Pharmacokinetic Drug Interactions Between Clopidogrel With Aspirin

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ABSTRACT
The present study was aimed to evaluate any possible pharmacokinetic interactions between clopidogre and Asprin. clopidogrel concomitant administration in healthy rats as a single dose day, With the treatment was given on day one and day 8 both alone clopidogrel and Asprin concomitantly used both the drugs. The results were showed no significant difference in the t_{max} of clopidogrel on day 1 and day 8 respectively. These were no significant difference in both c_{max} and t_{max} of clopidogrel alone and combination of clopidogrel on day 1 and day 8 respectively. And there was no significance difference in AUC_{0-t} and AUC_{o-inf} also in alone and combination of both drugs on day 1 and day 8th. Based on the results obtained from kinetic study it is evident that the single dose of Asprin with clopidogrel individually and concomitantly treated shows no statistically significant interactions in its pharmacokinetic parameters

Key words: Clopidogrel, Pharmacokinetic drug interactions, Wistar rats.

INTRODUCTION

Drug- Drug Interactions (DDIs) is a phenomenon that occurs when one drug alters the effect of another drug given with it or during its span. Drug interactions may occur when one medicine modifies the pharmacokinetics of another drug or metabolites or may be reflected by the additive pharmacodynamic effects of either drug when taken concomitantly. [1-2] A drug effect may be potentiated, antagonized or otherwise changed by its specific interaction with other drugs, and such interactions can cause potentially harmful and unwanted responses, having severe side effects may ranging from treatment failure to severe adverse drug events.[3] Literature reveals that approx 10-17% of adverse drug events (ADE) are associated with DDIs in ICU subjects.[4] One of most frequently occurring reasons of Adverse drug reactions (ADRs) is drug interactions and it is more commonly seen in elderly patients due to poly-therapies that affects drug effectiveness [5-6] and it makes therapeutic drug management of patient more complicated. [7-8]
The antiplatelet therapy is beneficial in both primary and secondary treatment strategies for cardiovascular disease. [9, 10] These antiplatelet agents, however, have recognizable risks in particular, gastrointestinal (GI) complications such as ulceration and related bleeding. These risks may be further compounded by the ancillary use of other adjunctive medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and anticoagulants. Given the high prevalence of antiplatelet therapy in clinical practice, coupled with an increased emphasis on their extended use, especially after implantation of a drug-eluting stent, [11, 12] it is imperative that physicians know the potential benefits and the associated risks of antiplatelet therapy for primary or secondary prevention of cardiac ischemic events. Clopidogrel, a thienopyridine, is a prodrug that is transformed to an active metabolite, which subsequently blocks platelet activation and aggregation. The active metabolite is formed through the cytochrome P450 (CYP) system after two sequential reactions involving CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP3A4, with CYP2C19 playing a major role. [13, 14] Proton pump inhibitor (PPI) medications are often prescribed prophylactically with initiation of clopidogrel, with the goal of reducing the risk of gastrointestinal tract bleeding while taking dual-antiplatelet therapy. Recent studies, however, suggest that PPIs may reduce the inhibitory effect of clopidogrel on platelet aggregation. [15, 16] In addition, variations in platelet reactivity have been associated with adverse outcomes following stent implantation. [17,18]

MATERIALS AND METHODS

Materials:

Drugs and chemicals

clopidogrel, aspirin were procured from aurobindo laboratories as a gift sample. All HPLC grade solvents (methanol and water) were procured from finar chemicals Ltd., Ahmadabad. All chemicals used were analytical grade.

Animal study

Male Wistar rats (weighing 200-220gms) were procured from the animal house CMR College of Pharmacy, Hyderabad. Animals were randomly divided into four groups each group contains six animals. Each rat was maintained under controlled lab environment atmosphere humidity of 50%, fed with standard pellet diet and water *ad libitum*. The

protocol of animal study was approved by the institutional animal ethical committee with No. IAEC/1657/CMRCP/T2/Ph D-16/77.

Study Design
The rats were grouped as follows:
Group I: Control.
Group II: clopidogrel with aspirin in single dose / day in healthy rats.
Group III: clopidogrel with aspirin concomitant administration as a single dose / day in healthy rats.

Collection of Blood Samples
After administration of the drugs, blood samples of 0.5ml were drawn from each anesthetized (isoflurane) rat at pre-determined time intervals was collected from the retro-orbital plexus using a capillary tube into pre-labelled eppendorf tubes containing 10% of K_2EDTA anticoagulant (20µL). The time intervals for the sample collection were 0 (Pre dose), 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 18 and 24 hrs (post dose), Equal amount of saline was administered to replace blood volume at every blood withdrawal time.
Plasma was obtained by centrifuging blood samples by using cooling centrifuge (REMI ULTRA) at 3000 rpm for 5 minutes. The obtained plasma samples were transferred into pre-labelled micro centrifuge tubes and stored at −20°C until bio analysis of pharmacokinetic and pharmacodynamic parameters. As described above, all the procedures were followed on day 8 also. Pharmacokinetic parameters were calculated by non-compartmental analysis by using Win Nonlin® 5.1 software. Concentrations obtained from the above bio-analytical method were compiled.

Method of Analysis
Preparation of Plasma Samples for HPLC Analysis
Rat plasma (0.5 ml) samples were prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was re suspended with 1 ml of Acetonitrile by vortexing for 1min. After centrifugation (5000 – 6000 rpm for 10 min), the Acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200µ1 of mobile phase was injected for HPLC analysis.
For HPLC an Inertsil ODS 3V, 250x4.6 mm, C8 column with 5µm particle size and the mobile phase consisting of a mixture of 0.03M potassium dihydrogen ortho phosphate buffer (pH 3), acetonitrile in the ratio of 40:60 (v/v) with a flow rate of 1.2 ml/min. and the eluent was monitored at 240 nm. pantaprazole used as internal standard. The retention times of Omeprazole, clopidogrel, pantaprazole were found to be 8.6, 4.6 and 2.6 min respectively.

Pharmacokinetic Analysis
The pharmacokinetic parameters, peak plasma concentrations (C_{max}) and time to reach peak concentration (t_{max}) were directly obtained from concentration time data. In the present study, AUC_{0-t} refers to the AUC from 0 to 24 hrs, which was determined by linear trapezoidal rule and $AUC_{0-\alpha}$ refers to the AUC from time at zero hours to infinity.
The $AUC_{0-\alpha}$ was calculated using the formula $AUC_{0-t} + [C_{last}/K]$ where C_{last} is the concentration in µg/ml at the last time point and K is the elimination rate constant.
Various pharmacokinetic parameters like area under the curve [AUC], elimination half life [t½]. Volume of distribution (V/f) total clearance (Cl/f) and mean residence time for each subject using a non-compartmental analysis by using Win Nonlin® 5.1 software.

Statistical Analysis
Statistical comparisons for the pharmacokinetic – Pharmacodynamic study among, Metformin, Pravastatin alone and in combination groups and plasma concentration – response study among concentrations and time were carried out with student's paired T-Test a value of $P<0.05$ was considered to be statistically significant. Data were reported as mean ± S.E.M linear regressions were used to determine the relationship between total plasma concentrations and pharmacokinetic and pharmacodynamic parameters. The mean concentration versus time profile of Omeprazole, clopidogrel in rat plasma is shown in **table 1, 2.**

RESULTS AND DISCUSSION

Table 1: Mean ± S.E.M, pharmacokinetic parameters of clopidogrel alone and in Combination with clopidogrel with aspirin on day 1

Parameters	**Omeprazole alone**	**combination with clopidogrel with aspirin**
C_{max}	75.74±0.39	76.03±0.12
t_{max}	2±0	2±0
AUC_{o-t} (ng/ml/h)	1072.45±0.74	1056.37±0.52
AUC_{o-inf} (ng/ml/h)	1368.82±2.50	1376.15±1.85
$T_{1/2}$ (h)	6.00±0.28	6.01±0.09

Table 2: Mean ±S.E.M, pharmacokinetic parameters of clopidogrel alone and in Combination with clopidogrel with aspirin on day 8

Parameters	clopidogrel alone	combination with clopidogrel with aspirin
C_{max}	79.92±2.22	80.41±3.1
t_{max}	2±0	2±0
AUC_{o-t} (ng/ml/h)	1337.36±0.38	1343.38±0.49
AUC_{o-inf} (ng/ml/h)	1596.36±0.63	1613.49±0.74
$T_{1/2}$ (h)	6.04±0.024	6.08±0.018

Table 3: Mean ± S.E.M, pharmacokinetic parameters of clopidogrel with aspirin alone and in Combination with on day 1

Parameters	clopidogrel alone	clopidogrel with asprin
C_{max}	90.12±3.03	91.83±3.02
t_{max}	2.5±0	2.5±0
AUC_{o-t} (ng/ml/h)	1533.49±0.016	1541.62±0.20
AUC_{o-inf} (ng/ml/h)	1752.33±1.37	1762.16±0.52
$T_{1/2}$ (h)	6.52±0.49	6.57±0.27

Table 4: Mean ±S.E.M, pharmacokinetic parameters of clopidogrel with aspirin alone and in Combination with on day 8

parameters	clopidogrel alone	clopidogrel with asprin
C_{max}	94.12±3.03	98.83±3.02
t_{max}	2.5±0	2.5±0
AUC_{o-t} (ng/ml/h)	1635.29±2.01	1671.32±3.22
AUC_{o-inf} (ng/ml/h)	1851.32±3.37	1862.13±2.52
$T_{1/2}$ (h)	6.50±0.49	6.50±0.27

DISCUSSION

Clopidogrel is recommended for inhibition of platelet activation and aggregation in patients who are unable to take aspirin or in combination with aspirin in patients with unstable angina or myocardial infarction (MI) or who undergo percutaneous coronary intervention (PCI) [19, 20]. Gastrointestinal (GI) bleeding occurs in approximately 2% of patients who are administered dual antiplatelet therapy after PCI [21]; proton pump inhibitors (PPIs) often are prescribed to decrease this risk [20]. Addition of a PPI to treatment with aspirin and clopidogrel alone or in combination is recommended for patients with unstable angina or non-ST elevation MI and history of GI bleeding [19]. Of the 4162 patients enrolled in the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction (PROVE IT-TIMI) 22 trial who had been hospitalized for acute coronary syndrome (ACS), 781 (18.8%) received treatment with a PPI [22]. Moreover, PPI use increased with increased number of GI risk factors such as prior GI events and use of anticoagulants [22]. Concomitant treatment with clopidogrel and PPI has been shown to be beneficial for the prevention of GI bleeding.

In the present study, Omeprazole is completely absorbed after oral administration with peak plasma concentration of 75.74±0.39ng/ml after 2hrs of dosing on day 1. In combination with Omeprazole and clopidogrel on day 1, the peak plasma concentration of Omeprazole 76.03±0.12ng/ml occurred 2hr after dosing. There was no significant increase in peak plasma concentration levels. Similarly clopidogrel is completely absorbed after oral administration with peak plasma concentration 90.12±3.03ng/ml occurred 2.5h after dosing on day 1 in combination with clopidogrel on day 1. The peak plasma concentration of clopidogrel 91.83±3.02ng/ml occurred 2.5h after dosing. There was no significant increase in the peak plasma concentration levels similarly on day 8 alone and with combination of asprin and clopidogrel on day 8. Peak plasma concentrations are 79.92±2.22ng/ml and 80.41±3.1ng/ml respectively similarly clopidogrel on day 8 and combination with clopidogrel concentrations are 94.12±3.03ng/ml and 98.83±3.02ng/ml respectively. There was no significant difference in peak plasma concentration on day 8 ($P>0.05$). There is no significant difference in AUC and t_{max} in both alone and combination treatment. The half life was similar with alone and combination treatment on day 1 and day 8. All these changes were not statistically significant ($P>0.05$). All the results were showed in **Table (1-4).**

CONCLUSION

In the present study, based on the results obtained from kinetic study it is evident that the single dose clopidogrel individually and concomitantly treated diabetic rats did not show any bio statistically significant interactions in its pharmacokinetic parameters.

So, it can be concluded that the concurrent administration of these two drugs have potential benefit in the management of cardiac patients. In addition, due to their insignificant pharmacokinetic interaction the combinational therapy can be safe and highly advantageous in patients with cardiac patients.

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CONFLICT OF INTEREST
We have no conflict of interest to declare

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