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Omeprazole and atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation in patients undergoing percutaneous coronary intervention in a tertiary health care system: A prospective drug–drug interaction study

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ABSTRACT

Background: Clopidogrel, a prodrug is found to be less effective in inhibiting the platelet aggregation when administered along with PPI's in patients undergoing cardiac stent, ST segment elevated Myocardial infarction (STEMI) followed by percutaneous coronary intervention (PCI). Clopidogrel binds to CYP2C19, a hepatic enzyme to get converted to its active metabolite in order to achieve desired pharmacological activity. The cytochrome P450 3A4 which is partially involved in the metabolism of clopidogrel also metabolizes statins, mainly atorvastatin to the greater extent.
Methodology: In the current study patients on PPI's with dual antiplatelet therapy and patients on PPI's and statins with dual antiplatelet therapy are considered to understand the potential drug–drug interactions (pDDI) among the South Asian population. Platelet aggregation was measured in 61 patients undergoing coronary artery stent implantation treated with clopidogrel and aspirin along with PPI's and statins.
Results: It was observed that omeprazole and atorvastatin, but not pantoprazole and rosuvastatin, inhibited the antiplatelet activity of clopidogrel. The percent platelet aggregation was 72 ± 6 ($p = 0.001$) and 43 ± 23 ($p = 0.027$) in the presence of clopidogrel with omeprazole and pantoprazole respectively. Aggregation was found to be 91 ± 4 ($p = 0.001$) and 12 ± 23 ($p = 0.031$) in the presence of clopidogrel with atorvastatin and rosuvastatin respectively.
Conclusion: A prominent drug–drug interaction was observed with patients on dual antiplatelet therapy along with omeprazole and atorvastatin.

1. Introduction

Clinical studies have indicated a potential drug–drug interaction (pDDI) between dual antiplatelets (clopidogrel and aspirin) and clinically prescribed PPI's like omeprazole, pantoprazole, esomeprazole [1] and statins such as atorvastatin and rosuvastatin. Health care in tertiary system consider drug–drug interactions as the major concern due to the alarming mortality rate associated with these interactions. The competitive binding of several classes of drugs to a single metabolic enzyme, cytochrome P450 which are most likely to be present in the liver and other hepatic tissues, leading to drug–drug interaction. These cytochrome P450 enzymes alter the pharmacology of one drug due to competitive binding of the other drug [2], thus leading to major pharmacokinetic interaction.

Drug–drug interactions have become an important issue in health care. It is now realized that many drug–drug interactions can be explained by alterations in the metabolic enzymes that are present in the liver and other extra-hepatic tissues. Many of the major pharmacokinetic interactions between drugs are due to hepatic cytochrome P450 (P450 or CYP) enzymes being affected by previous administration of other drugs. After coadministration, some drugs act as potent enzyme inducers, whereas others are inhibitors. However, reports of enzyme inhibition are very much more common. Understanding these mechanisms of enzyme inhibition or induction is extremely important in order to give appropriate multiple-drug therapies. In the future, it may help to identify individuals at greatest risk of drug interactions and adverse events [2].

Clopidogrel belongs to a thienopyridine class of drugs which inhibits platelet aggregation in the patients undergoing percutaneous coronary intervention and also reduces coronary stent thrombosis and myocardial infarction [3–5]. Clopidogrel converts to its active metabolite by

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forming a disulfide bridge with adenosine diphosphate (ADP) receptor and exhibits antiplatelet effect [6–9]. In animals, especially rats it's found that cytochrome P450 1A2 is responsible for the activation of clopidogrel [7], whereas in humans the activation of clopidogrel is mostly by the cytochrome P450 2C19 and partly by 3A4 [9].

Patients receiving antiplatelet therapy are most commonly prescribed with PPI's to reduce the gastrointestinal bleeding [10]. Cardiovascular events, stroke, myocardial infarction and mortality continue to occur in patients with vascular diseases because of competitive binding of the ADP receptor blocker clopidogrel and PPI's to the isoenzymes 2C19 [11,12]. Patients with acute coronary syndrome are most likely to have elevated levels of cholesterol which makes it necessary for statin therapy. Thus evaluation of each drug concentration in a poly prescription becomes atmost important.

The American College of Cardiology/American Heart Association 2007 Unstable Angina/Non-ST-Segment Elevation Myocardial Infarction guidelines recommend concomitant PPI therapy with aspirin and clopidogrel in patients with a history of gastrointestinal bleeding [13]. Consequently, the number of patients affected by a PPI–clopidogrel interaction could be substantial. In fact, a combined total of 100 million prescriptions are written for both PPIs and clopidogrel annually [14]. However, this does not include all omeprazole use since, at some strengths, it is available over-the-counter. It has been hypothesized that PPI use concurrently with clopidogrel will increase the risk of major adverse cardiac events [15].

Different isoforms of cytochrome P450 (CYP) metabolized different types of substrates (or drugs molecule) and make them soluble during biotransformation. Therefore, fate of any drug molecule depends on how they are treated or metabolized by CYP isoform. There is a need to develop models for predicting substrate specificity of major isoforms of P450, in order to understand whether a given drug will be metabolized or not. *In-silico* method for predicting the metabolizing capability of major isoforms (e.g. CYP 3A4, 2D6, 1A2, 2C9 and 2C19) has been explained [16].

2. Material and methodology

In the current study, patients with acute coronary syndrome and other cardiovascular diseases followed by stent implantation and percutaneous coronary intervention, where prospectively evaluated for platelet aggregation studies. Patients with prescription of clopidogrel and aspirin along with PPI's and statins were under consideration.

The institutional review board approved the protocol, and a written informed consent was signed by the patient/patient care taker, before commencing the study.

i. **Study site:** This study was conducted in JSS College of Pharmacy and Department of Cardiology, JSS Medical College and Hospital, Mysore.
ii. **Study design:** This was a prospective bioanalytical study.
iii. **Study period:** The study was conducted over a period of 19 months, from the month of June 2014 to December 2015.
iv. **Study subjects:** The study subjects were enrolled into the study based on the study criteria
v. **Study criteria**

a. **Inclusion criteria**

1. Male or female between the ages of 40 to 60 years, inclusive who are admitted in the hospital (in-patients)
2. Females must have negative results for pregnancy tests performed: at Screening on a urine specimen obtained within 2 weeks prior to initial study drug administration.
3. Body Mass Index (BMI) is 19 to 26, inclusive. BMI is calculated as weight in kg divided by the square of height measured in meters.
4. A condition of MI, stroke, heart attack etc. with percutaneous coronary intervention admitted in the cardiology/other department in the hospital.
5. Patients receiving the above said medications
6. Must voluntarily sign and date each informed consent, prior to the initiation of any screening or study-specific procedures.

b. **Exclusion criteria**

1. History of significant sensitivity to any drug.
2. Requirement for any over-the-counter and/or prescription medication other than above mentioned, vitamins and/or herbal supplements, on a regular basis.
3. Use of any medications (other than OTC/prescription), vitamins and/or herbal supplements, within the 1-week period prior to study drug administration.
4. Recent (6-month) history of drug or alcohol abuse.
5. Use of known inhibitors (e.g., ketoconazole) or inducers (e.g., carbamazepine) of cytochrome P450 3A (CYP3A) within 1 month prior to study drug administration.

vi. **Investigation and study protocols**

The institutional review board approved the study protocols and written informed consent was obtained from each subject before enrollment. In the study, 61 patients undergoing successful elective coronary artery stent implantation received an oral loading dose of 300 mg of clopidogrel (PLAVIX™) followed by 75 mg/day for 28 days.

Subjects enrolled for the study had undergone primary percutaneous coronary intervention followed by stent implantation, a standard diagnostic treatment. All patients were enrolled and studied prospectively. All the subjects received 300 mg of aspirin (NUSPRIN™) on admission and 200 mg/day thereafter throughout the study period.

Platelet aggregation activity was tested in blood samples withdrawn in the pathology laboratory 60 ± 5 min after administration of chewable aspirin (baseline).

Eight patients were on PPI's alone, among them five were prescribed with 40 mg of omeprazole (PRIOSEC™) twice daily and three were on pantoprazole (PANTODAC™) 40 mg twice daily. Seventeen patients were on dual antiplatelet therapy along with PPI's.

Out of eleven patients on statin therapy, six were taking 40 mg of atorvastatin (AVAS™) a day, and five were taking either 40 mg (n 5) of rosuvastatin (CRESTOR™) once daily. Platelet aggregation was measured before clopidogrel administration and 24 h later. Platelet aggregation measurements were repeated in ten patients on clopidogrel and aspirin alone and fifteen patients on clopidogrel plus PPI's and statins 24 days after successful stent implantation.

Subjects were divided into 5 quartiles depending on the patients who were on prescribed medications. Platelet aggregation induced by the ADP was measured at 24 h compared with the baseline at 0 h after the administration of clopidogrel loading dose was measured. Percentage aggregation was presented were presented categorically in the 5 quartiles. Mantel–Haenszel [2] analysis was used to test the linear trend. Patients of first quartile were compared with second till fifth quartiles using a 2-tailed Fisher's test. Variables were presented as mean ± SD.

Baseline demography and clinical characteristics of patients on dual antiplatelet therapy, PPI's and statins individually and in combinations are mentioned in Table 1.

Table 1
Baseline demographic and clinical characteristics of patients on dual antiplatelet therapy, PPI and statins individually.

	Total Study Population	Quartiles								
		1st	2nd		3rd		4th		5th	
		Patients on CLP & ASP	Patients on PPI's		Patients on statins		Patients on CLP & ASP with PPI		Patients on clopidogrel, aspirin, PPI and statins	
	(n 61)	(n 10)	(n 8)		(n 11)		(n 17)		(n 15)	
			OME (n 5)	PAN (n 3)	ATR (n 6)	ROS (n 5)	CLP & ASP with OME (n 9)	CLP & ASP with PAN (n 8)	CLP & ASP with OME & ROS (n 4)	CLP & ASP with OME & ATR (n 6)	CLP & ASP with PAN & ATR (n 3)	CLP & ASP with PAN & ROS (n 2)
Age, years	55 ± 19	48 ± 16	50 ± 15	45 ± 3	55 ± 10	60 ± 9	50 ± 14	60 ± 12	45 ± 7	65 ± 9	54 ± 8	48 ± 10
Male, n (%)	46 (75)	9 (90)	4 (80)	3 (100)	5 (83)	3 (60)	6 (67)	6 (75)	4 (100)	2 (33)	2 (67)	2 (100)
Body mass index (kg/m^2)	–	25.9 ± 3.8	25.1 ± 3.4	28 ± 2.9	24.6 ± 2.3	23.1 ± 3.3	25.3 ± 3.2	27 ± 2.1	24 ± 1	22 ± 3.3	21 ± 4.3	27 ± 2.2
Risk factors for CAD, n (%)												
Cigarette smoking	39 (31)	7 (70)	3 (60)	3 (100)	4 (67)	2 (40)	6 (67)	5 (63)	2 (50)	4 (67)	3 (100)	0 (0)
Hypertension	35 (28)	6 (60)	1 (20)	1 (33)	5 (83)	4 (80)	5 (56)	6 (75)	2 (50)	3 (50)	1 (33)	1
Diabetes mellitus	13 (8)	0 (0)	2 (40)	0 (0)	2 (33)	1 (20)	2 (22)	3 (38)	0 (0)	2 (33)	1 (33)	0
Dyslipidemia	33 (25)	5 (50)	2 (40)	1 (33)	4 (67)	3 (60)	5 (56)	3 (38)	1 (25)	5 (83)	3 (100)	2 (100)
Family history of MI	21 (34)	8 (80)	3 (60)	2 (67)	3 (50)	5 (100)	7 (78)	5 (63)	3 (75)	4 (67)	1 (33)	1 (50)
Initial clinical characteristics, n (%)												
Previous MI	18 (30)	5 (50)	4 (50)	1 (33)	5 (83)	3 (60)	4 (44)	4 (50)	0 (0)	1 (17)	0 (0)	1 (50)
Anterior infarction	18 (30)	6 (60)	3 (37.5)	2 (67)	6 (100)	1 (20)	6 (67)	2 (25)	1 (25)	3 (50)	0 (0)	0 (0)
In-hospital medical therapy, n (%)												
Statins	11 (18)	0 (0)	0 (0)	0 (0)	6 (100)	5 (100)	0 (0)	0 (0)	4 (100)	6 (100)	3 (100)	2 (100)
Antidiabetic	7 (11)	0 (0)	2 (40)	0 (0)	2 (33)	1 (20)	2 (22)	3 (38)	0	2 (33)	1 (33)	0
β-Blockers	14 (23)	7 (70)	1 (20)	1 (33)	1 (17)	0 (0)	1 (11)	0 (0)	1 (25)	1 (17)	0 (0)	0(0)

3. Evaluation of platelet aggregation

3.1. Preparation of Platelet rich plasma (PRP)

Platelet-rich plasma was prepared by centrifugation of 5 mol/L of ADP and was measured with a dual channel aggregometer (model 440, Chronolog, Havertown, PA). Chart recordings were monitored on a Kipp-Zone Chart Recorder (Fisher Scientific, Chicago, IL).

The whole blood collected from the patients was anticoagulated with sodium citrate (sodium citrate/whole blood ratio, 1:10) and centrifuged at 10,000 rpm for 10 min at room temperature for preparation of platelet rich plasma (PRP). The prepared PRP was incubated for 30 min at 37 °C in capped tubes with ^{14}C-serotonin. The cloudy yellow supernatant containing the platelets was separated without disturbing the WBC and RBC cell layers. Platelet-poor plasma (PPP) was prepared by centrifuging the remaining sample at 10,000 rpm for 20 min at room temperature [23]. Platelet function testing was completed within 3 h after blood collection. The PRP was adjusted to a platelet count of 200 to 350 × 10^3/μL (200–350 × 10^9/L) after addition of PPP. Clinical samples should be assayed with similar platelet counts in the normal range. Platelet counts less than 100 × 10^3/μL (100 × 10^9/L) are not optimal for these functional tests.

3.2. Evaluation

Platelet aggregation was determined by measuring the change in the optical density (i.e., light transmittance) of stirred PRP after addition of the aggregating agent to the aggregometer cuvette. Platelet aggregation occurs only if the PRP in the aggregometer cuvette is stirred, usually at the rate of 800 to 1200 rpm. A Teflon-coated magnetic stirrer was used. The aggregometer was standardized by placing the patient's PPP sample in one channel to represent 100% light transmittance and the patient's PRP sample in another channel representing 0% transmittance. The increase in light transmittance from 0% to 100% is reflected on the chart recorder as the aggregometer tracing. Usually, the baseline of the patient's PRP was adjusted to be at the 10% chart deflection level, and the patient's PPP baseline was adjusted to the 90% level on the chart recorder. The light transmittance of the PRP relative to the PPP blank was recorded automatically. When an aggregating agent is added to the PRP, platelet aggregates form, and this event results in an increase in the light transmittance, which is recorded and used as an index of platelet aggregation.

3.3. Calculations of platelet aggregation

The percentage of aggregation was determined as the percentage of chart deflection between 10% and 90% at its highest average point of deflection on the chart recorder as follows:

$$\text{Percentage of Aggregation} = (\text{Highest Average Point of Deflection} - 20)/80 \times 100$$

4. Statistical analysis

Platelet activity was expressed as a percentage of baseline value. Each patient served as his or her own control, and changes in platelet activity were evaluated with paired *t*-test.

5. Results

Of the 61 patients enrolled, 46 (75%) were male, mean age was 55 ± 19 years, and mean time from symptom onset to admission was 2.8 ± 2 h.

5.1. Evaluation of platelet aggregation

Platelet aggregation studies showed a decrease in the percentage aggregation after 24 h in all the seven combination of patient plasma samples. The percentage aggregation is shown in Table 2.

(B) The dual antiplatelets activity of clopidogrel and aspirin at 0 h (dotted columns) and at 24 h (line columns) in patients treated with or without PPI's and statins in different combinations. (C) Platelet aggregation at 0 h (dotted columns) and at 7 h (line columns) after clopidogrel and aspirin administration in patients with or without statins (40 mg).

5.2. Effect of dual antiplatelet therapy

On the day of study, ADP-induced percentage platelet aggregation of plasma collected after 7 ± 0.5 h of clopidogrel loading were inhibited to 30% of baseline 0 h (P 0.001) in the first quartile. Whereas platelet aggregation were inhibited only to 77% in patients on dual antiplatelets along with PPI's and statins. The effect of dual antiplatelet therapy was hindered in presence of omeprazole and atorvastatin when compared to pantoprazole and rosuvastatin.

5.3. Platelet aggregation effect

Subjects were divided into 5 quartiles and the ADP-induced platelet aggregation after 24 h was compared with that of 0 h baseline activity of each patient. Patients in the first quartile were not resistant to clopidogrel (30 ± 6% of baseline platelet aggregation). Whereas not much significant reduction in platelet aggregation in patients in the second quartile till fourth quartile (Table 2), to 72 ± 4%, 62 ± 3%, and 55 ± 9% of the respective baselines ($P < 0.005$ for all, Fig. 1a). Inhibition in platelet aggregation decreased significantly in the first quartile (P for trend 0.01). Patients on dual antiplatelet therapy with omeprazole and atorvastatin showed no significant reduction in the platelet aggregation, 77 ± 8% to baseline 96 ± 4% (P = ns). Platelet aggregation reduced considerably to 70 ± 2% to the baseline 0 h ($P < 0.005$) in patients with dual antiplatelets, pantoprazole and rosuvastatin.

5.4. Clinical conclusion

The number of cigarette smokers decreased significantly and gradually from the first through fifth quartile (Table 1), but otherwise no significant differences were found in the other baseline demographic, clinical characteristics and angiographic findings (Table 1). Also it was found that the other demographic parameters didn't differ, such as prescribed medications during hospital stay and in discharge summary (Table 1).

All the 61 patients in the investigation were monitored with a continuous clinical update. During the period four patients developed recurrent STEMI, one patient suffered from peripheral arterial occlusion, requiring immediate surgery. Two patients were reported with stent thrombosis followed by MI. Therefore, seven patients had repeated cardiovascular events, five of which occurred during treatment and two patients during the clinical follow-up. Among the seven patients, four patients resistant to clopidogrel (fourth quartile) and three were from the fifth quartile. In variance, major bleeding occurred in one of the patient from fourth quartile.

Patients were senior citizens with repeated cardiovascular events (P 0.008), and had a lower percentage reduction of platelet aggregation which persisted upto 24 h (90 ± 16% versus 72 ± 7%, P 0.001, Fig. 1).

Table 2
Platelet aggregation of patient plasma samples.

Sl. no.	Drug	No. of patients	Percentage aggregation (%)	
			0 h	24 h
1.	CLP & ASP	10	96	30
2.	CLP & ASP with OME	9	92	72
3.	CLP & ASP with PAN	8	94	62
4.	CLP & ASP with OME & ROS	4	90	55
5.	CLP & ASP with OME & ATR	6	96	77
6.	CLP & ASP with PAN & ATR	3	91	70
7.	CLP & ASP with PAN & ROS	2	92	56

Note: Average % aggregation in each group in ± SD.

6. Discussion

The overall goal of drug-metabolizing CYP enzymes is the conversion of lipophilic drugs into more hydrophilic compounds to facilitate elimination by the kidney. This study showed that clopidogrel is less effective in inhibiting platelet aggregation when co administered with omeprazole, a CYP2C19 inhibitor and atorvastatin, a CYP3A4 substrate. In contrast, when clopidogrel was administered with pantoprazole and rosuvastatin, a hydrophilic drug not metabolized by the CYP system, platelet aggregation inhibition was not altered. Furthermore, in vivo studies demonstrated that clopidogrel is converted to its active form mainly by CYP2C19 and partly metabolized by CYP3A4, the most prominently expressed CYP in the human liver. We conclude that omeprazole and atorvastatin, at doses routinely administered to patients, inhibits CYP450 isoenzymes activity in a dose-dependent manner, and thereby decreases the metabolic conversion of clopidogrel to its pharmacologically active form.

Individual patient care is atmost necessary because of many drugs competing to get metabolized by CYP3A4, making it very important to understand the efficacy of clopidogrel in presence of other medications. Platelet function testing is absolutely necessary to identify the aspirin resistant patients. Patients suffering from acute coronary syndromes [4], ST segment elevated MI and those on percutaneous coronary intervention; clopidogrel is prescribed for more than 3 months. In such cases determining potential drug–drug interaction with clopidogrel and other medications becomes extremely important.

Clopidogrel, after an oral dose of 75 mg, gets hydrolyzed from its methyl ester to inactive carboxylic acid derivative, which represents almost 85% of component related to clopidogrel in plasma. Therefore only remaining around 15% of the drug moiety is available for metabolism to its active form. Therefore the level of active clopidogrel is less than 10–15 folds as that of inactive carboxylic acid form.

Atorvastatin, a hydroxy acid is not responsible for the inhibition of CYP3A4, whereas its lactone form, which is more lipophilic in nature than the acid form competitively, inhibits the clopidogrel metabolism through CYP3A4. Atorvastatin acid has been responsible for 75% of HMG-CoA reductase activity. Atorvastatin lactone binds majorly to CYP3A4 than any other substrate and causes drug -drug interaction [19].

Since the rate of competitive inhibition depends on the concentration and relative affinity between two substrates to the binding site of CYP2C19 and CYP3A4. If present in equivalent concentration, omeprazole and atorvastatin are potent inhibitors of most of the substrates [19]. Clopidogrel mainly depends upon CYP2C19 to get converted to its active moiety, but partially binds to CYP3A4 for metabolism. Omeprazole inhibits CYP2C19 whereas atorvastatin inhibits CYP3A4, thus reducing the antiplatelet action of clopidogrel.

Atorvastatin binds CYP3A4 thirty times more tightly than any other substrates which are found in lower concentration. Atorvastatin inhibits the metabolism of clopidogrel because of a very low concentration of clopidogrel that binds to CYP3A4 than atorvastatin itself.

Since clopidogrel is an ADP receptor antagonist, a device which allows to measure platelet aggregation in which ADP is used to enhance platelet aggregation was required. Common turbidimetric aggregometers are dependent on analyst differences and also gives indirect results due to citrated platelet rich plasma containing various blood components. Another instrument which measures activated platelets by thrombin activating peptide to agglomerate fibrinogen beads [23],

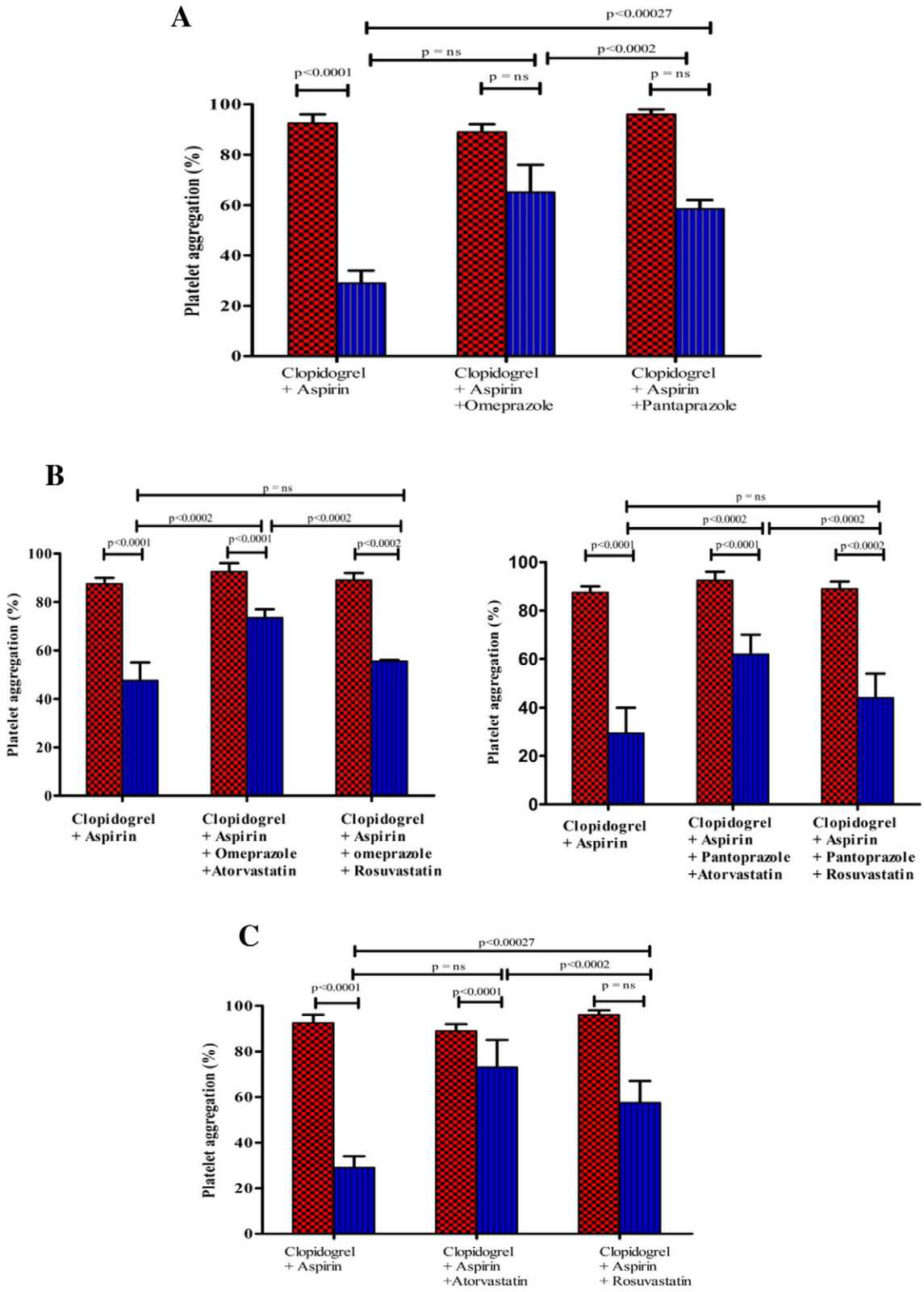

Fig. 1. The in vivo effect of PPI's and statins on the dual antiplatelets activity of clopidogrel and aspirin. (A) Platelet aggregation at 0 h (dotted columns) and at 24 h (line columns) after clopidogrel and aspirin administration in patients treated without or with PPIs (40 mg).

which provides automated, fast and reproducible results with small sample quantity was used in this study [11].

Cytochrome P450 isoenzyme, mostly 2C19 and partly 3A4 are responsible to convert clopidogrel prodrug to its active form. CYP3A4 is triggered mainly by polycyclic aromatic hydrocarbons which are present in cigarette smoke [13]. Lau et al. [25], explains about the varying clopidogrel response with the metabolism of cytochrome P450 enzyme. Various other factors like receptor signal pathway and ADP receptor polymorphism also varies the platelet aggregation response by clopidogrel.

In the current study, having a small sample size comparatively, provides an observatory but not confirmatory proof to conclude that the clopidogrel resistance is mainly because of the competitive binding to the enzyme CYP2C19 and CYP3A4. Also increase in the loading dose of clopidogrel may significantly reduce the percentage of recurrent cardiovascular cases. However resistance to clopidogrel was observed in STEMI patients.

7. Conclusion

Omeprazole showed considerably more clopidogrel resistance after eliminating the effects of interindividual variability in clopidogrel metabolism, compared to other PPI's and HMG-CoA reductase enhancer regimens.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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