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A new oral antiplatelet agent with potent antithrombotic properties: Comparison of DZ-697b with clopidogrel in a randomised phase I study

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Summary

DZ-697b is a new orally active antiplatelet agent that inhibits collagen and ristocetin-mediated platelet activation. It does not require metabolism to generate its active compound and has a safer profile than clopidogrel in pre-clinical studies. We compared the antithrombotic effects and bleeding time prolongations of three DZ-697b doses with clopidogrel 300 mg. In a four-treatment, three-period, crossover-design, 20 healthy subjects (31 ± 7 years, 85% males) were randomised to single oral doses of DZ-697b (60, 120 and 360 mg), and clopidogrel (300 mg) ($n=15$ in each treatment with crossing-over). Antithrombotic effects were assessed by measuring six-hour post-dose changes from baseline in thrombus size in the Badimon chamber and platelet adhesion using Diamed Impact-R platelet function assay. Bleeding times were also measured pre-dose and at six hours post-dose. DZ-697b caused dose-dependent reductions in thrombus size at both high- and low-shear rates (mean reductions at 60, 120 and 360 mg doses of: 13.0%, 18.7%, 26.4% and 11.4%, 12.7%, 22.1% respectively, $p<0.05$ for all). Effect of clopidogrel (reductions of 18.7% and 11.0% respectively, $p<0.05$ for both) was closest to DZ-697b 120 mg. Reductions in platelet adhesion were also dose-dependent. Bleeding time ratio from baseline were shorter with DZ-697b versus clopidogrel (1.3, 1.4 and 1.5 versus 1.9, $p<0.05$ for all). The oral agent DZ-697b shows potent, dose-dependent, antithrombotic effects that are comparable to 300 mg clopidogrel at the 120 mg dose. Despite having equal or greater anti-platelet potency than 300 mg clopidogrel, bleeding time prolongations are significantly shorter with DZ-697b.

Keywords

Antiplatelet agents; thrombosis; clinical studies; antiplatelet drugs

Introduction

Antiplatelet therapy plays a central role in the treatment of acute coronary syndromes (ACS) and ischaemic stroke and in the prevention of thrombosis after intracoronary stent placement. Clopidogrel has been shown to be more effective in reducing the risk of ischaemic stroke, myocardial infarction or vascular death than aspirin (1), and their combination is even more beneficial in preventing recurrent events in ACS patients (2). However, clopidogrel's platelet inhibitory effects and clinical benefits are not uniformly

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observed in all treated patients. This has led to the emergence of terms like clopidogrel “resistance” and “non-responders”, provoking investigations into the feasibility and efficacy of administering higher doses (3, 4) and even of clopidogrel reloading in patients already on chronic treatment (5–7). The evidence for improved clinical outcomes of these strategies is limited to date (8, 9). Newer therapies such as direct thrombin and factor Xa inhibitors, nitric oxide donors and more potent antiplatelet agents are in various stages of development (10–18). Among the new antiplatelet drugs, P2Y₁₂ inhibitor prasugrel was recently approved by the Food and Drug Administration (FDA) and ticagrelor (AZD6140) is in advance stages of development. These agents have shown more potency but also a higher risk of bleeding than clopidogrel (15–18). Clopidogrel and prasugrel are both thienopyridine derivatives that inhibit the P2Y₁₂ receptor and share the property of being prodrugs that require metabolism in order to become active. This requirement, at least with clopidogrel, can lead to variability in the degree and the onset-time of its action (19).

DZ-697b is a new, oral antiplatelet agent that inhibits collagen- and ristocetin-mediated platelet aggregation (20, 21). Unlike clopidogrel, DZ-697b does not require metabolism for the generation of an active compound, and preclinical evidence suggests that it has a lower risk of bleeding at therapeutic doses than aspirin (22). The overall profile in early studies suggests DZ-697b could provide a better balance between its therapeutic effect and bleeding risk than clopidogrel.

We investigated the antithrombotic properties and bleeding time prolongations of three doses of DZ-697b in comparison with a 300 mg loading dose of clopidogrel.

Materials and methods

Study design and population

The study protocol used a randomised, assessor-blind, four-treatment, three-period, crossover design. Healthy, non-smoking subjects between the ages of 18–55 years underwent screening, including detailed medical history, physical examination, laboratory blood tests and 12-lead electrocardiogram (ECG). Individuals with clinically significant abnormal test results or a body mass index outside the range of 19–31 kg/m² were excluded. Qualified subject (n=20) were randomised to one of four treatment sequences, each of which consisted of three of the following four possible treatments: 60 mg, 120 mg and 360 mg of DZ-697b and 300 mg of clopidogrel. Washout periods of 10–14 days separated treatments within each sequence (Fig. 1). This study design yielded 15 subjects in each of the four treatment groups. Sample size determination was not based on any formal power calculation. However, based on previous experience (12, 23, 24), the number of subjects was considered reasonable for the achievement of the study objective using a crossover design.

Participants abstained from consuming any systemic drugs or topical medications with significant systemic absorption for a minimum of 30 days prior to their dosing. On the morning of the first treatment session, subjects reported to the Mount Sinai School of Medicine (MSSM) Clinical Research Center (CRC) after fasting overnight. Following baseline assessment of all pharmacokinetic and pharmacodynamic parameters (including measurement of vital signs, ECG, blood tests, platelet function test with Impact-R, thrombus formation with perfusion chamber, plasma drug levels, and bleeding time), subjects were randomised to their treatment sequence and given the assigned treatment dose. After 6 hours (h) of the dosing, all of the pharmacokinetic and pharmacodynamic assessments performed at baseline were repeated. Subjects were then discharged from the CRC and began their first washout period, during which time they were prohibited from taking any medications, especially those with antiplatelet or anticoagulant activity. After 10–14 days of wash-out,

subjects returned to the CRC for their second treatment session. Protocol for the second and third treatment sessions was identical to the first.

The list of randomisation scheme was generated using permuted-block randomisation method at the beginning of the study and was accessible only to the study nurse and the principal investigator. All other investigators were kept blinded to any treatment-related information until the completion of data analysis at the end of the study. This included the fellows responsible for subject recruitment and those involved in data acquisition and/or analyses.

The study was approved by the Institutional Review Board of MSSM, and all subjects provided written informed consent prior to their participation in the study.

Blood sampling

A 19-gauge intravenous catheter was placed in the antecubital vein of the subjects on the morning of the study-day and used for all blood draws. After an initial 2 ml discard, blood samples were collected for plasma drug levels (lithium heparin tubes) and platelet function studies (3.2% sodium citrate tubes). The catheter was then connected to the Badimon chamber setup for the performance of the perfusion studies. The intravenous line was kept patent between the baseline and post-dose time-points using normal saline flushes.

Antithrombotic assessment

The antithrombotic effects of the drugs were assessed by measuring the difference in the size of the thrombus formed, before and 6 h after dosing, using the Badimon perfusion chamber (11, 12, 23, 24). This model consists of three small Plexiglass chambers in series, each containing a piece of porcine aorta stripped of the intimal layer to expose the underlying thrombogenic medial layer. The first chamber had a shear rate of 212 s^{-1} while the remaining two were $1,690 \text{ s}^{-1}$, mimicking venous and moderately stenotic arterial flow conditions respectively. A peristaltic pump at the distal end drew blood directly from an intravenous catheter and passed it over the porcine aorta at a constant rate of 10 ml/minute (min) for 5 min. The tissues were then fixed in 4% paraformaldehyde, processed for slide preparation and stained with combined Masson's trichrome elastin (CME). Image acquisition of the sections and measurement of thrombus area was carried out using Image Pro Plus software (version 4.5) and the data averaged to generate one low-shear and one high-shear result per time-point per subject. Labelling of slides and images were coded to conceal subject and treatment information in order to ensure blind assessment.

Platelet function test

Treatment effects on platelet adhesion under arterial and venous laminar flow conditions were assessed using a Diamed Impact-R device, according to manufacturer's instructions (Biomed/Bio-Rad Laboratories, Hercules, CA, USA) (25). This device can be used to assess primary haemostatic function and the effect of anti-platelet treatments. Blood samples (130 μl each) were subjected to shear forces of 500 s^{-1} and $1,800 \text{ s}^{-1}$ in separate polystyrene wells to mimic venous and arterial laminar flow conditions, respectively, resulting in adhesion of platelets to well surfaces. Excess blood was washed away and the platelets stained with May-Grünwald stain for image acquisition and analysis of the percent of well-surface covered with platelets. Samples were run in duplicates and the results were averaged, expressed as % surface coverage.

Bleeding time

Using the Surgicutt Bleeding Time procedure, bleeding times were measured at baseline just before drug administration on the first treatment day, and 6 h after dosing during each

treatment visit. While maintaining a pressure of 40 mmHg in a sphygmomanometer cuff, a disposable Surgicutt device was used to make a standardised incision (each 5 mm long and 1 mm deep) on the volar surface of the forearm, perpendicular to the antecubital crease. Time until cessation of bleeding (to the nearest 15 seconds) was recorded as the bleeding time.

Clotting parameters

Blood samples for prothrombin time (PT) and activated thromboplastin time (aPTT) were collected in 3.2% sodium citrate tubes and sent to the clinical laboratory of Mount Sinai Hospital, where the clotting parameters were measured using an automated coagulometer (STA-R, Diagnostica Stago, Parsippany, NJ, USA).

Pharmacokinetics

Blood samples for the measurement of plasma drug concentration were collected in pre-chilled lithium heparin tubes just prior to and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after dosing. Immediately following collection, samples were centrifuged at 1,500 g for 10 min at 4°C to obtain platelet-poor plasma, which was then separated into aliquots and stored at -20°C until analysis. All pharmacokinetic assessments were performed by BioDynamics Research Ltd. (Cambridge, UK).

Statistical analysis

The thrombus formation and pharmacodynamic parameters, along with respective changes from baseline, were summarised by treatment. A general linear mixed model for a four-treatment, three-period, incomplete block crossover design was used to assess the effect of DZ-697b on thrombus formation and pharmacodynamic parameters. Fixed effects included sequence, period and treatment, and subjects nested in sequence were included as random effects. The model also included the baseline value as a covariate. The paired t-test was performed comparing post-dose assessment with its pre-dose baseline for each treatment group. Scatter plots of DZ-697b plasma concentrations versus the pharmacodynamic parameters were generated, and if a relationship was apparent, regression analysis was used to further explore this relationship. Bleeding time and standard coagulation parameters, along with the ratios to baseline were summarised by treatment. The bleeding time ratio was also summarised by treatment using the geometric mean and 95% confidence interval (CI), computed by exponentiating the arithmetic mean and CI on the log transformed ratios. The bleeding time ratio was compared among the treatment groups using similar methodology as for the thrombus formation. Prior to statistical analysis of the bleeding time ratio, the bleeding time was log transformed.

All results are expressed as mean [95% CI] unless specified otherwise and were considered statistically significant if p-value was less than 0.05.

Results

Twenty healthy subjects were recruited from the Mount Sinai School of Medicine and randomised to the four treatment sequences with the cross-over design, resulting in a final count of 15 in each treatment arm. Characteristics of the study participants are presented in Table 1. All subjects completed the study without any major adverse events during the study or in the 30-day followup. Five minor adverse events were recorded including two incidents of diarrhea (after clopidogrel and after 360 mg DZ-697b treatments in the same subject), and three reports of skin bruising following minor, everyday trauma (one in clopidogrel and two in 360 mg DZ-697b treated subjects). All the adverse events resolved fully within five days of their onset.

Antithrombotic effect

Treatments with both DZ-697b and clopidogrel led to significant reductions in thrombus size compared to baseline (Table 2). Administration of DZ-697b resulted in dose-dependent decreases in thrombus that were greater under high-shear rate conditions (Fig. 2). Maximum effects were seen with 360 mg DZ-697b, which were significantly greater than clopidogrel (change in thrombus size of -26.4% versus -18.7% at high-shear and -22.1% versus -11.0% at low-shear respectively, $p < 0.01$ for both). Antithrombotic effects of DZ-697b 120 mg were closest to clopidogrel under high-shear flow (-18.7% versus -18.7%, respectively) whereas under low-shear conditions, 60 mg DZ-697b gave results closest to clopidogrel effect (-11.4% versus -11.0%, respectively). However, the differences between DZ-697b 60 mg, 120 mg and clopidogrel did not achieve statistical significance.

The decrease in thrombus size from baseline was greater with increasing DZ-697b plasma concentration (full partial correlation $r^2 = 0.551$ and 0.285 at high- and low-shear rates, respectively; overall slopes [95% CI] $1.082 [1.3644, 0.7989]$ and $0.424 [0.6503, 0.1970]$ $\mu\text{m}^2\text{ml/ng}$, respectively).

Antiplatelet effect

Platelet adhesion was inhibited in a pattern similar to the one seen in the perfusion studies, with dose-dependent DZ-697b effects that were greater under high-shear rate (Table 2). The 360 and 120 mg doses and clopidogrel treatment reduced platelet adhesion significantly under high-shear (-16.8%, -12.1% and -12.0%, respectively, $p < 0.05$ for each). Under low-shear rate, only the highest dose of DZ-697b had significant effect (-9.9 %, $p < 0.05$).

Bleeding times

All treatments led to prolongations in bleeding time with the maximum increase seen with clopidogrel. The bleeding time ratios with 60, 120 and 360 mg doses were 1.31, 1.44 and 1.50, respectively. Clopidogrel caused a greater prolongation in bleeding time than even the highest dose of DZ-697b (1.93, $p < 0.05$ versus all DZ-697 doses) (Table 2).

Clotting parameters

All clotting parameters were within the normal ranges at baseline assessment and no significant changes were observed in PT, aPTT or INR following treatment with either DZ-697b or clopidogrel. This was to be expected since both drugs inhibit platelets but have no effect on the coagulation pathway (Table 2).

Pharmacokinetics

Plasma concentrations of the three DZ-697b doses, measured over a period of 24 h, are presented in Figure 3. Each DZ-697b dose was rapidly absorbed with plasma concentrations peaking around 1 h. An overall dose-proportional increase in plasma concentration was observed with mean peak concentrations of 688 [583 – 793], 1341 [1178 – 1505] and 3981 [3198 – 4784] ng/ml showing a near identical ratio to that between the 60, 120 and 360 mg doses, respectively. Plasma drug levels were halved by 8 h (304 [266 – 343], 658 [583 – 733] and 1864 [1689 – 2039] ng/ml, respectively) and just under 10% of the respective peak concentration after 24 h (61 [47 – 74], 124 [97 – 150] and 360 [277 – 444] ng/ml, respectively).

Discussion

Our study shows that the new oral antiplatelet agent DZ-697b has potent and dose-dependent antithrombotic properties. Thrombus reduction and platelet inhibition by

DZ-697b in our study were comparable to 300 mg clopidogrel at 60 mg and 120 mg and significantly stronger than clopidogrel at 360 mg. The inhibitory effects were evident under both high- and low-shear rates, mimicking rheologies of arterial and venous flow conditions, respectively. Under high-shear conditions the effects were more pronounced, which is understandable given the greater role of platelets in arterial thrombosis as compared to thrombus formation in veins with low shear conditions.

Although DZ-697b dosing resulted in significant increase in bleeding time from baseline values, these prolongations were substantially shorter than what was observed after clopidogrel treatment. Even the highest dose DZ-697b did not prolong bleeding time as much as clopidogrel. An earlier study where oral administration of DZ-697b (3 – 200 mg/kg) was compared to aspirin (100–300 mg/kg) in guinea pigs showed similar findings, with DZ-697b exhibiting antithrombotic effects at doses much lower than those inducing gastric bleeding (22). In that study DZ-697b exhibited antithrombotic effects at doses over 10 mg/kg but did not affect gastric bleeding significantly until 200 mg/kg. Aspirin's antithrombotic effects were observed between 100–300 mg/kg with a significant increase in gastric bleeding noted at 200 mg/kg and above.

Successful antithrombotic therapy is a balancing act between maximising efficacy that yields reduction in adverse outcomes, and minimising bleeding risk that would lead to adverse events. This is partly the reason behind the success of clopidogrel and aspirin, each of which exerts significant, but relatively weak antiplatelet effect in comparison with agents like abciximab (26, 27) and prasugrel (17, 28), but balances it with an acceptable risk of bleeding that does not require cumbersome monitoring. However, like most therapeutic interventions, a small yet substantial proportion of clopidogrel- and aspirin-treated patients fall on the extremes of the distribution curve and include non-responders on one end and those with bleeding complications on the other. Furthermore, the need for metabolism to become biologically active only causes unavoidable delays in the onset of clopidogrel's action.

DZ-697b is active in its native form and differs from both clopidogrel and aspirin in its mechanism of action, exerting its effect by blocking collagen- and ristocetin-mediated phosphorylation of FcR γ -chain (20). The FcR γ -chain phosphorylation by collagen affects a wide array of platelet functions with the involvement of glycoproteins such as Gp-Ib and Gp-VI (29–33). Glycoprotein-Ib for instance, has been reported to physically couple with the FcR γ -chain leading to the adhesion and activation of platelets (34). The antithrombotic properties of DZ-697b, coupled with its shorter than clopidogrel bleeding time prolongations, give it the potential to be a future therapeutic option. A loading dose of 600 mg clopidogrel is increasingly administered in clinical practice nowadays, but the 300 mg dose was selected as a comparator in this study because to date that is the only loading dose approved by the FDA. Published reports show that higher doses of clopidogrel result in greater platelet inhibition than 300 mg (4, 7, 9) and should theoretically result in greater antithrombotic effect in the perfusion chamber model used in this study, although this has never been tested. However, the higher doses of clopidogrel are also likely to cause greater and more consistent prolongations in the bleeding time (35).

The new antiplatelet agent DZ-697b shows potent antithrombotic properties with lower bleeding time prolongations than 300 mg clopidogrel in healthy volunteers. The full potential of this new compound warrants further investigation in larger clinical studies.

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What is known about this topic?

- DZ-697b is a new, orally active anti-platelet agent with limited published data, primarily from small animal studies.
- DZ-697b acts by selectively inhibiting collagen- and ristocetin-induced platelet aggregation.
- In guinea pig model, DZ-697b showed anti-thrombotic effects at doses much lower than those inducing gastric bleeding in comparison with aspirin.

What does this paper add?

- This study is the first randomised human study with DZ-697b, presenting data related to efficacy and safety.
- This report presents information about the anti-thrombotic potential of this compound in comparison with a loading dose of clopidogrel following dosing in human subjects.
- It also reports the effect of DZ-697b dosing on bleeding time prolongation and compares it with a loading dose of clopidogrel.

Figure 1. Randomised, assessor-blind, four-treatment, three-period, crossover study design
Treatments within each sequence were separated by 10–14 days of washout.

Figure 2. Antithrombotic effects of DZ-697b and clopidogrel, assessed by comparing post-treatment thrombus size versus baseline in the Badimon perfusion chamber, at low and high-shear rates

Data is expressed as mean percent of baseline [95% CI]. * $p < 0.05$ versus baseline, † $p < 0.05$ versus baseline and clopidogrel.

Figure 3. Plasma concentrations of DZ-697b following administration of single oral doses (60, 120 and 360 mg) are presented over a 24 hour period
Results are presented as mean [95% CI].

Table 1

Baseline characteristics.

	DZ-697b		Clopidogrel	
	60 mg (n=15)	120 mg (n=15)	360 mg (n=15)	300 mg (n=15)
Age (years)	30.4 ± 7.7	31.4 ± 7.7	32.0 ± 6.4	29.6 ± 5.2
Males	12 [80 %]	13 [86.7 %]	13 [86.7 %]	13 [86.7 %]
BMI (kg/m ²)	26.0 ± 3.9	26.4 ± 3.5	24.8 ± 3.0	25.8 ± 3.5
Systolic B.P. (mm/Hg)	116.7 ± 10.7	120.4 ± 9.5	120.1 ± 10.9	116.3 ± 10.9
Diastolic B.P. (mm/Hg)	69.3 ± 6.7	70.6 ± 6.6	69.9 ± 7.3	68.9 ± 6.7
Platelet count (10 ³ /μl)	260.0 ± 41.5	255.9 ± 30.1	257.6 ± 44.7	257.9 ± 46.1
Glucose (mg/dl)	79.1 ± 6.5	81.1 ± 8.2	82.4 ± 8.3	82.3 ± 8.0
Total cholesterol (mg/dl)	159.2 ± 29.8	159.7 ± 36.6	160.6 ± 34.1	156.6 ± 34.1
Triglycerides (mg/dl)	91.4 ± 53.5	83.8 ± 49.1	85.9 ± 44.5	88.6 ± 48.6

Table 2

Efficacy and safety parameters.

	DZ-697b		Clopidogrel	
	60 mg (n=15)	120 mg (n=15)	360 mg (n=15)	300 mg (n=15)
Thrombus size (% change from baseline), mean [95% CI]				
High shear rate	-13.0 [-16.4, -9.6] *	-18.7 [-23.7, -13.3] *	-26.4 [-31.5, -21.1] †	-18.7 [-26.6, -10.9] *
Low shear rate	-11.4 [-16.5, -6.2] *	-12.7 [-16.3, -9.5] *	-22.1 [-28.0, -16.3] †	-11.0 [-15.2, -6.9] *
Platelet adhesion (% change from baseline), mean [95% CI]				
High shear rate	-7.3 [-16.4, 1.8]	-12.1 [-20.4, -3.8] *	-16.8 [-25.9, -7.6] *	-12.0 [-19.2, -4.7] *
Low shear rate	-4.7 [-14.5, 5.4]	-6.0 [-12.7, 0.9]	-9.9 [-15.0, -4.6] *	-5.8 [-13.4, 1.8]
Clotting parameters (ratio to baseline), mean [95% CI]				
PT	1.01 [0.99, 1.03]	1.01 [0.98, 1.03]	1.01 [0.99, 1.03]	1.01 [0.99, 1.03]
aPTT	0.99 [0.95, 1.03]	0.98 [0.95, 1.01]	0.99 [0.97, 1.02]	0.98 [0.95, 1.01]
INR	1.01 [0.99, 1.04]	1.02 [0.98, 1.06]	1.02 [0.98, 1.06]	1.01 [0.98, 1.03]
Bleeding time ratio geometric means [95% CI]	1.3 [1.1 – 1.6] †	1.4 [1.2 – 1.7] †	1.5 [1.2 – 1.9] †	1.9 [1.5 – 2.5] *

* p<0.05 versus pre-dose value,

† p<0.05 versus pre-dose and clopidogrel.