Thrombosis Research 125 (2010) 473–474

Contents lists available at ScienceDirect

Thrombosis Research

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

Letter to the Editors-in-Chief

Poor performance with a platelet counting technique to monitor clopidogrel inhibitory effects in the point-of-care setting

Platelets play a central role in the pathophysiology of acute coronary syndromes (ACS) and dual antiplatelet therapy with aspirin and clopidogrel has resulted in significant treatment advances. However, there is important variability in the responsiveness to clopidogrel treatment, and there is an increasing use of point-of-care methods to evaluate clopidogrel responsiveness at the individual level. Currently, there are several point-of-care techniques for testing of platelet inhibition in clinical use, each with their strengths and limitations [1,2].

On the basis of an observational study [3] suggesting that atorvastatin may attenuate platelet inhibition by clopidogrel, we performed a randomized, prospective study to investigate possible interactions between clopidogrel and statins, using *ex vivo* platelet function tests. The study and its main findings have been published elsewhere [4]. Briefly, this was a predetermined substudy of a 16-week, randomised, double-blind, parallel group, multicentre study performed in Sweden comparing the lipid-lowering efficacy of rosuvastatin and atorvastatin in patients (75% male, mean age 62 years) who had previously (on average 9 months before) undergone PCI (ClinicalTrials.gov Identifier NCT00235950). After having completed a statin dose titration period of 14 weeks, patients entered the present platelet substudy. The primary objective was the degree of inhibition of ADP-induced platelet activation achieved by clopidogrel maintenance treatment. Platelet function was assessed in all patients with the point-of-care method used in the previous observational study [3]. In most patients, the point-of-care platelet function test was carried out by medical staff, usually nurses, at cardiology clinics (nationwide). A subset of patients participated in detailed platelet function studies, including the point-of-care method and other platelet function tests, performed at a professional platelet laboratory localized in Stockholm [4].

After 14 weeks of randomised statin treatment, all patients in this platelet substudy received concomitant treatment with clopidogrel 75 mg OD during two weeks. Platelet function was evaluated before and after two weeks of clopidogrel co-treatment. Blood samples were collected for measurements of platelet function by either light transmission aggregometry (LTA) in platelet rich plasma (PRP), flow cytometry in whole blood [4] and the PlateletWorks point-of-care platelet counting technique [3,5,6] in the professional platelet laboratory patients, or the point-of-care platelet counting technique only in cardiology clinics patients. Selection of patients to the platelet laboratory in Stockholm was based on geographic reasons only.

LTA was used to study platelet macroaggregation, as described elsewhere [7]. Aggregometry in PRP is usually considered the "gold standard", and has been much used in studies of clopidogrel response variability and "resistance" [1]. Platelet microaggregation in whole blood was measured with the point-of-care MICROS cell counter (ABX Diagnostics, Montpellier, France) and the PlateletWorks test platform (Helena Laboratories, Beaumont, TX, U.S.A.) [3]. The cell counter uses traditional electronic impedance cell counting principles [5,6]. A reference platelet count is performed in 1 mL of fresh whole blood in a PlateletWorks tube containing K_3-EDTA as the anticoagulant, and in a tube containing citrate as anticoagulant and 20 μmol/L of ADP. In the presence of ADP, platelets associate and aggregate, and are no longer counted as individual platelets. The ratio of platelet counts in the agonist and reference tubes is calculated as percent platelet aggregation, and results are available within 4 min. This method has been validated against turbidimetric aggregometry and demonstrated a correlation coefficient of 0.83 in 225 paired samples [6]. Blood samples (5 mL) were collected by venipuncture without any anticoagulant, immediately aliquoted into the two PlateletWorks tubes and then mixed by repeated gentle inversion of the tubes. Measurements were to be performed within 10 min after aliquotation according to instructions from the manufacturer. This time was standardized to 8 min at the professional platelet laboratory.

In total, 46 patients randomised to rosuvastatin ($n=24$) and atorvastatin ($n=22$) treatment completed this substudy with detailed platelet function tests at the professional laboratory. In addition, 82 patients randomised to rosuvastatin and 78 patients randomised to atorvastatin treatment completed this substudy with point-of-care platelet function testing at cardiology clinics.

The platelet responsiveness to ADP stimulation measured by LTA at 14 weeks of randomized statin treatment (before clopidogrel) was similar ($76\pm6\%$ aggregation at 10 μM ADP) in the two treatment groups. Clopidogrel treatment inhibited 10 μM ADP-induced platelet aggregation $40\pm27\%$ in the rosuvastatin group and by $57\pm28\%$ in the atorvastatin group (Fig. 1). Before clopidogrel treatment, platelet microaggregation evoked by ADP with the MICROS/PlateletWorks method was $96\pm2\%$ and $96\pm3\%$ in the rosuvastatin and atorvastatin treated groups, respectively, in the professional platelet laboratory, but only $92\pm12\%$ and $91\pm16\%$, respectively, in patients tested at cardiology clinics. Among subjects tested at the professional platelet laboratory, clopidogrel inhibited ADP-induced aggregation by $41\pm28\%$ in the rosuvastatin group, and by $54\pm23\%$ in the atorvastatin group (Fig. 1). The correlation between clopidogrel inhibitory effects as measured in the platelet laboratory using LTA and the MICROS/PlateletWorks method was reasonably good ($r^2=0.456$). The regression equation between LTA and the platelet counting technique can be described as LTA = 9.21 + 0.830 x MICROS/PlateletWorks result. On average, the results in the professional platelet laboratory agreed very well with results obtained with LTA (Fig. 1), and with ADP-stimulated platelet activation measured by flow cytometry in whole blood [4].

In the subjects tested at cardiology clinics, however, clopidogrel inhibited ADP-induced aggregation by only $20\pm31\%$ and $16\pm37\%$, respectively, in patients on rosuvastatin and atorvastatin (Fig. 1). Thus, when used as a point-of-care method in cardiology clinics, the MICROS/PlateletWorks technique provided less pronounced ADP-induced aggregation before clopidogrel treatment, and much less pronounced, and non-significant, inhibition by clopidogrel. The numbers of non-responders to

0049-3848/$ – see front matter
doi:10.1016/j.thromres.2009.10.005

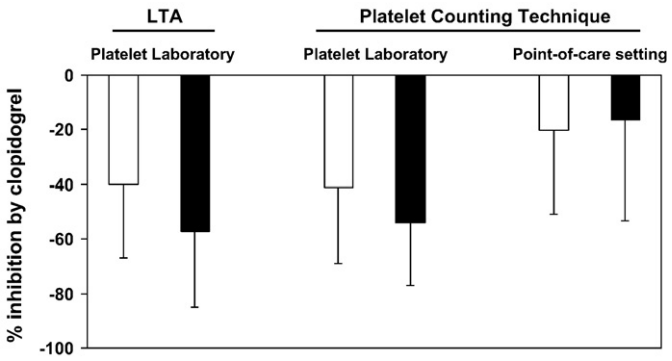

Fig. 1. Clopidogrel inhibition of ADP-induced platelet macroaggregation (measured by light transmission aggregometry [LTA] in PRP in a platelet laboratory) and microaggregation (measured by the platelet counting technique) in platelet laboratory patients and in cardiology clinic patients (i.e. a "point-of-care setting"). No statistical differences were observed regarding clopidogrel effects in patients treated with rosuvastatin (white bars) vs patients treated with atorvastatin (black bars). When handled in the platelet laboratory, the platelet counting technique yielded similar results as the LTA. When handled by staff with no previous experience in platelet testing, there was a lesser clopidogrel effect. Means ± SD, n = 22-24 for platelet laboratory patients and n = 78-82 for the "point-of-care setting" patients.

clopidogrel (defined as inhibition of platelet activity of less than 10%) according to the MICROS/PlateletWorks technique when performed by the platelet laboratory and by personnel in cardiology clinics were 2% (1 out of 46) and 11% (18 out of 160), respectively. Comparing the variances in the two settings showed no statistical difference ($p = 0.814$) [8], but there was a difference in the percent inhibition by clopidogrel with a mean of 45.7 (95%CI; 37.8-53.6) in the professional laboratory, and 17.1 (95%CI; 9.48-24.6) when the same method was used in the point of care setting ($p < 0.0001$). Thus, the MICROS/Plateletworks technique seems to be user-dependent, and did not perform well as a point-of-care method in cardiology clinics. Reasons for poor performance of the method in the point-of-care setting (despite training by company personnel) might include inadequate standardization of delays in measurement, and inefficient mixing of the assay tubes before measurement. Indeed, strong time dependence has previously been demonstrated for the assay [9].

In conclusion, in this randomised prospective study, using several sensitive and specific methods for the evaluation of ADP-induced platelet activation, the platelet counting technique performed well when executed at a professional platelet laboratory. However, when used at cardiology clinics, i.e. in a real point-of-care setting, the results were too low and not reliable. Validation of novel point-of-care methods in relevant settings is thus warranted.

Conflict of interest statement

The study was conducted in co-operation with AstraZeneca (AZ). Data were handled and statistical analyses performed by LJ at AZ. RM, PH and JÖ were responsible for study design and interpretation of data. RM and PH declare no conflicts of interest. JÖ has been an advisory board consultant for AZ and has received honoraria from and lectured for AZ, Pfizer, Sanofi-Aventis and other major pharmaceutical companies.

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5 June 2009