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Research Article

# ESTIMATION OF CLINICAL PHARMACOKINETIC INTERACTION BETWEEN ATORVASTATIN AND CLOPIDOGREL IN ATHEROSCLEROTIC HEART DISEASE PATIENTS

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**ABSTRACT**

Atherosclerotic heart disease includes acute coronary syndromes and hyperlipidmia. The prescription of patients with atherosclerotic heart disease mostly contains atorvastatin and clopidogrel. Aim of this study is to estimate clinical pharmacokinetic interaction between atorvastatin and clopidogrel in atherosclerotic heart disease patients. This study was conducted by estimating the carboxylic acid clopidogrel metabolite level in blood serum and estimating the effect of atorvastatin on blood serum level of clopidogrel metabolite level. In this non randomized, prospective, crossover, single centric and open labelled study was carried out on patients (n=11) of atherosclerotic heart disease with or without PCI. Blood samples were collected, on the basis of pharmacokinetic drug profile, after 1 hr of dosing of clopidogrel. After 24 hr blood samples were collected after 1hr of dosing of clopidogrel with atorvastatin. Serum was separated and stored at -40 °c until analysis. Estimation of carboxylic acid metabolite of clopidogrel was carried out by using validated and sensitive HPLC method. Concentration of carboxylic acid clopidogrel metabolite was observed significantly increased ($p<0.05$) in patients treated with clopidogrel and atorvastatin (7.02 ± 0.86 µg/ml) as compared to patients treated with clopidogrel alone (6.20 ± 0.91 µg/ml). Analysis revealed prominent increases in concentration of clopidogrel carboxylic acid metabolite in presence of atorvastatin and clopidogrel. This might be due to the inhibition of intestinal P-glycoprotein function by atorvastatin and so clopidogrel efflux was reduced. This might have led to increased concentration of clopidogrel in systemic circulation.

**Keywords:** Clopidogrel, Atorvastatin, Interaction and P- glycoprotein.

## INTRODUCTION

Atheromatous disease is ubiquitous and underlies the commonest causes of death (myocardial infarction caused by thrombosis which occurs on ruptured atheromatous plaque) and disability (stroke, heart failure) in societies. Hypertension is one of the most important risk factors for atheroma and other risk factors, especially dyslipidaemia, which, like hypertension, is amenable to drug therapy[1].

Modern treatment of acute coronary syndromes (ACS) with or without ST-segment elevation involves the use of at least five pharmacological groups during the acute phase, namely anti-platelet agents (including aspirin and clopidogrel), antithrombotic

agents (unfractionated heparin, enoxaparin or fondaparinux), beta-blockers and/or calcium channel blockers, ACE inhibitors, and statins. Long term treatment also involves the same combination of drugs, except anti-thrombins[2]. Generally atorvastatin and clopidogrel are most widely prescribed medicines in the patients with atherosclerotic heart disease which may reduce the incidence of arterial disease and prolong life[3]. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that is currently used in the treatment of hypercholesterolemia and mixed dyslipidemia.[4] While Clopidogrel hydrogen sulfate, methyl (+)-(S)-α-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-acetate hydrogensulfate, is a thienopyridine derivative that irreversibly blocks ADP receptor[5]. After percutaneous coronary intervention (PCI), atorvastatin is frequently used with the clopidogrel is to prevent stent thrombosis.[6] Interactions between clopidogrel and statins described in vitro have never been shown to have any clinical implications. In fact, the two drugs have been shown clinically to have an additional effect.[2] Study also suggests that both the drugs metabolize by CYP 3A4 enzyme, so drug interaction might be observed but there is ambiguity among the available reports[4-6].

Various evidences suggest that the oral bioavailability of some drugs is limited by the action of drug transporter proteins, which eject drugs that have diffused across the gut lining back into the gut. At present, the most well characterized drug transporter is 'P-glycoprotein' (P-gp) an efflux protein[7,8].

The important role of P-glycoprotein, in drug absorption and excretion in intestine, kidney and liver, has been revealed through reduction of absorption of orally administered drugs and promotion of urinary and biliary excretion[9,10] . There are many drugs have different impact on P-gp like substrate, inhibitors and inducers of P-gp[11].

Clopidogrel absorption and thereby active metabolite formation are diminished by P-gp–mediated efflux[12]. Results of several studies with in vitro models have shown that lovastatin, simvastatin, and atorvastatin are inhibitors for P-gp and may be substrates for this transporter as well[8]. Clopidogrel, the parent drug or its active metabolite remains undetectable in plasma. The major circulating compound, however, is an inactive carboxylic derivative, which its blood concentration is used to document the pharmacokinetic profile of clopidogrel[13].

In the light of above reports the aim of study is to estimate the plasma concentration level of carboxylic acid metabolite of clopidogrel in presence and absence of atorvastatin using analytical technique like HPLC and estimate the clinical pharmacokinetic drug interaction between atorvastatin and clopidogrel.

## MATERIALS AND METHOD

### 2.1 Chemicals and standard solutions

Phenytoin as an internal standard (I.S) was received as gift sample from Cadila Healthcare Ltd. (India). Atorvastatin (20 mg, Tab. Atorva, Cadila Healthcare Ltd., India), Clopidogrel (75 mg, Tab. Deplatt, Torrent Pharmaceuticals Ltd., India) were purchased from market. All reagents used were of analytical grade while acetonitrile was of HPLC grade.

A stock solution of CCA (1000 µg/ml) was prepared in methanol (95%). Working standards of the drug (0.5-100 µg/ml) were prepared by serial dilution of the stock solution in methanol. Working standard solution of the I.S. (7.5 µg/ml) was prepared in methanol. A 2N HCl solution was prepared in distilled water.

### 2.2 Sample collection

All patients were enrolled from Care Institute of Medical Sciences, Ahmedabad, India after ethical approval of same institute. All the patients (n=11) were administered with single dose of clopidogrel (Tab. Deplatt, 75 mg) at evening. On the basis of pharmacokinetic profile, clopidogrel achieved Cmax at approximately 1 hr of dosing. So blood sample was collected at 1 hr after dosing in disposable glass tubes (100mm×16 mm) without any additives. Next day at the same time atorvastatin (Tab. Atorva, 20 mg) was co-administered with clopidogrel and blood samples were collected at 1 hr after dosing. All the samples were allowed to clot at room temperature for 30 min. Serum was separated by centrifugation at 2000×*g* for 10 min and were stored at −40°C until analysis.

### 2.3 Chromatographic conditions

The HPLC system used consisted of two pumps of Shimadzu LC-10A solvent delivery system, a system controller (SCL 10AD), a UV–visible spectrophotometer detector (SPD-10AD) operated at wavelength of 220 nm, a

column oven (CTO-10A), a degasser (DGU-3A) and a data processor (C-R4A). The analytical column was a Shimpack CLC-ODS, 150mm×4.6mm I.D., 5µm particle size which was protected by a Shim-pack G-ODS guard column (1 cm×4.0mm I.D., 5 µm particle size). A mixture of 0.05M sodium phosphate buffer (pH 5.7; adjusted with phosphoric acid) and acetonitrile (56:44 v/v) was used as the mobile phase. The column oven temperature was set at 50˚C and the mobile phase was filtered, degassed and pumped at a flow rate of 1.7 mL/min.

**2.4 Sample preparation**

Aliquots of blank, calibration standard or unknown human serum samples (1 mL) were pipetted into 100mm×16mm disposable glass tubes, containing 100 µL of the working internal standard solution. The samples were mixed with 200 µL of the HCl solution (2.0 N) and extracted with 5 mL of ethyl acetate. After vortex mixing for 30 s and centrifugation (5 min at 6000×*g*) the organic phase was removed and evaporated to dryness under stream of nitrogen at 50˚C. The residue was reconstituted in 100 µL mobile phase and following brief mixing a volume of 20 µL was injected onto the HPLC system.

**2.5 Calibration curve and linearity**

Calibration curves of standard samples were prepared within the concentration range of 0.05–10 µg/mL using pooled human blank serum obtained from normal subjects. In disposable glass tubes (100mm×16 mm), after evaporation of 100 µL from each working solutions of the analyte, under a gentle stream of nitrogen at 50˚C, the residues were reconstituted in 1mL of drug-free human serum, mixed for 10 s on a vortex mixer and subjected to extraction and analysis as described above.

Calibration curves were obtained by linear least-squares regression analysis of plots of peak-area ratio to I.S. versus drug concentrations.

## 1. STATISTICAL ANALYSIS

Carboxylic acid metabolite of clopidogrel concentration changes in within group were compared using GraphPad Prism software (version 5.04) with 95% confidence level.

## 2. RESULT

A total of 11 patients diagnosed after screening from different inclusion and exclusion criteria and had received clopidogrel and atorvastatin were enrolled in the study.

Demographic data, vital parameters and lipid profile were also measured and recorded (Mean ± SD) during patient's enrolment. (Table 4.1) .

**Table 4.1: Demographics, Vital Parameters and Lipid Profile of Patients**

| Demographic | Data | Vital Parameters | Data |
|---|---|---|---|
| **Sex** | | **Heart rate** | 81.62 ± 8.65 |
| Male | 9 (81.82 %) | **BP** | |
| Female | 2 (18.18 %) | Systolic | 126.54 ± 9.46 |
| **Age** | 54.92 ± 5.74 | Diastolic | 79.62 ± 9.46 |
| **Height** | 166.85 ± 9.04 | | |
| **Weight** | 73.92 ± 9.54 | | |

| Lipid Profile | Data |
|---|---|
| Total cholesterol | 116.15 ± 57.71 |
| Triglyceride | 117.62 ± 39.14 |
| HDL | 29.54 ± 7.28 |
| LDL | 70 ± 38.51 |
| VLDL | 23 ± 4.56 |

**Previous cardiac history, risk factors and current co-morbid conditions were recorded in all the patients. (Table 4.2)**

**Table 4.2: Cardiac History, Diagnosis, Risk Factor and Concomitant Medication.**

| Cardiac History | | Diagnosis | |
|---|---|---|---|
| PCI | 7 (63.64 %) | CAD | 6 (72.73 %) |
| CABG | 3 (27.27 %) | UA | 2 (18.18 %) |
| IHD | 2 (18.18 %) | IHD | 1 (9.09 %) |
| MI | 1 (9.091 %) | MI | 2 (18.18 %) |
| **Risk Factors** | | **Concomitant Medications** | |
| | | Aspirin | 11 (100 %) |
| | | Ramipril | 4 (36.36 %) |
| HT | 5 (45.45 %) | Nikorandil | 9 (81.81 %) |
| DM-II | 1 (9.09 %) | Metformin+ Glipizide | 3 (27.27 %) |
| HT+DM- II | 2 (18.18 %) | Nitroglycerine | 4 (36.36 %) |
| Smoking | 1 (9.09 %) | Pantoprazole | 3 (27.27 %) |
| None | 2 (18.18 %) | Valsartan | 4 (36.36 %) |
| | | Alprazolam | 2 (18.18 %) |
| | | Spironolactone | 1 (9.09 %) |

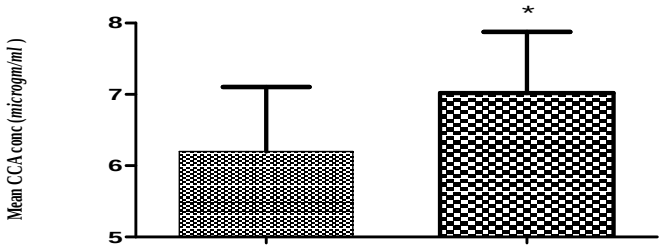


**Fig.4.1: Mean of Clopidogrel Carboxylic Acid Concentration (µg/ml)**

Concentration of carboxylic acid metabolite was observed significantly increased ($p<0.05$) in patients when treated with clopidogrel and atorvastatin (7.02 ± 0.86 µg/ml) as compared to when treated with clopidogrel alone (6.20 ± 0.91 µg/ml). (Fig. 4.1)

Here regression coefficient was found 0.999 of standard calibration curve. In below figure of chromatogram of HPLC shows peak no 4 of CCA and peak no 5 of I.S in single patient after dosing of clopidogrel and co administration of atorvastatin with clopidogrel (Fig 4.2). Calibration curve was prepared on a day of analysis results was as follows: Slope 25.60, coefficient of the linear regression analysis=0.999 and intercept=99.85.

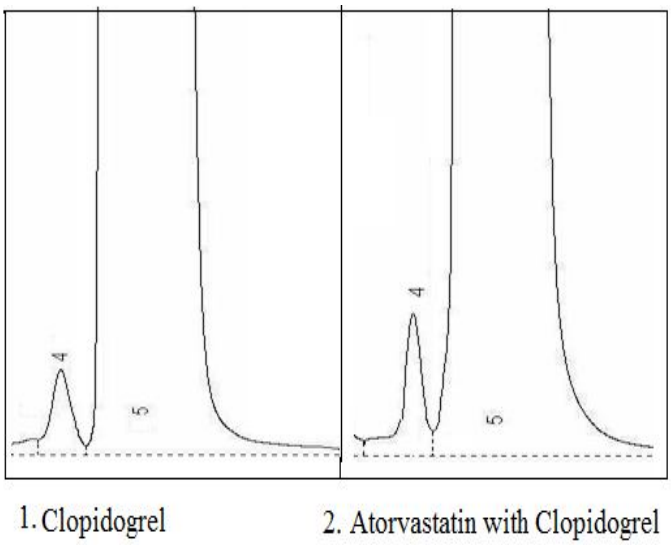


**Fig.2:Chromatograms**
1) Conc. of CCA in serum after Clopidogrel treatment.
2) Conc. of CCA in serum after co administration of Atorvastatin with Clopidogrel

## 5. DISCUSSION

Literature survey reveals that patients with atherosclerotic heart disease are treated with atorvastatin and clopidogrel[14].

Moreover studies also suggest that clopidogrel is efflux out back to gut by P-gp[12] while atorvastatin is inhibitor or substrate of P-gp[8]. Furthermore, various studies suggest that other statins like simvastatin and lovastatin are also inhibitor of p-gp but there is no any evidence in favour of simvastatin, while lovastatin shows action on p-gp[15-16].

Clopidogrel has only 50 % bioavailability[17] This study suggests might be increasing systemic concentration of clopidogrel in presence of atorvastatin. And there by it may be increasing bioavailability of clopidogrel.

Females have been shown to be a risk factor for clinically relevant adverse drug reactions with a 1.5 to 1.7-fold greater risk of developing an adverse drug reaction compared to male patients[18]. So dose adjustment is required when both drugs co-administered according to severity of disease in females.

### Limitations of Study

Our study was conducted with very small sample size. It may be possible that increasing the sample size may give some insight for pharmacokinetic drug interaction between clopidogrel and atorvastatin.

The second limitation of our study was that the enrolled patients were already on the atorvastatin and clopidogrel together with other concomitant medications. So it was not be possible to measure clopidogrel concentration alone.

## 6. CONCLUSION

Atorvastatin enhances the systemic bioavailability of clopidogrel. This might be due to the inhibition of intestinal P-gp function by atorvastatin and so clopidogrel efflux was reduced. This might have led to increased concentration of clopidogrel in systemic circulation. Therefore, concomitant use of clopidogrel with atorvastatin may require close monitoring for potential drug interactions.

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