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Regular Article

A phase 1 study of prasugrel in patients with sickle cell disease: Effects on biomarkers of platelet activation and coagulation☆

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A B S T R A C T

Introduction: Prasugrel, a $P2Y_{12}$ adenosine diphosphate (ADP) receptor antagonist effectively inhibits ADP-mediated platelet activation and aggregation, and may be useful in reducing vaso-occlusive crises in sickle cell disease (SCD). In this study, we assess the effect of prasugrel on biomarkers of platelet activation and coagulation in patients with SCD.
Materials and Methods: Twelve adult patients with SCD and 13 healthy subjects were examined before and after 12 ± 2 days of 5.0 or 7.5 mg/day oral prasugrel. Assessed cellular biomarkers included monocyte- and neutrophil-platelet aggregates, activated glycoprotein IIb-IIIa (GPIIbIIIa), P-selectin, CD40 ligand (CD40L), tissue factor (TF) expression on circulating platelets and on monocyte-platelet aggregates, and platelet-erythrocyte aggregates. Soluble biomarkers included CD40L, prothrombin fragment 1.2 (F1.2), thromboxane B_2 (TXB_2), P-selectin, and TF.
Results: Patients with SCD had increased platelet baseline activation compared to healthy subjects, as measured by percentages of monocyte-platelet aggregates, neutrophil-platelet aggregates, and platelets expressing CD40L. Likewise, baseline levels of soluble F1.2 and TXB_2 were elevated in patients with SCD compared to healthy subjects. After 12 days of prasugrel, patients with SCD had a significant reduction in platelet-monocyte aggregates that was not observed in healthy subjects. Following prasugrel administration, those with SCD maintained higher levels of monocyte-platelet aggregates and soluble F1.2, but had lower levels of platelet-erythrocyte aggregates and soluble TF compared to healthy subjects.
Conclusions: These results provide evidence for chronic platelet activation in the SCD steady state, activation that was in part attenuated by prasugrel, thereby suggesting that ADP may mediate platelet activation in SCD.
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Introduction

Sickle cell disease (SCD) is a common hemoglobinopathy that affects millions of people worldwide and has substantial medical, social, and economic impact [1]. Much of the morbidity and mortality of SCD arises from complications of vaso-occlusive crisis (VOC), which can result in ischemia and infarction of affected organs and which manifests clinically as episodic bouts of localized pain [2–4].

Microvascular vaso-occlusion and chronic inflammation are the hallmarks of VOC. Although the underlying pathophysiology remains unclear, it is thought to be the result of a complex and dynamic interplay between sickled red blood cells (RBCs), endothelial cells, leukocytes, platelets, and various plasma proteins. Evidence suggests that in patients with SCD, circulating platelets are chronically activated in the non-crisis steady state [5], and that this activation intensifies during VOC [6–8]. As sickled RBCs haemolyze, they release adenosine diphosphate (ADP) [6,9], which may drive platelet activation and aggregation [10]. In addition, activated platelets have increased expression of proteins such as CD40 ligand (CD40L) [5,8,11] and P-selectin [12], which may promote interaction with endothelial and inflammatory cells [11,12]. In addition to erythrocytes, platelet and leukocyte aggregates contribute to vessel occlusion [13]. Several studies have also found

Abbreviations: ADP, adenosine diphosphate; CD40L, CD40 ligand; EDTA, ethylenediaminetetraacetic acid; GPIIbIIIa, glycoprotein IIb-IIIa; PD, pharmacodynamics; PK, pharmacokinetics; RBCs, red blood cells; SCD, sickle cell disease; SD, standard deviation; TF, tissue factor; TXB_2, thromboxane B_2; VOC, vaso-occlusive crisis.

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coagulation markers including fibrin [7], tissue factor (TF), and thrombin [6,8] to be elevated in SCD in the non-crisis steady state and, to be further elevated during VOC in children and adult patients [7,12].

The potential role of platelets in VOC suggests that antiplatelet therapies may hold promise for prevention and treatment in SCD. Thus far, there have been two double-blind, placebo-controlled studies of the first-generation ADP-receptor antagonist ticlodipine. Though an *ex vivo* decrease in platelet aggregation was seen in a 4-week study of ticlodipine therapy, no difference in the frequency of VOC was observed [14]. However, in a larger study of ticlodipine lasting 6 months, a significant reduction in painful crises was seen in patients with SCD [15].

Prasugrel is a third generation platelet $P2Y_{12}$ ADP-receptor antagonist, which has been shown to effectively inhibit platelet activation and aggregation via irreversible inhibition of the $P2Y_{12}$ ADP receptor [10]. Given the potential role of ADP in the manifestation of VOC, prasugrel may reduce the frequency and severity of VOC and consistently decrease platelet activation to help reduce chronic low-level ischemia in these patients.

The pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of prasugrel have been well-characterized in healthy control subjects and in patients with coronary artery disease [16,17], but were unknown in patients with SCD. However, these characteristics have now been evaluated before and after 12 days of prasugrel administration (5.0 mg/day or 7.5 mg/day) in a Phase 1b, open-label, single-center trial that included patients with SCD and healthy control subjects. In patients with SCD, prasugrel produced dose-dependent exposure to its active metabolite, which in turn reduced platelet reactivity to ADP, as measured using a variety of assays [18]. In the current analysis of data from the same study, we examine levels of cellular and soluble biomarkers of platelet activation and coagulation at baseline and following 12 days of prasugrel administration in patients with SCD and compare these values to those found in healthy subjects.

Materials and Methods

Study design

This single-center, open-label trial was sponsored by Daiichi Sankyo Company, Ltd. and Eli Lilly and Company from July 2010 through February 2011 (NCT01178099). Patients with SCD and healthy controls were given a single 10-mg oral dose of prasugrel followed by administration for 12 ± 2 days of oral prasugrel, 5 mg/day for those weighing <60 kg and 7.5 mg/day for those weighing ≥ 60 kg.

Participants

Enrolled participants had to be between 18 and 60 years of age and weigh between 50 and 100 kg. Patients with SCD had to have the genotypes HbSS, HbSC, or $HbS\beta^0$ or $HbS\beta^+$ thalassemia, and were to have had no occurrence of VOC within 1 month of screening. Healthy control subjects were matched for gender, age (within 10 years), and weight (<60 kg and ≥ 60 kg within 10 kg). Please refer to the primary publication for further detail [18].

Cellular and soluble platelet and coagulation biomarkers

Within groups, we assessed the response to 12 ± 2 days of prasugrel administration, and between groups, we compared biomarker levels before and after prasugrel administration. Venous blood samples were collected at baseline (1 hour before the first dose of prasugrel) and on Day 12, 24 hours after the previous 5-mg or 7.5-mg dose of prasugrel.

Whole blood samples were collected into 3.2% citrate and, within 2 hours of collection, were assessed for biomarker expression by flow cytometry. As previously described, monocyte and neutrophil cell populations were distinguished within whole blood on the basis of their differential expression of CD14 and light scatter properties [19–21]. The presence of heterotypic platelet aggregates was then determined by assessing the binding of a platelet-specific antibody, CD61, to these cells. TF present on the surface of monocyte-platelet aggregates was measured using a CD142-specific antibody.

The level of circulating platelet-erythrocyte aggregates was determined by gating on CD41a-positive platelets and measuring staining of the erythrocyte-specific glycophorin marker CD235a to determine the percent of platelet-erythrocyte aggregates [9].

The activation state of circulating platelets was determined by the detection of activation-dependent changes in the platelet surface markers by flow cytometry [22]. Platelets were identified by size, light scatter pattern, and the binding of a CD61-specific antibody. The expression of P-selectin (CD62P), activated glycoprotein IIb-IIIa (GPIIbIIIa [PAC-1]), CD40L (CD154), and TF (CD142) on the surface of platelets was measured by staining with antibodies specific for these markers (all antibodies were from BD Biosciences, Oxford, UK).

Flow cytometric analysis was performed using a FACSCalibur cytometer and CellQuest Pro software (both BD Biosciences, San Jose, CA). Results were presented as percent of positive cells expressing a given marker above the background control level of staining. For platelet activation markers, this related to the proportion of platelets that may participate in aggregates or inflammatory processes.

Soluble CD40L (Quantikine Human CD40 Ligand Immunoassay, R&D Systems, Minneapolis, MN) and P-selectin (Human P-Selectin ELISA, R&D Systems, Minneapolis, MN) were assessed in plasma prepared from blood samples collected into ethylenediaminetetraacetic acid (EDTA) and centrifuged at 1000 g for 15 minutes within 30 minutes of collection. Samples for the CD40L assay were centrifuged for an additional 10 minutes at 10,000 g. Prothrombin fragment 1.2 (F1.2) was measured in plasma prepared from blood collected in 3.2% citrate using Dade-Behring Enzygnost F1 + 2 microkit (Marburg, Germany). Serum for thromboxane B_2 (TXB_2) was prepared from whole blood collected into glass tubes and allowed to clot for 1 hour at 37 °C [23] and assayed by immunoassay (Cayman Thromboxane B_2 EIA kit, Cayman Chemical, Ann Arbor, MI). Blood samples for soluble coagulation Factor III/TF measurement were collected in 3.2% sodium citrate. After centrifugation at 1500 g for 15 minutes, plasma was taken off and frozen at −80 °C within 30 minutes of collection. Thawed samples were tested using an immunoassay method (R& D Systems, Abingdon, UK).

Statistical analysis

Unless otherwise noted, values are presented as mean and standard deviation (SD) for continuous variables and as percentages for categorical variables. The two dose groups (5 mg/day and 7.5 mg/day) were combined for all analyses since there were only 4 subjects in each group receiving 5 mg/day and the doses were similar when considered on a mg/kg basis (0.107 ± 0.015 mg/kg for SCD patients and 0.104 ± 0.012 mg/kg for healthy subjects, $p = 0.66$). Platelet biomarker levels

Table 1
Baseline Demographic Characteristics for Healthy Subjects and Patients with Sickle Cell Disease.

Characteristic/measurement	Healthy Subjects (N = 13)	Patients with SCD (N = 13)[a]
Age (years), mean (SD)	26.7 (5.7)	29.1 (9.3)
Range (years)	19-42	19-50
Male gender, n (%)	7 (53.8)	8 (61.5)
Ethnicity, n (%)		
African descent	3 (23.1)	13 (100.0)
Asian	1 (7.7)	0 (0.0)
White	9 (69.2)	0 (0.0)
Weight (kg)		
Mean (SD)	64.5 (9.6)	63.3 (9.5)
Range (minimum – maximum)	50.5 – 82.1	52.2 – 87.2

Abbreviations: N = total number of subjects/patients; n = number of subjects/patients in that category; SCD = sickle cell disease; SD = standard deviation.
[a] N = 13 at baseline and 12 at study end.

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