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Research Article

ASSESSMENT OF ADVERSE EFFECT OF ATORVASTATIN WITH PLATELET $P2Y_{12}$-ADP RECEPTOR ANTAGONIST ON PLATELETS AGGREGATION AND RENAL FUNCTION IN CORONARY HEART DISEASE TREATED PATIENTS

Najat Abdulrazzaq Hasan[1]*, Mohammed Hasan Al Baghdadi[2] and Feryal Hashim Rada[1]

[1]Department of chemistry and biochemistry, College of Medicine, Al-Nahrain University PO box 14222, College of Medicine, Al-khademiya, Baghdad, Iraq.

[2]Ibn- Albitar Center for Cardiac Surgery, Ministry of Health.

ABSTRACT

Clopidogrel is a prodrug that is activated by the cytochrome P450-3A4 enzyme system. The active metabolite binds to the platelet $P2Y_{12}$- ADP receptor thereby inhibiting platelet aggregation. This study was done to evaluate the possible interactions of co-administration of clopidogrel with atorvastatin on antiplatelet potency of clopidogrel and on renal function in patients with coronary disease. Eighty patients with a coronary disease were included in this study. All patients received a dose of 75 mg/day of clopidogrel . Forty of them (group A) presented with recent treatment (<3 months) of clopidogrel , other forty patients (group B) presented with treatment (>1 years) of clopidogrel .The ADP-induced platelet aggregation, serum level of parent clopidogrel bisulfate and renal function tests were measured and studied at baseline (clopidogrel without atorvastatin) and at 2, 4, 6 weeks of clopidogrel with atorvastatin.The statistical results of Maximal Platelet aggregation % (MPA %) sought insignificant changes ($P > 0.05$) with atorvastatin co-treatment. The HPLC - analyses of serum parent clopidogrel bisulfate concentration showed insignificant changes ($P > 0.05$) with co-therapy. The Renal function tests at co therapy follow up intervals confirmed a significant decrease ($P< 0.005$) in mean serum levels of urea, cystatin c, and creatinine. Co-administration of atorvastatin does not affect the antiplatelet efficacy of clopidogrel at treatment timeline study, and it may have protective role for kidneys against the adverse effects induced by coronary angiogram diagnostic agent.

Keywords: Clopidogrel; Atorvastatin; Platelet and Renal function.

1. INTRODUCTION

Clopidogrel is thienopyridine antiplatelet agents that inhibit adenosine-5'diphosphate (ADP) – induced platelet aggregation[1] Asprodrugs, thienopyridines require metabolic conversion to the pharmacologically active compounds. These active metabolites contain a thiol group that forms an irreversible disulfide bond with cysteine(s) on platelet P2Y12 -ADP receptor[2] leading to inhibition of platelet activation and aggregation, once exposed, platelets are affected for their life span of approximately 7–10 days[3].

Dual antiplatelet therapy with aspirin and clopidogrel are standard of care for the treatment or prevention of ischemic events in individuals with acute coronary syndromes who are undergoing percutaneous coronary intervention[4] formation of clopidogrel's active metabolite requires two sequential CYP-mediated enzymatic steps[5.] HMG-Co A reductase inhibitors (statins) are lipid-lowering agents that are widely used in medical practice to lower plasma levels of low-density lipoprotein cholesterol. It is generally accepted that mechanisms beyond the reduction of plasma cholesterol contribute significantly to the anti atherogenic and tissue protective properties of statins[6.] Cholesterol-independent effects of statins include indirect anti-inflammatory effects such as activation of reduction-oxidation reaction-sensitive transcription factors[7.] Statins also protect tissues from various types of insults such as ischemia-reperfusion injury in the kidney and heart[8.]

1.1 Patients and Methods

Eighty patients, (30 female, 50 male), their ages ranging from (50-75) year, with coronary heart diseases that include acute myocardial infarction (AMI) on medical treatments, AMI on interventional therapy (cardiac catheterization and stenting) ,stable angina on medical treatments and unstable angina , recruited from IbnAlbitar Center for Cardiac Surgery.

Diagnoses are made based on clinical symptoms and tests like Electrocardiogram (ECG), Echocardiogram stress test and Coronary angiography. Patients with liver disease, renal failure, and heart failure have been excluded .According to the duration of clopidogrel (75mg /day) treatment, forty of them (group A) presented with recent treatment(<3 months), other forty patients (group B) presented with (> 1 years) treatment. Starting therapy with single daily dose of Clopidogrel 75mg followed by the addition of Atorvastatin 40mg once daily . Blood samples are aspirated on 2 week period starting from day-0 (Baseline), day-15,day-30 and day-45 to measure serum parent clopidogrel bisulfate level by HPLC analysis, platelet aggregation function was assay by measuring the inhibition of ADP-induced platelet aggregation of platelet-rich plasma (PRP), quantified using aggregometry, renal function tests that involve measuring serum levels of creatinine, urea, total Proteins by Photometric Colorimetric Test and serum levels of cystatin C (Cys C) that assayed by the quantitative sandwich enzyme immunoassay technique (ELISA).

1.1.1 Result

Baseline characteristics of 80 patients were taken showed in Table 1-. the platelet responsiveness to ADP stimulation, Maximal platelet aggregation (MPA%) at Baseline (clopidogrel only) and at day-15, day-30 and day-45 of clopidogrel with atorvastatin were non-significantly difference in the two groups ($P>0.05$) compared with the baseline mean, as well as insignificant difference ($P>0.05$) were appeared between group A and B at each follow up time intervals, Table 2, Figure 1.

The results of HPLC-analysis revealed that the mean levels of serum parent clopidogrel bisulfate concentration in group A and B at day-15, 30 and 45 were insignificantly change(P

> 0.05) compared with the baseline mean,Table 2 . The Results of renal function tests tended to show significant increase (P< 0.05) in mean serum levels of urea, creatinine, cystatin c at the baseline time for group A and insignificant change (P > 0.05) after co-treatment intervals. Whereas the results appeared within normal range in mean serum levels of total protein, Table 3.

The estimating glomerular filtration rate (eGFR) was calculated using Larsson formula (Larsson *et al.,* 2004):

eGFR (ml /min) = 77.24 × [cystatinC (mg/L)]$^{-1.2623}$

1.1.2 DISCUSSION

The statistical results of Maximal Platelet aggregation % (MPA %) of this study Figure1, Table 2, suggested that co-administration of clopidogrel (75 mg/day) with atorvastatin (40 mg/day) does not significantly (P > 0.05) affect the antiplatelet efficacy of clopidogrel in patients with symptomatic coronary artery disease.

The statistical results of HPLC analyses of serum parent clopidogrel bisulfate concentration, showed non-significant changes with atorvastatin co-treatment at day-15, 30-and 45-intervals compared with the baseline mean, this result support the result of MPA % of combine therapy and its reflect that the metabolism of clopidogrel bisulfate is not significantly inhibited by atorvastatin co treatment at the follow up intervals of this study.

A number of concerns were raised regarding the possibility of specific deleterious interactions between clopidogrel and statins when studies provided controversial results that short-term therapy (5-24) hours with clopidogrel in conjunction with atorvastatin could attenuate the antiplatelet efficacy of clopidogrel, suggesting that the co-administration of CYP3A4-metabolized statins with clopidogrel may competitively inhibit metabolic activation of clopidogrel in the liver[11].

Lau *et al.,* 2003, used a novel point of-care method to study platelet micro aggregation and only 9 with pravastatin and 19 with atorvastatin-treated patients were studied, found that atorvastatin, but not pravastatin treatment was associated with a dose-dependent reduction of the antiplatelet activity of clopidogrel at 6–8 days after clopidogrel initiation[12.] However Serebruany *et al.,* 2004, showed that atorvastatin did not affect platelet biomarkers after clopidogrel administration, compared with other statins or no statin, within 24 hours in patients undergoing coronary stenting[13.]

Mitsios *et al*., 2004, showed that the therapeutic efficacy of clopidogrel in patients with acute coronary syndrome was not significantly affected by concomitant administration of atorvastatin for 5 weeks, and clopidogrel did not affect the therapeutic efficacy of atorvastatin[20] Farid *et al.,* 2008, concluded that co-administration of atorvastatin 80 mg/day with prasugrel or clopidogrel did not negatively affect the antiplatelet response to either drug after a loading dose or during maintenance dosing[10.]

Malmstrom*et al.,* 2009, who concluded that treatment with CYP3A4 metabolized statins (20,40, 80 mg/day), atorvastatin or simvastatin, did not attenuate the platelet inhibitory effect at 2 weeks of clopidogrel maintenance treatment compared with the non-CYP3A4 metabolized ,rosuvastatin[9.]

The elevated level of renal function parameters in group A that recently undergoing angiography and not in patients with stable angina on medication therapy were may be due to the used of diagnostic agents, in particular Iohexol ,an X-ray contrast medium for intravascular use .

Although thepathogenesis of Radio contrast nephropathy (RCN) induced with iohexol remainsincompletely understood, tubular hypoxic injury, due to a reduction of renal medullary blood flow, and direct tubular cytotoxicity play a substantial role[14.] The risk of

developing nephropathy after radiocontrast exposure may be as high as 50%, depending on numerous risk factors. Preexisting renal dysfunction and dehydration are the most predictive contributors to RCN, whereas volume of contrast exposure, contrast osmolality, congestive heart failure, diabetes, anemia, andadvanced age also increase risk[15].

The elevated levels of mean serum urea, in group A seen in patients undergoing angiography while in group B seen in diabetic patients and in patients with high protein diet. Increase in blood levels of urea, creatinine and cystatin c induced by diagnostic agent were decreased byatorvastatin co-therapy so treatment with atorvastatin provided marked functional protection for the kidney.

Recent studies demonstrated that atorvastatin has antioxidant effects and reduces the activity of Reactive Oxygen Species (ROS).The treatmentwith atorvastatin in arterial hypertensive patientsimprove lipid profile and decreases the oxidative stress bynormalizing the activity of enzymes catalase, superoxide dismutase (SOD), glutathione peroxidase (GSH-PX)andrestoring the normal level of glutathione (GSH) and malondialdehyde[16]. Atorvastatin acts in the kidney as a potent free radical scavenger and inhibits Mitogen ActivatedProtein Kinase (MAPK) and Nuclear Factor –$_K$B (NF-$_k$B) signaling pathways activated by ROS and thus prevent tubule cell apoptosisinduced by gentamycin [17].

The cardio protective effects of atorvastatin involved the up-regulation of cyclooxygenase(COX-2) and increased production of prostacyclin (PGI2) ,suggesting that in kidney cells, statin stimulates over expression of COX-2 ,resulting in increased PGI2[18] .The vasodilator effect of PGI2 on the afferent arterioles, allowingmore blood flow through the glomerulus and auto regulates intraglomerular pressure[19].

1.1.3 CONCLUSION

The anti-platelet activity of clopidogrel was maintained when co-administered with atorvastatin for 15, 30, 45 days in patients with coronary artery disease and stent implantation. Atorvastatin may have protective role for kidneys against the adverse effects induced by coronary angiogram diagnostic agent because treatment with atorvastatin effectively scavenged the free radicals and offered considerable protection to kidneys.

Table 1: Distribution of the patients' characteristics in the studied groups

	Group–A	Group-B	P-value
Number (n)	40	40	NS*
Mean age (year), ± SD (Range)	60 ± 15 (45–75)	63 ± 13 (50–75)	NS*
Female sex %	42.5 %	32.5 %	NS*
Male sex %	57.5 %	67.5 %	NS*
Hypertension %	75 %	80 %	NS*
Diabetes mellitus %	30 %	35 %	NS*
Hypercholesterolemia %	50 %	55 %	NS*
Stable angina %	25 %	25 %	NS*
Unstable angina %	25 %	25 %	NS*
NSTEMI %	30 %	30 %	NS*
STEMI %	20 %	20 %	NS*
Drug Eluting Stent (DES %)	67 %	70 %	NS*

NS* = Non-significant difference; P>0.05,

NSTEMI = Non-ST segment elevation myocardial infarction

STEMI = ST segment elevation myocardial infarction

Table 2: The changes in maximal platelet aggregation percent (MPA %) and serum levels of parent clopidogrel bisulfate after 45 min intake of oral 75 mg/tablet through 6 weeks treatment presented as mean ± SD for the studied groups

		Baseline (day-0)	day-15	day-30	day-45
1- Mean MPA %± SD	*-Group-A*	30 % ± 12	32.07 % ± 11	31.8 % ±11.4	30.5 % ± 11.6
	-P-value *vs. Baseline*	--------	NS*	NS*	NS*
	-Group-B	28 % ± 11.2	30.68 % ± 10	29.7 % ±10.3	28.64 % ± 10.2
	-P-value *vs. Baseline*	--------	NS*	NS*	NS*
2- MeanS.Clopidogrel bisulfate conc.(ng/ml)± SD	-Group-A	2.135 ± 0.5	2.315 ± 0.53	2.239 ± 0.45	2.17 ± 0.4
	-P-value *vs. Baseline*	---------	NS*	NS*	NS*
	-Group-B	2.146 ± 0.45	2.328 ± 0.55	2.268 ± 0.35	2.195 ± 0.42
	-P-value *vs. Baseline*	----------	NS*	NS*	NS*

NS* = Non-significant difference, P>0.05

Table 3: Differences in levels of serum renal function biomarkers through 6 weeks treatment presented as mean ± SD for the studied groups

		Baseline(B) (day-0)	Day-15	Day-30	Day-45
1-Mean S. Total Protein (gm/dl) ± SD	Group-A	7.03 ± 1.5	7.27 ± 1.15	7.2 ± 1.06	7.14 ± 1.08
	P-value *vs.*B	-----	NS*	NS*	NS*
	Group-B	7.06 ± 0.86	7.21 ± 1.2	7.1 ± 0.83	6.97 ± 1.15
	P-value *vs.* B	-------	NS*	NS*	NS*
2-Mean S. Urea (mg/dl)± SD	Group-A	43.03 ± 8.5	40.22 ± 10.7	38.5 ± 8.45	35.25 ± 9.3
	P-value *vs.* B	--------	NS*	P < 0.01▼	P < 0.001▼
	Group-B	44.5 ± 11.2	42.45 ± 9.32	40.33 ± 9.5	36.12 ± 9.4
	P-value *vs.*B	---------	NS*	P < 0.05▼	P < 0.001▼
3-Mean S. Creatinine (mg/dl) ± SD	Group-A	1.38 ± 0.5	1.31 ± 0.4	1.24 ± 0.35	1.15 ± 0.4
	P-value *vs.*B	--------	NS*	P < 0.05▼	P < 0.01▼
	Group-B	1.03 ± 0.45	1.2 ± 0.5	1.13 ± 0.42	1.05 ± 0.34
	P-value *vs.*B	---------	NS*	NS*	NS*
4-Mean S. Cystatin C (mg/L) ± SD	Group-A	1.02 ± 0.3	0.94 ± 0.25	0.9 ± 0.22	0.88 ± 0.2
	P-value *vs.*B	--------	NS*	P< 0.05▼	P< 0.01▼
	Group-B	0.79 ± 0.2	0.86 ± 0.23	0.83 ± 0.19	0.8 ± 0.23
	P-value *vs.*B	--------	NS*	NS*	NS*
5-Mean eGFR (ml/min)± SD	Group-A	80.33± 22.16	88.51± 21.4	92.23 ± 24.5	96.77 ± 22.3
	P-value *vs.*B	--------	NS*	P< 0.05▼	P< 0.01▼
	Group-B	104.01±25.5	96.44± 24.7	97.72± 22.37	102.37± 23.4
	P-value *vs.*B	--------	NS*	NS*	NS*

NS* = Non-significant difference (P>0.05); *vs.*=versus, n=patients number; For each group (n=40); Group-A =treatment < 3 months; Group-B= treatment > 1 year ;Baseline (day-0) for group A&B=clopidogrel (75mg/day) without atorvastatin treatment; Day 15,30,45 intervals=clopidogrel (75mg) +atorvastatin (40mg) treatment for groups A & B.

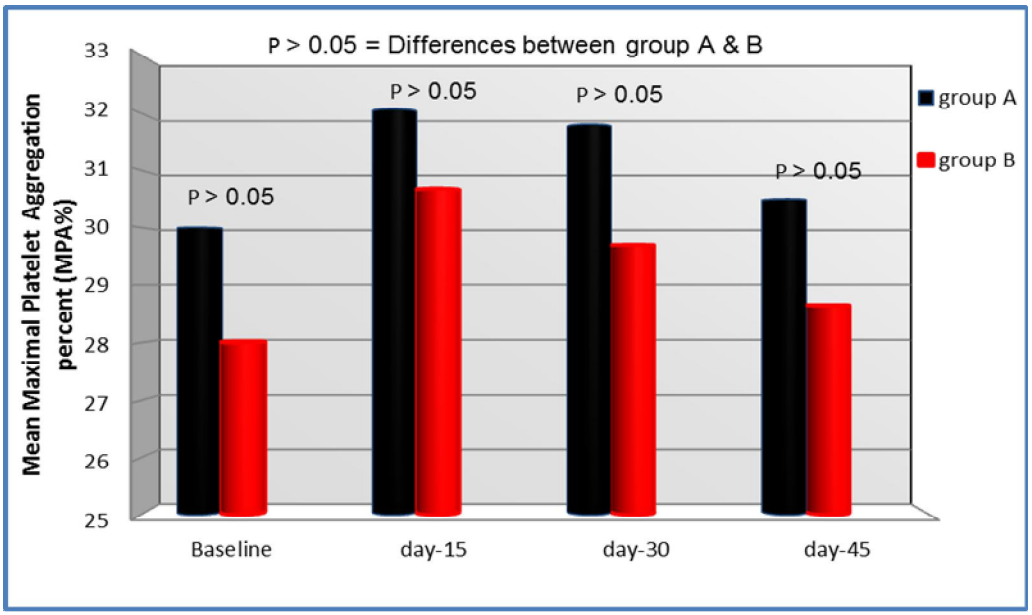

Fig. 1: The graphic demonstrate a non-significant effect of atorvastatin intake with clopidogrel for 6 weeks treatment on mean levels of maximal platelet aggregation percent (MPA %) for the studied groups

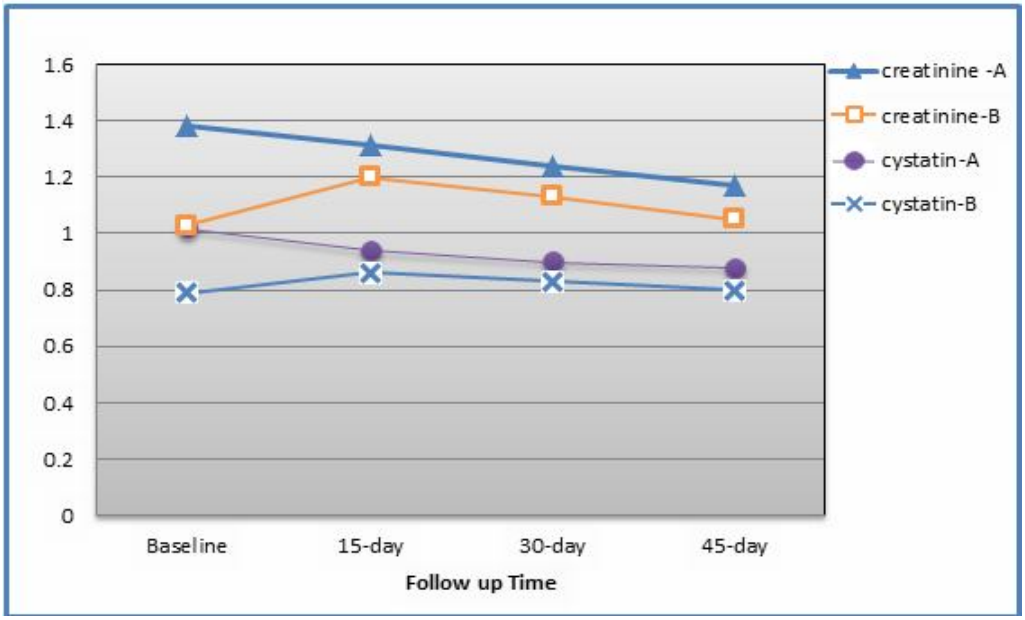

Fig. 2: The mean levels of serum Cystatin C and Creatinine for the studied groups

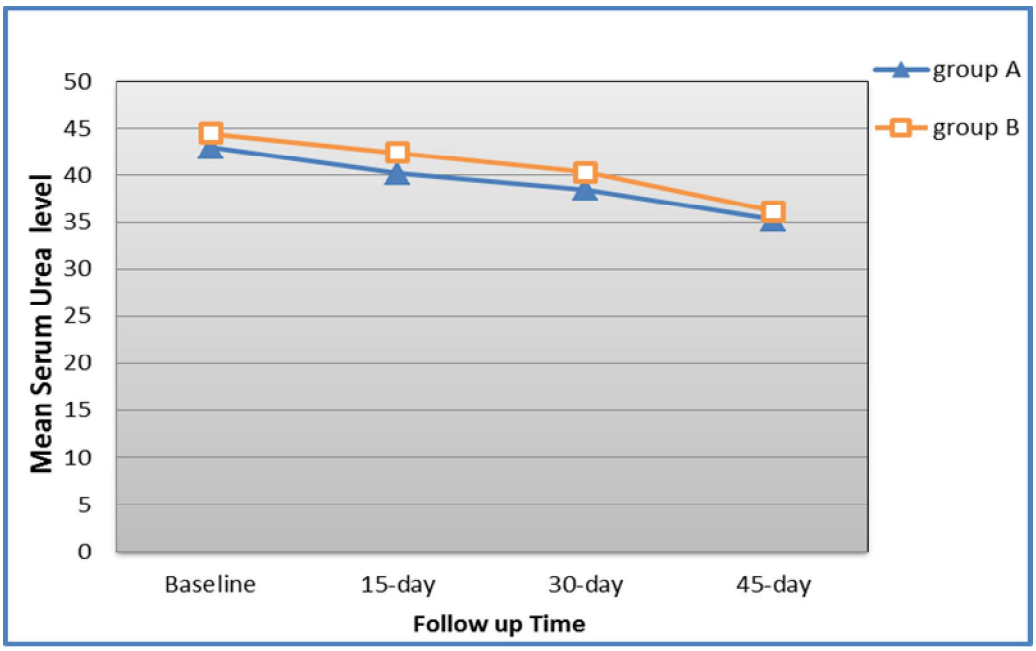

Fig. 3: The mean levels of serum Urea for the studied groups

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