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Relationship between hyporesponsiveness to clopidogrel measured by thrombelastography and in stent restenosis in patients undergoing percutaneous coronary intervention

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ABSTRACT

Objectives: The relationship between hyporesponsiveness to clopidogrel and in stent restenosis (ISR) was analyzed, and the cut-off value of hyporesponsiveness to clopidogrel for ISR was evaluated.

Design and methods: 861 consecutive patients enrolled and patients' inhibition rates in arachidonic acid (AA) and adenosine 5′-diphosphate (ADP) pathways were measured by thrombelastography (TEG) system. Patients were divided into ISR and non-ISR groups according to the results of coronary angiography. Correlation between hyporesponsiveness to clopidogrel and ISR was analyzed.

Results: 249 patients were in ISR group and 612 patients were in non-ISR group. The frequency of clopidogrel hyporesponsiveness in ISR group was significantly higher than that in non-ISR group ($P < 0.01$). Inhibition rates in AA and ADP pathways in ISR group were lower than those in non-ISR group ($P < 0.01$). The inhibition rate in ADP pathway was inversely correlated with ($r = -0.225$, $P = 0.001$) the severity of ISR. After being adjusted for traditional covariates, the inhibition rate in ADP pathway ($\beta = -0.191$, $R^2 = 0.011$, $P = 0.013$) remained independently associated with the severity of ISR; clopidogrel hyporesponsiveness was an independent risk factor of ISR (HR 6.62, 95% CI 2.84–15.49, $P = 0.001$). ROC curve analysis showed that the predictive cut-off value of the inhibition rate in ADP pathway for ISR was 10.1%.

Conclusions: The inhibition rate in ADP pathway is inversely related to the ISR severity. Clopidogrel hyporesponsiveness is an independent risk factor for ISR and can predict the risk of ISR.

Introduction

Ischemic events following percutaneous coronary intervention (PCI) depend on platelet activation and thrombin generation. Antiplatelet therapy with clopidogrel and aspirin is the current standard of care for patients undergoing PCI. Despite the proven benefits of adding clopidogrel to aspirin therapy, a significant percentage of patients will experience both short and long term post-stenting ischemic events.

Hyporesponsiveness to antiplatelet drugs especially clopidogrel puts patients undergoing PCI at a higher risk of recurrent ischemic events [1]. A significant number of studies have been conducted in acute coronary syndrome (ACS) patients. Previous studies have demonstrated that acute in-stent thrombosis and subacute in-stent thrombosis are associated with short term post-stenting ischemic events [2] while very late stent thrombosis [2] and in stent restenosis (ISR) are associated with long term post-stenting ischemic events. Hyporesponsiveness to clopidogrel is expected to play an important role in the occurrence of stent thrombosis [3,4]. Therefore, insufficient inhibition of platelets may be one of the pathogenic mechanisms for short term post-stenting ischemic events by increased thrombosis formation [5,6]. ISR usually occurs 6 months or later post stenting, and plays an important role in long term post-stenting ischemic events [7]. However, the pathogenesis for recurrent long term post-stenting ischemic events caused by hyporesponsiveness to clopidogrel is unclear, and the correlations between long term post-stenting recurrent ischemic events,

Abbreviations: AA, arachidonic acid; ACS, acute coronary syndrome; ACEI, angiotensin-converting enzyme inhibitor; ADP, adenosine 5′-diphosphate; AMI, acute myocardial infarction; ARBs, angiotensin receptor blockers; AUC, areas under the curve; BMI, body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; CAG, coronary angiography; CRE, serum creatinine; CRF, chronic renal failure; CRP, C reactive protein; DM, diabetes mellitus; EF, eject fraction; eGFR, estimated glomerular filtration rate; ESS, endothelial shear stress; FBG, fasting blood glucose; HR, heart rate; HRs, hazard ratios; IQR, inter quartile range; ISR, in stent restenosis; LAD, left anterior descending artery; LCX, left circumflex; LDL-C, low density lipoprotein-C; LM, left main; MACE, major adverse cardiac events; PCI, percutaneous coronary intervention; PTCA, percutaneous transcoronary angioplasty; RCA, right coronary artery; ROC, receiver operating characteristic; SBP, systolic blood pressure; SD, standard deviation; ST, stent thrombosis; TC, total cholesterol; TEG, thrombelastography; TG, triglyceride.

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hyporesponsiveness to clopidogrel and ISR are unclear, and need to be further studied.

In the present study, we first try to analyze the relationship between hyporesponsiveness to clopidogrel and ISR, and then evaluate the cut-off value of hyporesponsiveness to clopidogrel for ISR.

Patients and methods

Study population

From January 2010 to June 2013, a total of 861 consecutive patients with coronary artery disease (CAD) (age >40 years old) and a complete clinical history who underwent PCI with at least one implanted stent on dual antiplatelet treatment with aspirin at a dose of 100 mg and clopidogrel at a dose of 75 mg once daily after stenting; and came back with ischemic symptoms, evidence of myocardial ischemia (inducible or spontaneous) or for regular examination for the purpose of second coronary angiography (CAG) in our hospital were considered eligible. Patients with acute infection, chronic hepatic dysfunction, nutritional derangements, malignancy, severe valvular heart disease, severe heart failure or other severe medical illnesses with life expectancy <12 months, or with cardiogenic shock were considered ineligible for the study.

Our patients were divided into ISR group and non-ISR group according to the results of CAG.

All patients consented in writing to their participation in the study, and the study agreement was approved by the Chinese People's Liberation Army General Hospital research ethics committee and complied with the Declaration of Helsinki.

Data collection

The clinical characteristics of all patients were recorded on admission. These included age, gender, heart rate (HR), body mass index (BMI), systolic and diastolic blood pressures (SBP, DBP), ejection fraction (EF), diabetes mellitus (DM), primary hypertension, hyperlipidemia, previous myocardial infarction (MI), previous stroke, chronic renal failure (CRF), smoking history, and cardiovascular medication. C reactive protein (CRP) was measured by a turbidimetric assay. Routine labs were measured. The fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), low density lipoprotein-C (LDL-C) and serum creatinine (CRE) were analyzed by immunoturbidimetry (Roche modular 7600 automatic biochemistry analyzer). For all patients, renal function was assessed using the baseline estimated glomerular filtration rate (eGFR).

Platelet function testing

Measurement of platelet function was conducted between 8:00 and 9:00 am on admission, 1 h following oral taking of a maintaining a dose of 75 mg clopidogrel and a 100 mg aspirin once daily using thrombelastography (TEG). Samples were obtained under negative pressure using citrate (9NC coagulation sodium citrate 3, 2%) and heparin anticoagulation tubes (BD Company, USA). Blood samples were analyzed using TEG mapping assay (Haemoscope Corp., USA) in the TEG5000. The whole blood was available and the process achieved nearly point-of-care testing (POCT) by this method. All platelet aggregometry tracings were confirmed by a single reader, at a central clinical laboratory (Center for Platelet Function Studies, People's Liberation Army General Hospital, Beijing, China).

In this study, thienopyridine hyporesponsiveness was defined as less than 30% inhibition of platelet aggregation with 2 μmol/L ADP, and aspirin hyporesponsiveness was defined as less than 50% inhibition of platelet aggregation with 1 mmol/L AA [2]. Inhibition of platelet aggregation was defined as: (100 − ((MA pi–MA f) / (MA t–MAf)) × 100%. In this formula: MA pi = MA ADP or MA AA, MA f = MA fibrin, MA t = MA thrombin including MACK or MACKH, MA means maximal amplitude (which means maximal platelet aggregation activity), MACKH means giving the maximal response for thrombin generation in the presence of citrate, kaolin and heparinase, and MACK means giving the maximal response for thrombin generation in the presence of citrate and kaolin.

Coronary angiography and treatments

CAG was performed on all patients after admission. Restenosis was defined as the presence of a stenosis >50% of lumen diameter located in the native vessel segment treated with a stent. Culprit lesion morphology, location, and procedural characteristics were recorded. Results for each enrolled patient were recorded by observers who were blind to the results of laboratory tests. According to the results of CAG, patients were advised to perform percutaneous transcoronary angioplasty (PTCA), percutaneous coronary artery stent implantation, coronary artery bypass graft (CABG) or intensive medical therapy.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation (SD) or median (with inter-quartile range (IQR)). The *t* test was used if continuous variables were normally distributed, while the Wilcoxon two-sample test was used if continuous variables were not normally distributed. Categorical data were summarized as frequency. The chi-square test was used to compare categorical variables. Multivariate Cox regression analysis was applied to determine predictors of ISR. Hazard ratios (HRs) were reported with corresponding 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curve was constructed for distinction between ISR and non-ISR patients. The relationships between baseline clinical, laboratory characteristics and the severity of ISR were described using Pearson correlation coefficients and a linear regression model. All P values were two-sided, and a P value < 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences, version 17.0 (SPSS, Chicago, Illinois).

Results

Patients' baseline characteristics

Baseline clinical characteristics and laboratory examinations were fully documented for 861 patients (Fig. 1). Of the 861 patients (720 men and 141 women), 249 patients were in ISR group and 612 patients were in non-ISR group. The average interval from the first PCI to the second CAG was not different (13 ± 3.9 vs. 12.5 ± 4.1 months, P > 0.05). The general conditions, risk factors, medication and laboratory test data of the two groups were shown in Table 1.

As compared with non-ISR group, patients in ISR group had a higher presentation of unstable angina pectoris (UAP) and acute myocardial infarction (AMI) (P < 0.01). The ratio of previous myocardial infarction in ISR group was higher than that in non-ISR group (P < 0.05), while other characteristics including age, gender, HR, BMI, SBP, DBP, and EF and laboratory examinations that included TG, TC, LDL-C, FBG, CRE, and eGFR were not different between the two groups (P > 0.05). In addition, no differences were found in medication between the two groups.

The level of plasma CRP was higher in ISR group than that in non-ISR group ((0.43 (IQR0.1–0.67) vs. 0.3 (IQR0.1–0.35) P < 0.05)). Additionally, the plasma CRP level in clopidogrel hyporesponsiveness group (n = 195) was higher than that in clopidogrel normal responsiveness group (n = 666) (0.51 (IQR0.1–0.74) vs. 0.3 (IQR0.1–0.46) P < 0.05).

Angiographic and procedural characteristics

The angiographic and procedural characteristics in ISR and non-ISR groups for the first PCI procedure were described in Table 2. The most

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