ORIGINAL RESEARCH

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Effects of High Loading Dose of Clopidogrel in ST Elevated Myocardial Infarction Treated with Fibrinolytic Agent

Abstract

Purpose: The purpose of the present study was to evaluate the effects of different loading doses of clopidogrel on ST segment resolution on ECG, changes in cardiac enzyme levels and serum levels of high-sensitivity C-reactive protein (Hs-CRP) in patients with ST elevated myocardial infarction (STEMI) treated with fibrinolytic therapy.

Methods: Patients admitted to our cardiology clinic with a diagnosis of STEMI and treated with fibrinolytic therapy were included: Group 1 (n=58) received a 300 mg loading dose of clopidogrel, Group 2 (n=55) a 450 mg loading dose and Group 3 (n=59) a 600 mg loading dose. A 75 mg/d maintenance dose of clopidogrel was given in all groups.

Results: All demographic characteristics and baseline laboratory parameters were statistically similar among three groups ($p>0.05$). When ST resolution periods were compared, most patients in Group 3 had ST resolution at 30 minutes; Group 2 at 60 minutes and Group 1 at 90 minutes ($p<0.05$). Peak levels of creatine kinase (CK) and CK-MB were as follows: Group 3, 8th hour, Group 1 and 2, 12th hour. Peak levels of those enzymes were significantly lower in Group 3 than in Group 1 and 2 (<0.05). Although basal hs-CRP levels of all groups were similar, the increase in hs-CRP levels at 48 hours was lower with higher clopidogrel loading doses ($p<0.05$).

Conclusion: In this study comparing three different clopidogrel loading doses, the higher doses provided earlier ECG resolution, earlier and lower peak CK and CK-MB levels and lower levels of hs-CRP.

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Clopidogrel with 300 mg loading dose and followed by 75 mg maintenance dose is advised in ST elevated myocardial infarction (STEMI) patients who are under 75 years and treated with fibrinolytic therapy [1,2]. Effect of high dose clopidogrel loading in patients with STEMI treated with primary angioplasty was evaluated well previously and 600 mg loading dose was found to be more effective; however, effects of high loading doses of clopidogrel in STEMI patients treated with fibrinolytic therapy have not yet been studied thoroughly.

The aim of our study was to determine the effects of different additional loading doses of clopidogrel on ST-segment resolution on ECG, serum levels of cardiac enzymes and high-sensitivity C-reactive protein (Hs-CRP) in STEMI patients who received fibrinolytic therapy.

Methods

Patient Population

Patients who presented with ischemic discomfort lasting >20 minutes at rest within 12 hours with ST-segment elevation of at least 0.2 mV in at least two contiguous precordial leads were enrolled. The study was limited to only anteriorly located myocardial infarction to homogenize the investigated population. The exclusion criteria were conduction or rhythm abnormalities (bundle branch block, idioventricular rhythm, etc.), any contraindication to thrombolytics, early coronary angiography due to recurrent ischemia or failed thrombolysis, those on aspirin and/or thienopyridines treatments, any contraindication to aspirin or clopidogrel, past history of MI or coronary revascularization; presence of heart failure (Killip class II/III) or cardiogenic shock, hepatic failure, renal failure (serum creatinine >2.5 mg/dL), thrombocytopenia ($<100,000/\text{mm}^3$) and patients older than 70 years. Major bleeding was defined as intracranial bleeding or clinically overt bleeding associated with a decrease in hemoglobin of >5 g/dl according to the TIMI criteria [3]. The study protocol was approved by the institutional review board, and written informed consent was taken from all patients.

Study Protocol

A total of 180 patients were included initially in the study. The first 60 consecutive patients were allocated to Group 1, the second 60 consecutive patients were allocated to Group 2 and the last 60 consecutive patients were allocated to Group 3. Two patients in Group 1, five patients in Group 2 and one patient in Group 3 were excluded because of recurrent ischemia. Physicians performing the follow-up assessment were not aware of the group assignment. Standard protocol for reperfusion therapy

was a 300 mg loading dose of aspirin followed by 150 mg/day, unfractionated heparin 60 U/kg i.v. bolus (maximum 4000 U) followed by an infusion at 12 U/kg/h (maximum 1000 U/h), administration of tissue plasminogen activator (tPA) with 15 mg i.v. bolus followed by 0.75 mg/kg infusion during first 30 minutes and then 0.5 mg/kg infusion during 60 minutes (maximum 100 mg). In addition, Group 1 patients (n=55) received a loading dose of 300 mg clopidogrel and a maintenance dose of 75 mg/d clopidogrel, Group 2 (n=58) received a 450 mg loading dose and a 75 mg/d maintenance dose and Group 3 (n=59) received a 600 mg loading dose and a 75 mg/d maintenance dose. Different loading doses of clopidogrel were given at the time of randomization in the emergency department before sending patient to coronary care unit.

Blood Measurements

Blood samples were taken from left antecubital vein from all patients. All collected blood samples were analysed by OLUMPUS AU 600 and Bayer Advia 120 Cell CBC Counter Hematologia. Routine biochemical parameters (glucose, lipid profile, urea and creatinine) and complete blood counts (hemoglobin, hematocrit and leucocytes) were analyzed daily. Baseline and 48 hour hs-CRP levels were measured by a high sensitivity nephelometric method. Determined values were recorded separately for each patient.

ECG Analysis

Standard 12-lead electrocardiograms (ECG) were obtained at baseline and at 30 minute periods during the first 120 minutes after initiation of fibrinolytic therapy with an ECG recorder (Nihon Kohden, Tokyo, Japan). ST-segment elevation was analyzed manually with lens-intensified calipers to the nearest of 0.025 mV at 20 milliseconds after the end of QRS complex with the PR segment as the reference baseline from leads I, aVL, and V1 through V6 for anterior infarction. The percent resolution of ST deviation from baseline to 30, 60, 90 and 120 minutes was calculated. More than 50 % resolution in ST segment elevation was accepted as a successful criteria for reperfusion.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows version 12.0 (SPSS 12.0, Chicago, USA) software package program. Continuous variables were expressed as mean±standard deviation and categorical variables as percentage (%). Differences between study groups in baseline characteristics were assessed with the use of two-sided Fisher's exact tests and chi-

TABLE 1. Baseline demographic characteristics and laboratory findings of the study groups.

	Group 1 (n=58)	Group 2 (n=55)	Group 3 (n=59)	P value
Mean age (year)	58.3±9.7	60.1±9.6	58.7±9.8	NS
Male (n, %)	36 (62)	33 (60)	35 (59)	NS
Symptoms-to-lytic time (h)	2.6±1.3	2.8±1.5	2.7±1.6	NS
Family history (n, %)	23 (40%)	20 (36%)	23 (39%)	NS
Current smoker (n, %)	35 (60%)	35 (64%)	36 (61%)	NS
Hypertension (n, %)	32 (55%)	33 (60%)	36 (61%)	NS
Diabetes mellitus (n, %)	14 (24%)	11 (20%)	14 (24%)	NS
Body mass index (kg/m ²)	27.2±2.8	26.9±2.9	27.1±2.7	NS
Total cholesterol (mg/dL)	233.6±51	217.8±50.4	231±44.3	NS
HDL cholesterol (mg/dL)	49.4±8.2	49.9±6.9	51.7±7.7	NS
LDL cholesterol (mg/dL)	154.6±38.3	141.8±37.7	150.7±36.5	NS
Triglyceride (mg/dL)	173.4±66.4	157.6±49.2	158.5±39.3	NS
Urea (mg/dL)	35±10.1	36.9±11.9	32.9±7.8	NS
Glucose (mg/dL)	150.6± 60.2	153.8±64.9	152.3±68.1	NS
Hemoglobin (g/dL)	13.1±1.1	13.3±1.2	13.5±1.3	NS
Hematocrit (%)	40.7±3.6	41.7±4.4	41.4±3.9	NS
Leucocyte (K/uL)	12.9±1.4	10.5±1.4	12.3±1.3	NS
Creatine (mg/dL)	1±0.1	1±0.1	1±0.1	NS

square tests for categorical variables. One-way anova and post hoc Bonferroni test were used for continuous variables between study groups. Results were presented as 95% confidence intervals and statistical significance was accepted as p value less than 0.05.

Results

There was no statistically significant difference among all three groups with regards to demographic characteristics and baseline laboratory parameters ($p>0.05$) (Table I). CK and CK-MB levels at 0, 4 and 8 hour periods were similar in all groups ($p>0.05$). Peak levels of CK and CK-MB levels were reached at 8 hour in Group 3 while peak levels of cardiac enzymes were reached at 12 hour in Group 1 and Group 2. In addition, peak levels of cardiac enzymes were lower in Group 3 than in Group 1 and 2. There was a statistically significant difference between Group 1 and Group 3 in CK and CK-MB levels at 12, 16, 20

and 24 hours. While CK-MB levels were significantly different in Group 2 and 3 at 12, 16, 20 and 24 hours; CK levels were significantly different only at 12 and 16 hours ($p<0.05$) (Table 2).

The times required for complete resolution of ST-segment elevation were compared between groups: while ST resolution was completed at 30 minutes in more than half (58%) of Group 3 patients to whom 600 mg clopidogrel were given, it was completed in Group 2 at 60 minutes and Group 1 at 90 minutes. Consequently, there existed a significant difference between groups in terms of ST resolution periods ($p<0.05$) (Table 3). In other words, earliest ST resolution was achieved in Group 3 patients to whom a loading dose of 600 mg clopidogrel was given, then in Group 2 patients to whom a loading dose of 450 mg was given and lastly in Group 1 patients to whom a loading dose of 300 mg was given (Table 3).

TABLE 2. Comparison of the study groups according to CK and CK-MB levels between 0-24th hours.

CK-MB levels of groups between 0-24 th hours						
Hour	Group 1 (n=58)	Group 2 (n=55)	Group 3 (n=59)	Pa value	Pb value	Pc value
0.	27±16	31±16	33±17	NS	NS	NS
4.	114±105	128±92	95±55	NS	NS	NS
8.	171±71	173±84	147±84	NS	NS	NS
12.	184±122	185±107	122±67	NS	0.002	0.003
16.	130±50	121±55	89±44	NS	0.004	<0.001
20.	87±34	78±30	62±25	NS	0.025	<0.001
24.	51±21	46±14	29±13	NS	0.035	0.001
CK levels of groups between 0-24 th hours						
0.	218±125	270±161	282±130	NS	NS	NS
4.	1056±1133	1105±790	867±544	NS	NS	NS
8.	1758±1032	1739±905	1477±721	NS	NS	NS
12.	1848±946	1741±714	1264±635	NS	0.007	0.001
16.	1493±804	1291±552	944±461	NS	0.017	<0.001
20.	1032±535	878±365	693±351	NS	0.080	<0.001
24.	624±358	538±208	459±264	NS	NS	0.012

Pa: P value between Group 1 and Group 2

Pb: P value between Group 2 and Group 3

Pc: P value between Group 1 and Group 3

TABLE 3. Comparison of ST segment resolution periods between the study groups.

Target ST segment resolution	Group 1 (n=58)	Group 2 (n=55)	Group 3 (n=59)	P value
30 min (n, %)	10 (%17)	15 (%27)	38 (%64)	<0.001
60 min (n, %)	14 (%24)	32 (%58)	19 (%32)	<0.001
90 min (n, %)	34 (%59)	8 (%15)	2 (%3)	<0.001

Although baseline hs-CRP levels between groups were similar, a significant difference was found at 48 hours. As the loading dose of clopidogrel increased, the peak levels of hs-CRP decreased (dose-related inverse relationship) and this difference was statistically significant among all three groups ($p<0.001$) (Table 4).

Major hemorrhagic complication occurred in only one patient in Group 1 as a central nervous system hemorrhage.

TABLE 4. Comparison of the study groups according to Hs-CRP levels.

hs-CRP (mg/L)	Group 1 (n=58)	Group 2 (n=55)	Group 3 (n=59)	P value
Basal	7.2±3.4	6.9±3.7	7.0±3.6	NS
48 th hour	31.6±9.8	16.8±5.3	10.2±4.4	<0.001

One patient in both Group 1 and Group 2 had a small hematoma in the upper extremity and one patient in Group 3 had an epistaxis that was managed by local pressure.

Discussion

In this study, the effects of 450 mg and 600 mg clopidogrel loading doses were determined. These doses are above the

guidelines, which advise a 300 mg loading dose. The effects of high loading doses on ST resolution, changes in enzymes and serum levels of an inflammatory parameter such as hs-CRP were also investigated. According to our data, increased loading dose of clopidogrel provided earlier ST resolution in ECG, earlier and lower peak cardiac enzyme levels and lower serum hs-CRP levels.

In the COMMIT study, which included 45852 STEMI patients treated with fibrinolytics and anticoagulants, aspirin and aspirin plus clopidogrel were compared. In that study, a 9% decrease in primary end points and a 7% decrease in mortality rates were found in clopidogrel group at the end of one month [4]. There was no clopidogrel loading dose in the COMMIT study. In addition, short- and long-term beneficial effects of loading doses on reperfusion in acute coronary syndromes and reductions in reinfarctions and mortality rates were reported in studies evaluating clopidogrel loading doses. In the CLARITY-TIMI 28 trial, STEMI patients treated with fibrinolytic therapy were randomized to either a 300 mg loading dose plus 75 mg daily clopidogrel or a placebo. When the data was evaluated after 48-192 hours, the investigators reported that reinfarction, death rate, TIMI 0 and TIMI 1 rates were lower in clopidogrel group [5]. Also in the ECG CLARITY-TIMI 28 trial, it was shown that the rate of ST segment resolution was significantly higher in the group to which clopidogrel was given [6].

Previous studies investigated the effects of high doses of clopidogrel (i.e., 450 and 600 mg) in STEMI patients treated by primary angioplasty (PTCA) and they reported that higher doses had positive effects [7,8]. But studies evaluating the effects of higher doses of clopidogrel on STEMI treated with fibrinolytic therapy are limited. There are no other studies in literature comparing 300 mg loading dose of clopidogrel with higher loading doses.

Although previous studies reported beneficial effects of 450 and 600 mg clopidogrel loading doses on STEMI treated by primary PTCA, there was no additional benefit when loading dose was increased to 900 mg. For this reason, in our study, subjects were given 450 mg and 600 mg loading doses.

No group was evaluated for 900 mg loading dose for ethical reasons. In the ARMYDA-2 trial, it was reported that 600 mg clopidogrel loading dose resulted in an earlier and more potent inhibition of ADP-induced platelet activation than 300 mg loading dose without a significant side effect and hemorrhage in STEMI patients undergoing primary coronary artery stenting [7]. Similarly, in the ISAR-CHOICE study, antiplatelet and pharmacokinetic effects of different clopidogrel doses were compared and it was reported that 600 mg clopidogrel loading dose provided a higher plasma concentration and

more platelet inhibition than 300 mg loading dose, while increasing the loading dose to 900 mg did not make any difference [8].

Previous studies reported that STEMI patients with successful reperfusion had lower peak CK and CK-MB levels, shorter time to achieve peak enzymes values and, consequently, shorter time needed for the normalization of these cardiac enzymes when compared with unsuccessful reperfusion in STEMI [9,10]. In our study, peak CK and CK-MB levels were lower in Group 3 and time to achieve peak enzymes levels was shorter than for other groups.

It was thought that as the relation between atherosclerosis and inflammation was defined clearly, the inflammatory markers in circulation could help determining cardiovascular event risk. In comparison with other inflammatory markers, the role of CRP in determining the risk of cardiovascular disease stands out [11]. The results, which are numerous, broad, randomized and prospective in method, make us believe that basic hs-CRP levels can be used to determine future cardiovascular risk [12-14]. It has already been proven that hs-CRP levels have a prognostic value for patients with acute coronary syndrome, in the short as well as in the long term [15,16]. Clopidogrel, as a platelet ADP P2-Y12 receptor antagonist, was investigated in numerous studies for its anti-inflammatory properties in addition to its antithrombocyte effects. A study by Zairis *et al.*, which included 319 patients admitted with STEMI and then treated with thrombolytic therapy, reported that the group with higher CRP levels had a significantly lower incidence of ST-segment resolution and higher of in-hospital mortality rate [17].

Study Limitations

The most important limitations of our study are the small patient population and absence of a sophisticated randomization protocol. Although our patient population was large enough to detect the positive effects of clopidogrel, it was inadequate to detect possible hemorrhagic complications. Another limitation of our study is that coronary reperfusion after fibrinolytic therapy was not assessed with coronary angiography. Other limitations of our study include the limited (48 hour) follow-up period, the inclusion of patients presenting only with anterior STEMI, the use of only t-PA as thrombolytic therapy and absence of clinical endpoints.

Conclusion

In the present study, with three different loading doses of clopidogrel, the higher dose was found to provide more effi-

cient reperfusion and anti-inflammatory effects. Based on these results, the 600 mg loading dose of clopidogrel, which is the maximum dose used in our study, should yield more favorable results for both reperfusion and treatment of inflammation. Large scale studies with longer follow-up periods are needed to understand the efficiency of clopidogrel loading doses higher than 300 mg in STEMI patients treated with fibrinolytic therapy and our study is important to encourage these larger studies.

References

1. Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. *Nat Rev Drug Discov* 2003; 14: 201-28.
2. Cannon CP, Braunwald E. Unstable angina and non-ST elevation myocardial infarction. Zipes DP, Libby P, Bonow RO, Braunwald E (editors). *Braunwald's Heart Disease*. 7th ed. Philadelphia: Saunders, 2005: 1243-79.
3. Rao AK, Pratt C, Berke A, et al. Thrombolysis in Myocardial Infarction (TIMI) trial-phase I: hemorrhagic manifestations and changes in plasma fibrinogen and the fibrinolytic system in patients treated with recombinant tissue plasminogen activator and streptokinase. *J Am Coll Cardiol* 1988; 11: 1-11.
4. Chen ZM, Jiang LX, Chen YP, Xie JX, Pan HC, Peto R, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: randomised placebo-controlled trial (COMMIT). *Lancet* 2005; 366: 1607-21.
5. Marc S. Sabatine, Carolyn H. McCabe, C. Michael Gibson, Christopher P. Cannon Design and rationale of Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis in Myocardial Infarction (CLARITY-TIMI) 28 trial. *American Heart Journal* 2005; 149: 227-33.
6. Scirica BM, Sabatine MS, Morrow DA, Gibson CM, Murphy SA, Wiviott SD, et al. The Role of Clopidogrel in Early and Sustained Arterial Patency After Fibrinolysis for ST-Segment Elevation Myocardial Infarction The ECG CLARITY-TIMI 28 Study. *J Am Coll Cardiol* 2006; 48: 37-42.
7. Patti G, Colonna G, Pasceri V, Pepe LL, Montinaro A, Di Sciascio G. Randomized trial of high loading dose of clopidogrel for reduction of periprocedural myocardial infarction in patients undergoing coronary intervention: results from the antiplatelet therapy for reduction of myocardial damage during angioplasty (ARMYDA-2) study. *Circulation* 2005; 111: 2099-106.
8. Von Beckerath N, Taubert D, Pogatsa MG, Schomig E, Kastrati A, Schomig A. Absorption, metabolism, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel: results of the intracoronary stenting and antithrombotic regimen: Choose Between 3 High Oral Doses for Immediate Clopidogrel Effect (ISAR-CHOICE) Trial. *Circulation* 2005; 112: 2946-50.
9. Halkin A, Stone GW, Grines CL, Cox DA, Rutherford BD, Esente P, et al. Prognostic implications of creatine kinase elevation after primary percutaneous coronary intervention for acute myocardial infarction. *J Am Coll Cardiol* 2006; 5: 951-61.
10. Aksakal E, Sevimli S. The correlation between early period parameters with left ventricular ejection fraction at the end of the first month in acute myocardial infarction patients with undergoing successful primary percutaneous transluminal coronary angioplasty. *MJAU* 2005; 37: 25-30.
11. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med* 2002; 252: 283-94.
12. Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis? *Arterioscler Thromb Vasc Biol* 2001; 21: 603-10.
13. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* 2004; 350: 1387-97.
14. Allantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2004; 109: 837-42.
15. Heeschen C, Hamm CW, Bruemmer J, Simoons ML. Predictive value of C-reactive protein and troponin T in patients with unstable angina: a comparative analysis. CAPTURE Investigators. Chimeric c7E3 AntiPlatelet Therapy in Unstable angina Refractory to standard treatment trial. *J Am Coll Cardiol* 2000; 35: 1535-42.
16. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. *Fragmin during Instability in Coronary Artery Disease*. *N Engl J Med* 2000; 343: 1139-47.
17. Zairis MN, Manousakis SJ, Stefanidis AS, Papadaki OA, Andrikopoulos GK, Olympios CD, et al. C-reactive protein levels on admission are associated with response to thrombolysis and prognosis after ST segment elevation acute myocardial infarction. *Am Heart J* 2002; 144: 782-9.