Thrombosis Research 144 (2016) 56–61

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

High platelet reactivity on aspirin in patients with acute ST elevation myocardial infarction

Jean-Guillaume Dillinger [a], Alaa Saeed [a,1], Vincent Spagnoli [a,1], Claire Bal dit Sollier [b,1], Georgios Sideris [a,1], Stephane Manzo Silberman [a,1], Sebastian Voicu [a,1], Ludovic Drouet [b,1], Patrick Henry [a,*,1]

[a] Department of Cardiology, Lariboisiere Hospital, AP-HP, Paris Diderot University — INSERM U 942, Paris, France
[b] Department of Angio-hematology and IVS, Lariboisiere Hospital, AP-HP, Paris Diderot University, Paris, France

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 2 May 2016
Accepted 4 May 2016
Available online 10 May 2016

Keywords:
Aspirin
Myocardial infarction
Platelet reactivity
P2Y12 inhibitors

ABSTRACT

Background: Despite dual antiplatelet treatment, major ischemic events are common following ST elevation myocardial infarction (STEMI). We aimed to assess high platelet reactivity on aspirin (HPR-aspirin) and its association with P2Y12i (HPR-P2Y12i) during the acute phase of STEMI.
Methods: We included all consecutive patients admitted for STEMI treated by primary angioplasty in our center for 1 year. All patients received a loading dose followed by a maintenance dose of aspirin (75 mg/day) and prasugrel (ticagrelor or clopidogrel if contraindicated). Platelet reactivity was assessed 4 ± 1 days and 75 ± 15 days after admission using light transmission aggregometry with arachidonic acid (LTA-AA–HPR-aspirin) and VASP (HPR-P2Y12i) to define HPR as well as serum Thromboxane-B2 and LTA-ADP. Major cardiac and cerebrovascular events were recorded for 1 year.
Results: We included 106 patients – mean age was 61 y.o., 76% were male and 20% had diabetes. STEMI was anterior in 52% and LV ejection fraction at discharge was 51 ± 9%. 50% of patients were treated with prasugrel and 34% with ticagrelor. At day 4 after STEMI, HPR-aspirin was found in 26% patients and HPR-P2Y12i in 7%. HPR-both aspirin and P2Y12i was found in 4%. Diabetes and age were predictors of HPR-aspirin. HPR-aspirin was persistent 75 days later in 36% patients. At 1 year, 7.9% patients had experienced major adverse cardiovascular and cerebrovascular events (MACCE). HPR-aspirin and HPR on both aspirin and P2Y12i were significantly associated with MACCE.
Conclusion: HPR-aspirin is frequent just after STEMI and associated with MACCE especially when associated with HPR-P2Y12i.

1. Introduction

Dual antiplatelet therapy (DAPT) with aspirin and P2Y12 receptor antagonists (P2Y12i) to inhibit platelet reactivity (PR) is an effective pharmacologic therapy administered to patients with acute coronary syndrome (ACS) [1]. Guidelines now recommend DAPT with the new P2Y12i when possible as the mainstay treatment strategy to prevent stent thrombosis and subsequent ischemic events in ST-elevation myocardial infarction (STEMI) [2].

Despite the proven benefits of DAPT even with the new P2Y12-inhibitors, ischemic complications and Major Adverse Cardiac and Cerebrovascular Event (MACCE) continue to occur after STEMI especially during first months. The recent PLATO and TRITON trials found that these events occurred at a rate around 5% one month after the index event and around 10% to 12% at 1 year [3,4] and the largest absolute number of definite stent thrombosis occurred in the acute and subacute phases.

High on aspirin platelet reactivity (HPR-aspirin) and high on P2Y12i platelet reactivity (HPR-P2Y12i) (mainly clopidogrel) have been shown to be linked to MACCE after coronary angioplasty in stable coronary artery disease (CAD) and after ACS [5–7]. HPR-P2Y12i could be more frequent in ACS compared to patients with stable CAD due to the increased platelet reactivity before treatment, interference with other treatments, problems of absorption or unstable hemodynamics [8]. HPR-aspirin and HPR-P2Y12i could therefore explain the high rate of ischemic events found after ACS despite optimized antiplatelet treatment.

The aim of this study was to evaluate the rate of HPR-aspirin and HPR-P2Y12i during the first days following STEMI compared to the

* Corresponding author at: Department of Cardiology, Lariboisiere Hospital, Assistance Publique, Hôpitaux de Paris, 2 rue A Pare, 75010 Paris, France.
E-mail address: patrick.henry@lrb.aphp.fr (P. Henry).
[1] All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

http://dx.doi.org/10.1016/j.thromres.2016.05.002
0049-3848/

chronic phase, to determine the predictors of HPR and the link with clinical events.

2. Methods

2.1. Study population

This prospective open single-center study enrolled consecutive patients admitted in the cardiac care unit of Lariboisiere Hospital (Paris) for STEMI between January 2013 and December 2013. Patients were eligible if admitted with ischemic symptoms (prolonged chest pain) and persistent ST-segment elevation of ≥ 1 mm in two contiguous electrocardiographic main leads or ≥ 2 mm in two contiguous electrocardiographic precordial leads. All patients underwent urgent coronary angiography confirming the diagnostic of STEMI (occlusion of a coronary artery or evidence of thrombus) and primary angioplasty was performed in most cases. An initial bolus of heparin was administered and sometimes followed by bivalirudin. Exclusion criteria were out-of-hospital cardiac arrest, known coagulopathy, significant hematological disorders on admission (platelet count < 100,000/mm^3, hematocrit < 25%), severe liver disorders, or treatment with thrombolytic or glycoprotein IIb/IIIa antagonists within the previous seven days.

2.2. Design

The patients were treated initially with an aspirin loading dose of 250–500 mg intravenously in the ambulance, followed by a daily morning dose of 75 mg. The aspirin used for the study was powder containing 75 mg of non-enteric coated lysine acetylsalicylate (Kardegic®, Sanofi–Aventis, France). P2Y12i treatment was selected in the following order: #1prasugrel – #2 ticagrelor in patients aged >75 y.o., weight < 60 kg or with a history of bleeding – #3 clopidogrel in patients with a history of severe bleeding or with concomitant treatment with anticoagulant. Loading dose of P2Y12i (60 mg of prasugrel, 180 mg of ticagrelor or 600 mg clopidogrel) was given in the ambulance (80% of the time) or just after coronary angiography. A maintenance morning dose was administered for prasugrel (10 mg), twice a day for ticagrelor (180 mg/day) and clopidogrel (75 mg). DAPT was planned for one year according to ESC guidelines [2]. Platelet reactivity was assessed on day 4 ± 1 following admission. Two measurements were performed with an interval of 2 days for the first 35 patients to assess reproducibility for LTA. Platelet aggregation testing was re-assessed at 75 ± 15 days after admission. Both physicians and patients were blinded to the PR results. No change in the aspirin or P2Y12i regimens (molecule, dose or frequency of administration) was recommended unless a clinical event occurred. Informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local Ethics Committee (NCT02038309).

2.3. Blood sampling and aggregation tests

Treatments were systematically taken in front of a nurse until blood sampling at day 4 and compliance was assessed by interview at day 75. All blood samples were fasting and performed in the morning at trough level (between 8:00 and 9:00 am) before ingestion of the morning dose of aspirin (i.e., 24 h after the last aspirin intake) and P2Y12i (i.e., 24 h after the last intake of prasugrel or clopidogrel and 12 h after the last intake of ticagrelor). Samples were delivered to the laboratory within 1 h and processed within 2 h. CK and troponin measurements (CK ARCHITECT C 8000, Abbott, USA - AxSYM Troponin I ADV, Abbott, USA – 95% CI: <0.08 μg/L) were performed every 6 h from admission until two samples showed a decrease in the level. Aspirin efficacy was assessed by light transmission aggregation triggered by arachidonic acid 0.5 mg/mL (LTA-AA) and Thromboxane B2 [9,10]. P2Y12i efficacy was assessed by VASP and LTA-ADP 20 μmol/L.

2.4. Light transmission aggregometry (LTA)

Platelet rich plasma light transmission aggregometry has been routinely performed for several years in our laboratory. Briefly, to assess aspirin efficacy, platelet-rich plasma was obtained by centrifugation of citrated whole blood at 156 *g* for 12 min at 18 °C and platelet-poor plasma by further centrifugation at 1000 *g* for 20 min. In vitro platelet aggregation was measured at 37 °C in an aggregometer (Model 490-4D, Chrono-Log Corporation, Kordia, Netherlands) following the optical aggregometry method of Born. To assess biological efficacy of aspirin, aggregometry was induced by adding arachidonic acid (AA 0.5 mg/mL - LTA-AA). To assess biological efficacy of P2Y12i, aggregometry was induced by adding adenosine diphosphate (ADP – 20 μmol/L - LTA-ADP). For both, LTA aggregation was expressed as the maximum aggregation intensity (MAI) in light transmission in platelet rich plasma using the autologous platelet poor plasma as the 100% reference. Mean standard deviation (SD) was 0.85 for AA and 0.71 for ADP. Laboratory personnel were blinded to the treatment.

2.5. Serum thromboxane B2 immunoassay

Whole blood samples were collected into dry glass tubes to coagulate and were maintained for 1 h at 37 °C. The blood coagulum was then rapidly centrifuged at 1000 *g* for 10 min to obtain a cell-free extract. Aliquots of this extract were immediately frozen at −80 °C and used to determine the serum concentrations of thromboxane B2 (TXB2) – an index of *in vitro* platelet COX-1 activity. TXB2 determination was performed by immunoassay (TXB2 EIA Kit, Cayman Chemical Company).

2.6. Vasodilator-stimulated phosphoprotein phosphorylation (VASP) index

VASP index was measured for additional assessment of biological efficacy of P2Y12 inhibitors. For the VASP assays, samples were drawn into a citrated tube, capped, and stored at ambient temperature. VASP phosphorylation in response to prostaglandin E_1 with and without P2Y12 was determined by whole-blood flow cytometry after platelet membrane permeabilisation using a commercially available kit from Diagnostica Stago/Biocytex (Marseille, France) [11]. The VASP index was calculated from the median fluorescence intensity (MFI) of samples incubated with prostaglandin E1 (PGE1) and P2Y12 according to the formula: $\text{VASP index} = [(\text{MFI}_{(\text{PGE1})} - \text{MFI}_{(\text{PGE1 + ADP})}) / \text{MFI}_{(\text{PGE1})}] / 100$.

2.7. Follow-up

The follow-up visit took place 1 year after the index event. MACCE was defined as the first occurrence of any event from the composite of death, recurrent myocardial infarction (MI), stroke or unplanned hospitalization for chest pain. Recurrent MI before cardiac markers had returned to normal was defined as symptoms and re-elevation of troponin or CK of at least 50% over a previous value that was decreasing. MI after cardiac biomarkers had returned to normal was defined as elevation of biochemical markers above the upper limit of normal with either ischemic symptoms at rest, ECG changes or pathological findings of an acute MI. A stroke was defined as a focal loss of neurological function caused by an ischemic or hemorrhagic event with residual symptoms at least 24 h after onset or leading to death. Unplanned hospitalization was defined as a non-scheduled hospitalization in a cardiologic unit with chest pain as the main symptom. Minor bleeding was not recorded. Transfusions were recorded.

2.8. Statistical methods

HPR-aspirin was defined as maximum platelet aggregation intensity (MAI) ≥ 20% measured by LTA-AA [6] and was the reference method for prediction and survival analysis. A production of thromboxane-