differences, gender difference (Wang *et al*., 2013), age and weight difference and utilization of different food items during the study course. Lilja *et al*. (2004) specified that grape fruit juice increased plasma concentration of simvastatin and its active metabolite. Zhai *et al*. (2013) reported about the decrease in plasma concentration by consumption of chilli pepper.

Simvastatin is highly lipophilic in nature that makes distribution of simvastatin too much rapid. According to its nature it must follow two or multi-compartment model. It was reported that it is prodrug and its active metabolite simvastatin acid is hydrophilic and both inactive and active forms show reversible conversion to each other with in the body (Prueksaritanont *et al*., 2005; Grozdanis *et al*., 2008). It was assumed that two compartment model can better elaborate the simvastatin pharmacokinetics with first order absorption but it showed alternate pattern that might be due to its rapid distribution phase that involved in doubtful robustness and error chances increased (Kim *et al.,* 2003).

Drug-drug interactions resulted in alteration in plasma concentration of one drug which is ultimately affecting the pharmacokinetics of drugs. The present study indicated that clarithromycin significantly increase the simvastatin C_{max} from 2.47±0.34 ng.mL^{-1} to 5.66±1.18 ng.mL^{-1} and AUC from 15.10±3.73 ng.hr.mL^{-1} to 58.49 ±15.73 ng.hr.mL^{-1}. There was significantly decrease in V_d from 50.59±9.02 L.kg^{-1} to 20.82±3.99 L.kg^{-1} and Cl_B from 23.17±5.06 L.hr^{-1}.kg^{-1} to 10.06±5.49 L.hr^{-1}.kg^{-1}. As total body clearance and volume of distribution decreased side by side, therefore no impact on the elimination half-life ($t_{1/2\beta}$). This parameter also showed statistically non-significant behaviour (1.55±0.29 versus 1.69±0.62 hr) as indicated in table 2.

The variations in pharmacokinetic parameters were found due to the effect of clarithromycin which increased the plasma concentration of simvastatin.

Simvastatin showed dose related adverse outcomes. Risk of myotoxicity due to simvastatin increased at high doses. It has been reported that 80 mg of daily intake of over 12 months of simvastatin can lead to serious adverse events (Bottorff, 2006). The present project explained the drug interaction in foregoing lines has been demonstrated through achieving the raised plasma concentration of simvastatin after concomitant administration with clarithromycin. After concomitant intake of simvastatin and clarithromycin, the peak plasma concentration of simvastatin increased up to 23.4±11.2 ng.mL^{-1}, however single oral administration of simvastatin presented the peak plasma concentration (C_{max}) 4.16±1.20 ng.mL^{-1} (table 2).

The preceding findings was confirmed by Lilja *et al.* (2004) and Kiani and Imam (2007) that grape fruit has moderate CP3A4 inhibition characteristic and increase the concentration of drugs in plasma that shared the same metabolic pathway. Regular intake of even 250 mL or one glass of grape fruit juice considerably enhanced the plasma concentration of simvastatin and simvastatin acid (active metabolite) in chronic usage of simvastatin. Similarly, calcium channel blockers such as verapamil, amlodipine and diltiazim mildly tent to inhibit CYP3A4. Statins and calcium channel blockers routinely co-prescribed to cardiac patients. Literature reported their

Table 1: Plasma concentrations ($ng.mL^{-1}$) of simvastatin alone and with clarithromycin in eight healthy adult male subjects

Time in hours	Simvastatin alone ($ng.mL^{-1}$)	Simvastatin with Clarithromycin ($ng.mL^{-1}$)
0.5	0.72 ± 0.15^{NS}	0.62 ± 0.28
1	1.40 ± 0.33^{NS}	1.47 ± 0.34
1.5	2.59 ± 1.32^{NS}	3.49 ± 1.95
2	3.37 ± 0.95*	9.85 ± 8.38
2.5	4.16 ± 1.20*	23.4 ± 11.20
3	3.18 ± 1.27*	16.0 ± 5.71
4	2.12 ± 1.02*	12.9 ± 8.80
5	1.49 ± 0.69*	6.59 ± 4.30
6	0.94 ± 0.45*	2.83 ± 2.20
8	0.65 ± 0.25*	1.40 ± 0.95
10	0.24 ± 0.17^{NS}	0.65 ± 0.54

Data are mean values (± SD), *= Significant $p<0.05$ difference from respective value, NS= Non-Significant $p>0.05$ difference from respective value

Table 2: The pharmacokinetic parameters of simvastatin alone and with clarithromycin in eight healthy male subjects

Parameters	Units	Simvastatin 20 mg alone	Simvastatin 20 mg with Clarithromycin 250 mg
C_{max}	$ng\ mL^{-1}$	2.47 ± 0.33*	5.66 ± 1.18
T_{max}	hr	2.04 ± 0.29^{NS}	2.44 ± 0.89
K_{abs}	hr^{-1}	0.55 ± 0.14^{NS}	0.47 ± 0.18
$t_{1/2abs}$	hr	1.47 ± 0.25^{NS}	1.69 ± 0.62
B	$ng\ mL^{-1}$	6.07 ± 1.14*	15.36 ± 3.14
β	hr^{-1}	0.46 ± 0.08^{NS}	0.47 ± 0.18
$t_{1/2\beta}$	hr	1.56 ± 0.29^{NS}	1.69 ± 0.62
V_d	$L\ kg^{-1}$	50.59 ± 9.02*	20.82 ± 3.99
Cl_B	$L\ hr^{-1}\ kg^{-1}$	23.17 ± 5.06*	10.06 ± 5.49
$AUC_{(0-10)}$	$ng\ hr\ mL^{-1}$	15.10 ± 3.73*	58.49 ± 15.73
MRT	hr	4.16 ± 0.54^{NS}	4.87 ± 1.78

Data are mean values (± SD), C_{max} Peak plasma concentration, T_{max} time to reach C_{max}, K_{abs} absorption rate constant, $t_{1/2abs}$ absorption half-life, B Zero time drug concentration of elimination phase, β Elimination rate constant, $t_{1/2\beta}$ Elimination half-life, V_d Volume of distribution, Cl_B Total body clearance, $AUC_{(0-10)}$ area under the plasma concentration–time curve from time 0 to 10 hours, MRT Mean residence time. *= Significant $p<0.05$ difference from respective value, NS= Non-Significant $p>0.05$ difference from respective value

Table 3: The pharmacokinetic variables of simvastatin in eight healthy adult male subjects following a single dose of 20 mg simvastatin (control) and with clarithromycin 250 mg

S. No.	Parameters	Units	*Simvastatin alone (control)	Simvastatin with **Clarithromycin	Difference observed in parameters
1	C_{max} Percentage of control	$ng\ mL^{-1}$	2.470 ± 0.343 100%	5.662 ± 1.179 229%	↑ 2.3 folds
2	$AUC_{(0-10)}$ Percentage of control	$ng.hr\ mL^{-1}$	15.10 ± 3.727 100%	58.49 ± 15.73 387%	↑ 3.9 folds

Data are mean values (± SD), C_{max} Peak plasma concentration, $AUC_{(0-10)}$ area under the plasma concentration–time curve from time 0 to 10 hour post medication. *Simvastatin 20mg, **Clarithromycin 250 mg, ↑ increase in concentration.

interaction and emphasizes on the need to closely monitor the patients receiving these combinations (Choi *et al*., 2009; Park *et al*., 2010 and You *et al*., 2010).

Simvastatin and clarithromycin share the same metabolic pathway that is cytochrome P450 3A4, as one is substrate for CYP3A4 (simvastatin) and other is inhibitor of CYP3A4 (clarithromycin), therefore possible interaction of both is observed in the present study. The interaction might be considered due to involvement of same enzymatic metabolic pathway. The present project have provided awareness about the pharmacokinetic interactions of simvastatin with clarithromycin to avoid dose related adverse outcomes, without sacrificing therapeutic worth of simvastatin.

CONCLUSION

This study suggests avoid the concomitant prescription of simvastatin and clarithromycin to minimize the undesirable side effects resulting from high simvastatin concentration. Further studies can be made in future to search out the mechanism of interaction between simvastatin and clarithromycin is due to the involvement of same enzymatic metabolic pathway for both drugs.

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