Cangrelor increases the magnitude of platelet inhibition and reduces interindividual variability in clopidogrel-pretreated subjects

H.J. Bouman, J.W. van Werkum, C.M. Hackeng, N. Clappers, J.M. ten Berg

Background. Inadequate platelet inhibition despite aspirin and clopidogrel therapy during and after a percutaneous coronary intervention is associated with an impaired clinical outcome. Cangrelor, a direct and reversible P2Y₁₂ inhibitor that is currently in development, has the potential to achieve higher levels of inhibition of ADP-induced platelet aggregation than clopidogrel. The aim of the present study was to compare the magnitude of platelet inhibition in clopidogrel-pretreated patients before and after the *in vitro* addition of a subtherapeutic dose of cangrelor.

Methods. Blood samples were drawn from patients pretreated with clopidogrel and aspirin who were undergoing elective percutaneous coronary intervention (n=39). Platelet function analysis with 'classical' light transmittance aggregometry (both peak and late aggregation [at 6 min]) was performed before and after the *in vitro* addition of cangrelor (0.25 µmol/l) to platelet-rich plasma (PRP). After an incubation period of five minutes, platelet aggregation was induced by 5 and 20 µmol/l ADP.

Results. The *in vitro* addition of 0.25 µmol/l cangrelor to the PRP from clopidogrel-treated subjects resulted in an additional reduction in ADP-induced platelet aggregation. For ADP con-

centrations of 5 and 20 µmol/l, peak aggregation showed a decrease of 75 and 85%, respectively (p<0.001 for both), while late aggregation was almost completely diminished (p=0.003 and p<0.001, respectively). Furthermore, the interindividual variation in inhibition of ADP-induced platelet aggregation by clopidogrel was greatly reduced after the addition of cangrelor.

Conclusion. We demonstrate that the *in vitro* addition of even a subtherapeutic dose of cangrelor to the PRP of clopidogrel-pretreated patients results in an additional reduction of ADP-induced platelet aggregation. Moreover, cangrelor was able to diminish the interindividual variation observed in clopidogrel-inhibited platelet aggregation. (*Neth Heart J* 2009;17:195-8.)

Keywords: P2Y₁₂, clopidogrel, cangrelor, platelet aggregation

Clopidogrel is an orally administered prodrug that requires metabolic conversion to an active metabolite to inhibit platelet aggregation via the P2Y₁₂ receptor.¹ However, numerous studies have revealed that the efficacy of this metabolism varies widely from subject to subject, resulting in a poor platelet inhibition in 5 to 44% of treated patients.^{2,3}

Recent studies have demonstrated an association between the magnitude of post-clopidogrel treatment platelet aggregation and the occurrence of atherothrombotic events in patients undergoing percutaneous coronary intervention (PCI).⁴⁻⁶ On the background of these findings, it is important to note that the results of the recently published TRial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel (TRITON study) clearly demonstrated that the proposed proof of principle 'a higher P2Y₁₂ inhibition is associated with a reduction in atherothrombotic events', appears to be true.⁷ Specifically, intensified P2Y₁₂ inhibition during and in the early days after PCI reduces myocardial infarction, as long as care is taken in certain patient groups who are *a priori* at high risk of bleeding.⁷

H.J. Bouman
J.W. van Werkum
J.M. ten Berg

Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands

C.M. Hackeng
Department of Clinical Chemistry, St. Antonius Hospital, Nieuwegein, the Netherlands

N. Clappers
Department of Cardiology, UMC St Radboud Nijmegen, the Netherlands

Correspondence to J.W. van Werkum
Department of Cardiology, St. Antonius Hospital, PO Box 2500,
3435 CM Nieuwegein, the Netherlands
E-mail: w.van.werkum@antonius.net

Cangrelor (formerly known as AR-C69931MX, The Medicines Company, Parsippany NJ USA) is a direct and reversible antagonist of the P2Y₁₂ receptor that does not require conversion to an active metabolite for its antiplatelet action.⁸ Small phase II dose-finding studies using two different concentrations of cangrelor (2 µg/kg/min and 4 µg/kg/min) in patients with an acute coronary syndrome have revealed a high magnitude of inhibition of platelet aggregation (IPA) after initiation of the cangrelor infusion.^{9,10} However, platelet function was measured with impedance aggregometry, a method that has not been very well studied with respect to P2Y₁₂ inhibition.¹¹ Therefore, we aimed to study the effect of cangrelor on platelet aggregation in patients pretreated with aspirin and clopidogrel using the 'gold standard' light transmittance aggregometry (LTA).

Methods

Patients on aspirin and clopidogrel therapy scheduled for an elective PCI were enrolled. All subjects gave written informed consent. Risk factors and comorbidities were carefully obtained using a standard medical questionnaire. Physical examination of patients was performed by research physicians. Smoking was defined as any cigarette smoking in the last month. Hypertension was defined as a systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg. Diabetes mellitus was defined according to the World Health Organisation criteria. Hypercholesterolaemia was defined as a fasting LDL-cholesterol concentration 3.4 mmol/l or the need for statin therapy.

Blood samples were drawn from the arterial sheath before heparinisation, into citrated (3.2%) tubes (Sarstedt, Nümbrecht, Germany). The samples were then centrifuged at 120 g for ten minutes to recover platelet-rich plasma (PRP) and further centrifuged at 850 g for 15 minutes to recover platelet-poor plasma (PPP). PRP was adjusted to achieve a final platelet count between 250 and 300 × 10⁹/l using the volunteer's PPP. Two-hundred-and-fifty µl adjusted PRP was pipetted into four cuvettes, of which two were spiked with 25 µl 0.9% NaCl and two were spiked with 25 µl cangrelor in a final concentration of 0.25 µmol/l (this concentration represents the 2 µg/kg/min infusion) and incubated for five minutes. Both peak and late aggregation (at 6 minutes) were quantified after stimulation with adenosine diphosphate (ADP; final concentrations 5 and 20 µmol/l).¹² Platelet aggregation was measured on an ATRACT 4004 aggregometer (LABiTec, Arensburg, Germany) and PPP served as the reference for 100% aggregation.

Statistical analysis

Discrete variables are reported as counts (percentages) and continuous variables as mean ± SD. A paired Student's t-test was used to compare aggregation values before and after the addition of cangrelor. The F-test was used to compare standard deviations.

Statistical analyses were performed using SPSS 11.5 and a p value of <0.05 was considered statistically significant.

Results

A total of 39 patients on aspirin and clopidogrel therapy were enrolled. Patient characteristics are depicted in table 1.

Stimulation of PRP, obtained from patients pretreated with aspirin and clopidogrel, with 5 and 20 µmol/l ADP resulted in a peak aggregation of 25±13% and 42±18, respectively. Late aggregation values were 13±13% and 26±24% after stimulation with 5 and 20 µmol/l ADP, respectively. Furthermore, despite adequate dosing of clopidogrel, a wide interindividual variability in the magnitude of platelet aggregation was observed between patients (figure 1).

The *in vitro* addition of 0.25 µmol/l cangrelor resulted in a great reduction of ADP-induced platelet aggregation. For ADP concentrations of 5 and 20 µmol/l, peak aggregation showed a decrease of 75 and 85%, respectively (p<0.001 for both concentrations of ADP), while late aggregation was almost completely diminished, despite stimulation with ADP.

Also, the interindividual variation in both 5 and 20 µmol/l ADP-induced platelet aggregation was greatly reduced after the addition of cangrelor (F-test probability value comparing standard deviations p<0.001).

Table 1. Patient characteristics.

Patient characteristics	(n=39)
Men	33 (85)
Age (years)	62±9
BMI (kg/m ²)	27±4
Hypertension	6 (15)
Hypercholesterolaemia	5 (13)
Current smoker	5 (13)
Previous MI	19 (49)
Previous revascularisation	19 (49)
Medication	
- Clopidogrel	39 (100)
- Aspirin	39 (100)
- Statin	33 (85)
- β-blocker	33 (85)
- ACE inhibitor	12 (31)
Platelet count (x 10 ⁹ /l) in EDTA	307±85
Mean Platelet Volume (fL)	6.8±0.6

Values are presented as numbers (%) or mean ± SD. BMI=body mass index, MI=myocardial infarction.

Figure 1. Peak and late ADP-induced platelet aggregation (5 and 20 $\mu\text{mol/l}$) before (BC) and after (AC) the *in vitro* addition of 0.25 $\mu\text{mol/l}$ cangrelor.

Discussion

The purpose of this study was to evaluate the inhibitory effects of cangrelor with 'classical' LTA in patients pretreated with aspirin and clopidogrel. Although we used a lower concentration of cangrelor than currently studied in the ongoing CHAMPION studies (this is 4 $\mu\text{g/kg/min}$ infusion ~ final concentration 0.5 $\mu\text{mol/l}$), the addition of this subtherapeutic concentration of cangrelor yielded a large decrease of peak aggregation and an almost completely diminished late aggregation. Moreover, cangrelor was able to diminish the wide interindividual variation in platelet inhibition that was observed with clopidogrel therapy.

Recent studies have clearly demonstrated that a subset of patients who receive dual antiplatelet therapy with aspirin and clopidogrel do not reach adequate levels of platelet inhibition at the time of PCI.³ And, more importantly, these patients are at a higher risk for the (re)occurrence of atherothrombotic events.^{4,6}

Evidence that higher P2Y₁₂ inhibition results in an improved clinical outcome is provided by the recently published TRITON study, which showed that the more potent platelet inhibition achieved by prasugrel results in an approximately 20% reduction of atherothrombotic events as compared with clopidogrel.⁷

Although prasugrel has a more rapid onset of action compared with clopidogrel, cangrelor has the additional advantage over clopidogrel and prasugrel of being a reversible and direct-acting P2Y₁₂ antagonist, reaching optimal platelet inhibition in only minutes after the start of the infusion. In combination with a short half-life of several minutes, this results in a rapid 'on-off' feature that enables cangrelor to temporarily suppress platelet activation on top of an oral platelet inhibitor during and after PCI. These features might improve clinical outcome concerning atherothrombotic events and avoid an increase in bleeding complications accompanying chronic intensified platelet inhibitor therapy.

Two large-scale phase III, randomised clinical trials (CHAMPION-PCI and CHAMPION-PLATFORM) comparing clopidogrel with cangrelor and cangrelor on top of clopidogrel respectively, are currently assessing whether the superiority of cangrelor over clopidogrel in platelet inhibition will be translated into an improved clinical efficacy.

In conclusion, the *in vitro* addition of even a subtherapeutic concentration of cangrelor to the PRP of aspirin and clopidogrel pre-treated patients strongly reduces the magnitude of ADP-induced platelet aggregation. Moreover, cangrelor was able to diminish the interindividual variation observed in clopidogrel-inhibited platelet aggregation.

Disclosure of conflict of interests

The authors state that they have no conflict of interest. The cangrelor was kindly provided by The Medicines Company. ■

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