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Pharmacodynamics, pharmacokinetics, and safety of the oral reversible $P2Y_{12}$ antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin

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KEYWORDS
Platelets;
Drugs;
Atherosclerosis

Aims This double-blind, parallel-group study was conducted to assess the pharmacodynamics, pharmacokinetics, and safety of AZD6140, the first oral, reversible adenosine diphosphate (ADP) receptor antagonist.

Methods and results Patients ($n = 200$) with atherosclerosis were randomized to receive AZD6140 50, 100, or 200 mg twice daily (bid) or 400 mg daily (qd) or clopidogrel 75 mg qd for 28 days. All groups received aspirin 75–100 mg qd. AZD6140 (100 and 200 mg bid, 400 mg qd) rapidly and nearly completely inhibited ADP-induced platelet aggregation after initial dosing (day 1) and at day 28. On day 1, peak final-extent inhibition of platelet aggregation (IPA) was observed 2–4 h post-dose with AZD6140, whereas clopidogrel minimally inhibited platelet aggregation (mean percentage IPA $< 20\%$, all time points). Four hour post-dose at steady state, the three higher doses of AZD6140 produced comparable final-extent mean percentage IPA ($\sim$90–95%), which exceeded that with AZD6140 50 mg bid or clopidogrel ($\sim$60%). AZD6140 was generally well tolerated. All bleeding events, except one in a patient receiving 400 mg qd, were minor and of mild-to-moderate severity.

Conclusion AZD6140 100 and 200 mg bid were well tolerated and were superior to AZD6140 50 mg bid and clopidogrel 75 mg qd with regard to antiplatelet efficacy.

Introduction

Oral antiplatelet agents—particularly aspirin and the thienopyridines clopidogrel and ticlopidine—constitute a cornerstone of therapy for vascular disease given the integral role of platelets in the progression of atherosclerosis and in acute clinical events including myocardial infarction, ischaemic stroke, and sudden death.[1–3] Aspirin and the thienopyridines each significantly reduce the risk of these events when given as daily therapy.[4–6] Risk reduction is greater with adjunctive use of clopidogrel and aspirin than with aspirin alone,[5,7,8] a finding consistent with the drugs' complementary mechanisms of action. Aspirin inhibits cyclo-oxygenase to reduce the production of the platelet activator thromboxane A_2. The thienopyridines inhibit multiple pro-aggregatory actions of the platelet agonist adenosine-5′-diphosphate (ADP) by blocking the $P2Y_{12}$ platelet ADP receptor.[9]

Although these antiplatelet agents have improved the management of atherosclerotic disease, additional therapeutic options are needed. Many patients experience thromboembolic events despite daily antiplatelet therapy, and aspirin and clopidogrel resistance have been observed.[1,9–13] Additional limitations of the thienopyridines, which are prodrugs, include high interpatient variability in plasma concentrations and antiplatelet effects, relatively modest inhibition of the *ex vivo* platelet aggregation response to ADP, irreversible binding to $P2Y_{12}$ receptors such that recovery of platelet function is precluded, and toxicities including thrombotic thrombocytopenic purpura and neutropenia.[14–16]

The oral, reversible $P2Y_{12}$ receptor antagonist AZD6140 is being developed for the prevention of thromboembolic events in patients with atherosclerosis. AZD6140 is the first of a new chemical class of antiplatelet agents, the cyclopentyltriazolopyrimidines. Like the thienopyridines, AZD6140 blocks the platelet $P2Y_{12}$ receptor to inhibit ADP's prothrombotic effects. Unlike the thienopyridines, which are irreversible antagonists, AZD6140 binds reversibly

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to the $P2Y_{12}$ receptor and nearly completely inhibits ADP-induced platelet aggregation *ex vivo*. Also unlike the thienopyridines, AZD6140 is orally active without the requirement for metabolic activation. AZD6140 has one known active metabolite that is present in blood at about one third the concentration of the parent in studies in healthy volunteers (AstraZeneca data on file, Phase I studies SC-532-5169, SC-532-5171). This metabolite is approximately as potent as AZD6140 at blocking the $P2Y_{12}$ receptor *in vitro* and is thought to contribute to antiplatelet effects after oral dosing with AZD6140.

In studies in healthy volunteers, AZD6140 given as single oral doses of 100–400 mg had linear pharmacokinetics, nearly completely inhibited platelet aggregation 2 h post-dose with a lessening of inhibition over the 24 h post-dose period, and was well tolerated.[17] This randomized, double-blind, parallel-group study was conducted to assess the pharmacodynamics, pharmacokinetics, safety, and tolerability of AZD6140 with aspirin relative to those of clopidogrel with aspirin in patients with atherosclerotic disease. A range of AZD6140 doses was assessed with the aim of identifying doses for further investigation in larger clinical studies.

Methods

Patients

Males and postmenopausal or surgically sterile females ages 25–85 years were eligible for the study, if they had received aspirin 75–100 mg daily during at least 2 weeks before randomization for confirmed atherosclerotic disease as demonstrated by (i) a history of coronary artery disease with coronary artery stenosis $\geq$50% on coronary angiogram or previously documented myocardial infarction occurring $\geq$3 months before randomization and/or (ii) peripheral artery occlusive disease (i.e. effort-induced claudication of presumed atherosclerotic origin and ankle/brachial systolic blood pressure ratio $\leq$0.85 in either leg at rest) or history of peripheral artery occlusive disease with peripheral vascular surgery or other intervention and/or (iii) carotid, vertebral, or intracerebral artery stenosis $\geq$50%, or previously documented ischaemic non-disabling stroke with a modified Rankin score $\leq$1, or a transient ischaemic attack with cerebral artery stenosis $\geq$50% occurring $\geq$3 months before randomization. Exclusion criteria included acute coronary syndrome within 3 months or any percutaneous intervention with balloon or stent within 4 months before randomization, conditions associated with increased risk of bleeding, screening creatinine level $\geq$1.2 times the upper limit of normal, haemoglobin $\geq$5% below the lower limit of normal, platelet count $<125 \times 10^9$/L, known active liver disease or screening laboratory tests indicative of liver disease, and use of an anticoagulant within 10 days or antiplatelet therapy other than aspirin within 7 days before randomization.

Procedures

This randomized, double-blind, double-dummy study performed at 13 sites across Denmark, Hungary, and Norway was conducted in a manner consistent with Good Clinical Practice guidelines and the Declaration of Helsinki. The study was approved by the Ethics Committees in participating countries, and all patients provided written informed consent. Eligible patients were randomized to receive AZD6140 (50 mg bid, 100 mg bid, 200 mg bid, or 400 mg qd) or clopidogrel 75 mg qd for 28 days. Randomization was done by assigning patients sequentially to a code from a computer-generated randomization list generated by AstraZeneca Research and Development, Charnwood. Blinding of all study treatments was ensured by the provision of one capsule (clopidogrel or placebo) and three tablets (AZD6140 or placebo) daily for all treatment arms using a double-dummy design. All patients received, in addition to AZD6140 or clopidogrel, aspirin 75–100 mg qd maintained at a stable dose for a given patient. Clinic visits occurred at screening, randomization (day 1), and on days 7, 14, 21, and 28.

Medications prohibited during the study included heparin, low-molecular-weight heparin, oral anticoagulants, fibrinolytic agents, glycoprotein IIb/IIIa inhibitors, prostacyclin (PGI_2), antiplatelet therapies other than the aspirin taken with study medication, digoxin, cytochrome P450 inhibitors or substrates with a narrow therapeutic index, and non-selective non-steroidal anti-inflammatory drugs.

Measures

Inhibition of platelet aggregation

The main pharmacodynamic measure was the inhibition of ADP-induced platelet aggregation as measured in duplicate by optical aggregometry of platelet-rich plasma (PRP) obtained from blood samples taken pre-dose, post-dose at 2, 4, 8, and 12 h on days 1, 14, and 28, and post-dose at 24 h on days 14 and 28. On day 28, no drug was administered at the 12 h time point for the bid regimens. Both final extent of aggregation and maximal extent of aggregation were measured in response to 20 μM ADP. Final extent of inhibition of platelet aggregation (IPA) was determined 6 min after the addition of 20 μM ADP. Final-extent IPA is mediated primarily by the $P2Y_{12}$ receptor, whereas maximal-extent IPA depends on both $P2Y_1$ and $P2Y_{12}$ receptors and, therefore, is only partly modifiable by $P2Y_{12}$ receptor antagonists.[18] Effects of study medication on the inhibition of 4 μg/mL collagen-induced platelet aggregation were also assessed with the same methods used to assess the inhibition of ADP-induced platelet aggregation.

Blood samples for the measurement of platelet aggregation were drawn from an indwelling venous cannula for repeat sampling or by direct venipuncture. On each sampling occasion, the first millilitre of blood was discarded, and saline was used to keep the cannulae patent. One 15 mL venous blood sample was collected in a plain syringe. From this sample, 7.2 mL was transferred into two tubes, each containing 0.8 mL trisodiumcitrate dihydrate.

PRP was obtained by centrifugation at room temperature for 10 min at 180 *g*. After centrifugation, the upper turbid layer of PRP was removed, and the residual blood was centrifuged for 10 min at 1500 *g* to obtain platelet poor plasma (PPP). The platelet count of the PRP was measured, and PPP was used to adjust the platelet count to 250 000 platelets/μL. Preparation of PRP began within 15 min of obtaining blood samples so that aggregation studies could start 1 h ($\pm$10 min) after the blood sampling.

Bleeding time

Bleeding times were assessed pre-dose on day 1 and 8 h post-dose on day 28 with the Simplate method at a distending venous pressure of 40 mmHg applied with a standard sphygmomanometer cuff. Blood was blotted in a systematic manner with a filter paper disc every 30 s through 30 min and, if blood flow had not ceased, every 60 s thereafter through 60 min.

Pharmacokinetics

The pharmacokinetics of AZD6140 and its active metabolite AR-C124910XX were assessed from blood samples taken pre-dose and post-dose at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h on days 1, 14, and 28 and post-dose at 24 h on days 14 and 28. Blood samples for measurement of drug concentrations were drawn through the same cannula used for pharmacodynamic sampling. The blood was taken into lithium heparin tubes and placed on ice until centrifugation (1500 *g*, 4°C, 10 min) within 30 min of sampling to collect the plasma, which was transferred to plain polypropylene tubes and frozen upright at or below -20°C until analysis at York Bioanalytical Solutions, York, UK. Human heparinized plasma samples were assessed for total concentrations of AZD6140 and

AR-C124910XX by protein precipitation with acetonitrile followed by analysis with reversed-phase liquid chromatography and negative-ion atmospheric pressure chemical ionization tandem mass spectrometry (-APCI/LC/MS/MS). The quantification range and limit of quantification of the method for AZD6140 were 1.0–500 and 1.0 ng/mL, respectively. The corresponding values for the method for the active metabolite were 2.5–500 and 2.5 ng/mL.

Safety and tolerability

The primary tolerability measure was the incidence of adverse events, defined as any untoward medical occurrences developing or worsening after administration of study medication regardless of suspected cause. Reports of adverse events could originate from investigator observations at clinic visits, volunteer self-reports, and volunteer reports as the result of direct questioning by study personnel. Adverse events involving bleeding were classified as major or minor. A major bleeding event was defined as one that occurred in a critical site (e.g. intracranial, intraocular, spinal, pericardial, joint, or retroperitoneal), was clinically overt and led to the transfusion of $\geq$2 units of packed red cells of whole blood, was clinically overt and associated with a fall in haemoglobin $\geq$20 g/L, or was fatal. All other bleeding events were classified as minor. All adverse events including bleeding events were classified by the investigator as being mild (easily tolerated), moderate (causing discomfort that interferes with normal activities), or severe (incapacitating, preventing normal activities).

Other safety and tolerability assessments included 12-lead electrocardiograms (ECGs) obtained at screening as well as pre-dose and 3 h post-dose on days 1, 14, and 28, clinical laboratory tests (haematology, clinical chemistry, urinalysis) on days 1, 7, 14, 21, and 28, and vital signs on days 1, 7, 14, 21, and 28.

Statistics

A total of 200 patients were randomized to study treatment. With 40 patients per group, the study had precision for estimating the mean percentage IPA for each dose group in the study within $\pm$10% and the ability to distinguish differences of $\sim$8–12% between the mean of any AZD6140 group and the mean of the clopidogrel group. The primary outcome variable of interest was the final extent percentage inhibition of ADP-induced platelet aggregation, as this measure best reflects $P2Y_{12}$ receptor blockade. No formal hypothesis testing was undertaken, but confidence intervals were provided for the primary outcome variable to assist in the interpretation of results. All other data were summarized with descriptive statistics. All patients, who were randomized to treatment and received at least one dose of study medication, were included in the analysis of platelet aggregation, bleeding time, pharmacokinetics, and safety outcome variables.

Inhibition of platelet aggregation

Mean percentage inhibition of ADP- and collagen-induced platelet aggregation (final extent and maximal extent) for each measured time point on days 14 and 28 was summarized as percentage change from the pre-dose baseline aggregation value on day 1. Percentage-inhibition values in the range of 0–100% were recorded; any value falling outside that range was truncated to the appropriate limit. Differences in least squares mean values between treatment groups were calculated from an analysis of covariance (ANCOVA) with factors for treatment group and country and a covariate for baseline platelet aggregation. The ANCOVA model accounted for the expected heterogeneity of variance within each treatment group. The treatment group comparisons of primary interest were the four pairwise comparisons with clopidogrel. No adjustment to the significance levels and corresponding confidence intervals were made.

Bleeding times

Mean and median bleeding times on days 1 and 28 were calculated. Bleeding times of 60 min or longer were recorded as $\geq$60 min rather than the exact value.

Pharmacokinetics

For AZD6140 and its active metabolite, area under the plasma concentration vs. time curve (AUC), maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), apparent terminal half-life ($t_{1/2}$), and total plasma oral clearance (CL/F) were estimated using actual sampling times in non-compartmental analyses. The AUC was calculated using the linear trapezoidal rule. The $t_{1/2}$ was calculated as ln (2)/k. The CL/F was calculated as dose/AUC. Pharmacokinetic parameters were summarized for each treatment group as a function of gender or age ($\leq$65 years, $>$65 years).

Safety and tolerability

Adverse events, clinical laboratory tests, vital signs, and results of 12-lead ECGs were summarized for each treatment group with descriptive statistics for all patients who received at least one dose of study medication.

Results

Patients

From September 2003 to December 2003, 201 patients were randomized, of whom one withdrew before receiving study medication and 200 received study medication (AZD6140 50 mg bid $n = 41$, 100 mg bid $n = 39$, 200 mg bid $n = 37$, 400 mg qd $n = 46$, clopidogrel 75 mg qd $n = 37$) (*Figure 1*). Of the 200 patients who received study medication, 185 completed the study and 15 prematurely withdrew. Reasons for withdrawal were adverse events ($n = 10$), failure to meet eligibility criteria ($n = 1$), and other miscellaneous reasons ($n = 4$) (*Table 1*). Pharmacodynamic, pharmacokinetic, and safety/tolerability data were summarized for all 200 patients who received at least one dose of study medication.

Demographics and baseline clinical characteristics were comparable among treatment groups (*Table 1*). All patients had atherosclerotic disease, although the distributions of patients having particular disorders varied slightly among treatment groups (*Table 1*). Concomitant medications, the most common of which were statins and beta-blockers (*Table 1*), were balanced across treatment groups.

Inhibition of platelet aggregation

AZD6140 inhibited ADP-induced platelet aggregation (final extent) at 2 h post-dose after initial dosing (day 1) and at steady state (day 28) (*Figure 2*). With AZD6140 100 mg bid, 200 mg bid, and 400 mg qd, the magnitude of inhibition at steady state was greater than that with either AZD6140 50 mg bid or clopidogrel (*Figure 2* and *Table 2*). Least squares mean differences between the three higher doses of AZD6140 and clopidogrel in percentage IPA ranged from 25 to 30% on day 14 and from 24 to 31% on day 28 (*Table 2*). The three higher doses of AZD6140 did not substantially differ from one another with respect to mean percentage IPA. A similar pattern of results was observed for maximal-extent IPA although, as expected, inhibition was lower for maximal extent than final extent (*Figure 2*). *Figure 3* shows mean and median percentage IPA (final extent and maximal extent) and 10th, 25th, 75th, and 90th percentile values pre-dose and 4 h post-dose on day 14.

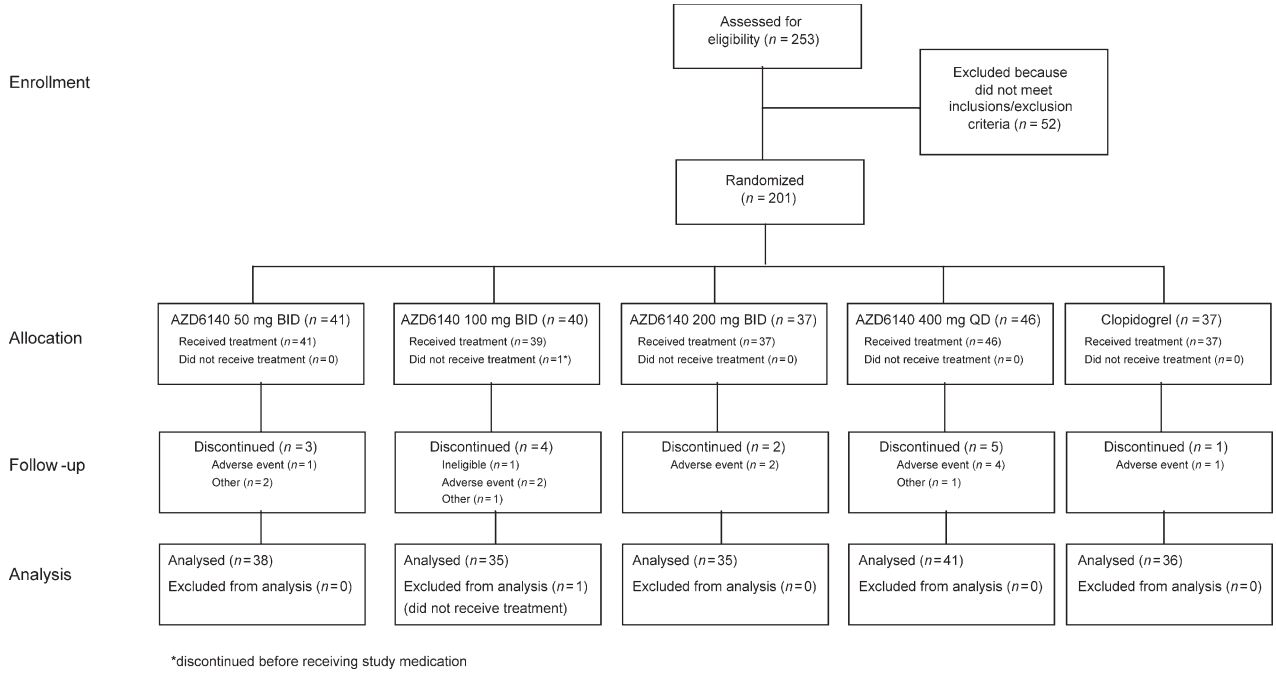


Figure 1 Patient disposition showing enrolment to the completion of study.

Table 1 Patient disposition, demographics, and baseline clinical characteristics

	AZD6140				
	50 mg bid ($n = 41$)	100 mg bid ($n = 39$)	200 mg bid ($n = 37$)	400 mg qd ($n = 46$)	Clopidogrel ($n = 37$)
Patient disposition, *n*					
Randomized	41	40	37	46	37
Entered treatment	41	39	37	46	37
Discontinued early	3	4	2	5	1
Completed study	38	35	35	41	36
Male, *n* (%)	27 (66)	29 (74)	28 (76)	36 (78)	26 (70)
White race, *n* (%)	41 (100)	39 (100)	37 (100)	46 (100)	37 (100)
Mean (SD) age (years)	64 (10.3)	63 (9.3)	64 (7.9)	64 (10.1)	61 (9.4)
Mean (SD) weight (kg)	79 (14.3)	79 (12.9)	83 (18.7)	81 (12.5)	81 (16.9)
Hypertension, *n* (%)	20 (49)	20 (51)	21 (57)	27 (59)	15 (41)
Diabetes, *n* (%)	11 (27)	4 (10)	9 (24)	3 (7)	8 (22)
Atherosclerotic disease, *n* (%)					
Coronary artery disease	26 (63)	20 (51)	28 (76)	31 (67)	29 (78)
Peripheral artery disease	9 (22)	10 (26)	5 (14)	7 (15)	4 (11)
Cerebrovascular disease	4 (10)	3 (8)	3 (8)	3 (7)	4 (11)
Cardiovascular history, *n* (%)					
Myocardial infarction	26 (63)	20 (51)	24 (65)	29 (63)	23 (62)
Stroke	6 (15)	7 (18)	4 (11)	12 (26)	3 (8)
Transient ischaemic attack	3 (7)	1 (3)	3 (8)	1 (2)	2 (5)
Current smoking status, *n* (%)					
Non-smoker	21 (54)	25 (64)	28 (76)	31 (67)	25 (68)
Smoker	19 (46)	14 (36)	9 (24)	15 (33)	12 (32)
Most common concomitant medications, *n* (%)[a]					
Statins	31 (76)	28 (72)	31 (84)	30 (65)	31 (84)
Beta-blockers	21 (51)	19 (49)	24 (65)	27 (59)	24 (65)
Angiotensin-converting enzyme inhibitors	11 (27)	15 (38)	17 (46)	14 (30)	11 (30
Organic nitrates	8 (20)	5 (13)	14 (38)	10 (22)	9 (24)
Dihydropyridine derivatives	9 (22)	7 (18)	10 (27)	12 (26)	5 (14)
Sulfonamides (diuretics)	5 (12)	6 (15)	6 (16)	9 (20)	5 (14)
Angiotensin II antagonists	6 (15)	2 (5)	2 (5)	10 (22)	4 (11)

[a]Concomitant medications used by >20% of patients in any treatment group are listed. Aspirin, which was used by all patients, is not included in this listing.

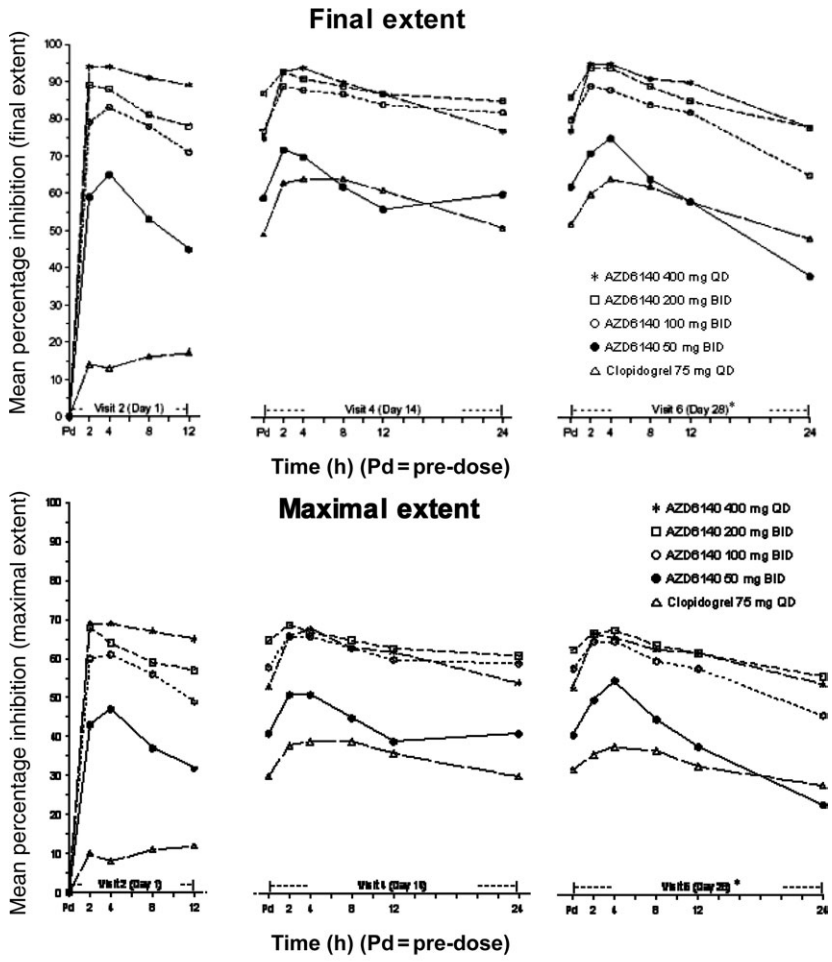


Figure 2 Mean percentage inhibition of ADP-induced platelet aggregation (final extent, top graph; maximal extent, bottom graph) in patients with atherosclerotic disease treated with AZD6140 50 mg bid, 100 mg bid, 200 mg bid, or 400 mg qd or clopidogrel 75 mg qd for 28 days.

Table 2 Least squares mean and median differences between AZD6140 and clopidogrel for percentage inhibition of ADP-induced platelet aggregation (final extent)

Comparison with clopidogrel	n[a]	Least squares mean difference (SEM) and 95% CI	Median difference and 95% CI
Day 14, 4 h post-dose			
AZD6140 50 mg bid	75	7 (5.1) −4, 17	7 −3, 18
AZD6140 100 mg bid	73	25 (4.2) 16, 33	23 14, 33
AZD6140 200 mg bid	72	27 (4.3) 18, 36	25 18, 34
AZD6140 400 mg qd	81	30 (4.1) 22, 38	29 19, 38
Day 28, 4 h post-dose			
AZD6140 50 mg bid	73	11 (4.5) 2, 20	13 3, 23
AZD6140 100 mg bid	70	24 (3.7) 16, 31	25 16, 33
AZD6140 200 mg bid	68	30 (3.5) 23, 37	30 20, 40
AZD6140 400 mg qd	76	31 (3.4) 24, 38	31 23, 40

[a]The combined numbers of patients for each treatment group comparison on therapy.

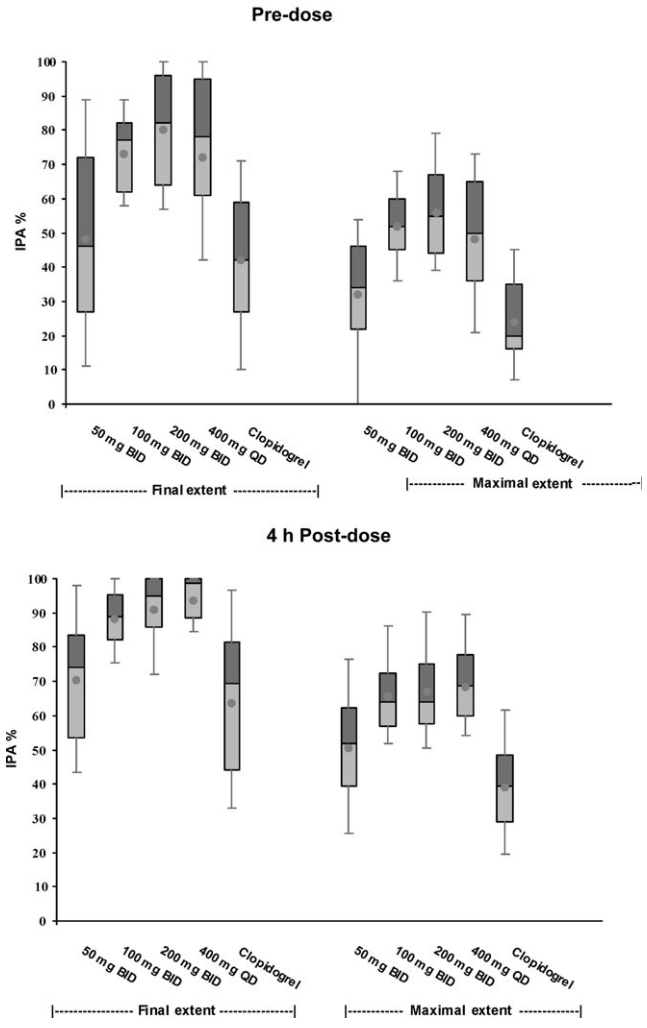


Figure 3 Mean and median percentage IPA (final extent and maximal extent) and 10th, 25th, 75th, and 90th percentile pre-dose (top graph) and 4 h post-dose (bottom graph) on day 14. The line within the box represents the median. The circle represents the mean. Upper and lower edges of the box represent the 75th and 25th percentiles. Upper and lower whiskers represent the 90th and 10th percentiles, respectively.

The pattern of results for the inhibition of collagen-induced platelet aggregation was comparable to that for the inhibition of ADP-induced platelet aggregation (*Figure 4*). The magnitude of inhibition of collagen-induced platelet aggregation was similar to that of maximal-extent ADP-induced aggregation. This finding is in keeping with the sensitivity of ADP-induced platelet aggregation, but not collagen-induced platelet aggregation, to ADP-receptor antagonists.

Bleeding times

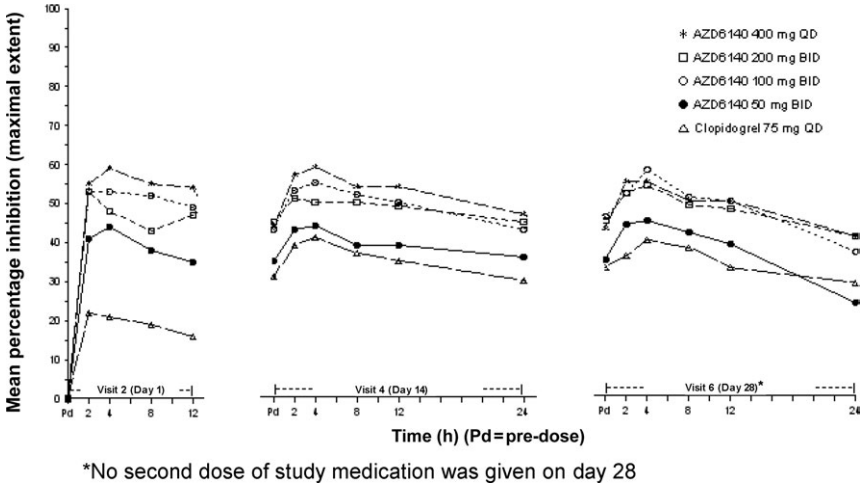
Bleeding times on day 28 were increased relative to the day 1 baseline in all treatment groups (*Table 3*). AZD6140 (all doses) appeared to increase bleeding times to a greater extent than clopidogrel, but no obvious dose–response relationship was observed.

Pharmacokinetics

Plasma concentrations of AZD6140 and its active metabolite AR-C124910XX increased linearly and dose proportionally on day 1 and were stable and predictable at steady state, which was achieved by day 14 (*Table 4* and *Figure 5*). At day 28, AZD6140 200 mg bid and 400 mg qd exhibited slightly greater than dose-proportional pharmacokinetics with dose-normalized AUCs that were ~50% more than dose-proportional and with correspondingly lower CL/F relative to the 50 mg and 100 mg bid regimens. Exposure to AR-C124910XX at steady state was ~35% of exposure to AZD6140. AZD6140 and AR-C124910XX C_{max} and AUC did not vary significantly as a function of sex (male, female) or age (≤65 years, >65 years).

Pharmacokinetic/pharmacodynamic relationship

The onset of maximum IPA effect was rapid and corresponded with the time of maximum plasma concentrations. Increases in dose beyond 100 mg bid resulted in only small additional increases in IPA. The 100 mg bid regimen had peak blood levels of about 800 ng/mL. The 200 mg bid dose, which had concentrations more than two-fold



*No second dose of study medication was given on day 28

Figure 4 Mean percentage inhibition of collagen-induced platelet aggregation (maximal extent) in patients with atherosclerotic disease treated with AZD6140 50 mg bid, 100 mg bid, 200 mg bid, or 400 mg qd or clopidogrel 75 mg qd for 28 days. Final-extent data (data not shown) were comparable to the maximal-extent data.

Table 3 Actual bleeding time minutes (day 28 vs. day 1)

Treatment group	*n*	Median (range)
AZD6140 50 mg bid		
Day 1	41	5.0 (2.0–60.0)
Day 28	38	14.8[a] (3.0–60.0)
AZD6140 100 mg bid		
Day 1	39	5.0 (1.0–10.0)
Day 28	36	17.8[a] (4.8–60.0)
AZD6140 200 mg bid		
Day 1	37	5.5 (2.8–9.5)
Day 28	36	23.0[a] (4.0–60.0)
AZD6140 400 mg qd		
Day 1	46	5.1 (2.5–16.5)
Day 28	43	15.5[a] (2.3–60.0)
Clopidogrel 75 mg qd		
Day 1	37	5.0 (0.0–15.0)
Day 28	36	10.5[a] (3.6–60.0)

[a]At least one bleeding time >60 min was recorded.

higher than the 100 mg bid dose, yielded only a small increase in IPA.

Safety and tolerability

The most common adverse event was bleeding, the incidence of which increased with the three higher doses of AZD6140 compared with AZD6140 50 mg bid and clopidogrel (*Table 5*). One major bleeding event (gastrointestinal haemorrhage with drop in haemoglobin) in a patient receiving AZD6140 400 mg qd was reported. The remaining bleeding events were classified as minor and of mild-to-moderate severity. Bleeding excepted, adverse events reported in at least 10% of patients in any treatment group were dyspnoea, dizziness, headache, and red blood cells in the urine (*Table 5*). The incidence of dyspnoea appeared to increase with increasing dose of AZD6140 (reported in 10% of patients with AZD6140 50 mg bid and 100 mg bid, 16% of patients with 200 mg bid, and 20% of patients with 400 mg qd). Among the 23 AZD6140-treated patients with dyspnoea, 29 instances of dyspnoea (21 considered mild and eight considered moderate) were reported. None of the incidents of dyspnoea was considered to be serious, and none was associated with congestive heart failure or bronchospasm.

No deaths occurred during the study. In the AZD6140 groups, the numbers of patients withdrawing prematurely from the study because of adverse events were one (for a minor bleeding event) for 50 mg bid, two (for dyspnoea/diarrhoea, a minor bleeding event) for 100 mg bid, two (for a minor bleeding event, an overdose) for 200 mg bid, and four (for three minor bleeding events, one major bleeding event) for 400 mg qd. One clopidogrel-treated patient withdrew prematurely because of an adverse event (polyarthritis).

No notable time- or treatment-related changes in any haematology, clinical chemistry, or urinalysis parameters were observed with the exception of changes in mean uric acid levels (increases of 5–10% in all AZD6140 groups and a decrease of ~10% in the clopidogrel group). No treatment-related changes in vital signs or 12-lead ECGs were observed.

Table 4 Mean (coefficient of variation %) pharmacokinetic parameters of AZD6140 and its active metabolite AR-C124910XX

	AZD6140 50 mg bid			AZD6140 100 mg bid			AZD6140 200 mg bid			AZD6140 400 mg qd		
	Day 1	Day 14	Day 28	Day 1	Day 14	Day 28	Day 1	Day 14	Day 28	Day 1	Day 14	Day 28
AZD6140												
n	41	39	38	39	34	33	37	32	35	46	42	39
t_{max} (h)	3.66 (41)	2.54 (56)	3.33 (56)	3.05 (50)	2.82 (74)	2.52 (55)	3.09 (57)	2.61 (69)	2.74 (82)	2.03 (63)	2.41 (149)	2.12 (71)
C_{max} (ng/mL)	287 (70)	375 (50)	387 (57)	594 (55)	810 (41)	798 (59)	1224 (35)	2278 (31)	2200 (41)	3374 (41)	3653 (41)	3827 (42)
AUC (ng h/mL)	1640 (50)	2666 (47)	2688 (56)	3648 (56)	5530 (48)	5337 (45)	7581 (35)	16 364 (39)	15 104 (39)	NA	31 723 (43)	31 338 (53)
CL/F (L/h)	NA	22.9 (45)	23.7 (48)	NA	21.6 (42)	22.6 (44)	NA	13.7 (34)	15.3 (39)	NA	15.0 (41)	15.6 (39)
AR-C124910XX												
n	41	39	38	39	34	33	37	32	35	46	42	39
t_{max} (h)	4.23 (33)	3.31 (65)	3.25 (61)	3.69 (32)	3.00 (42)	3.22 (45)	3.71 (43)	3.31 (63)	3.16 (69)	3.17 (41)	3.24 (49)	3.26 (53)
C_{max} (ng/mL)	73 (109)	114 (48)	118 (61)	135 (50)	261 (41)	239 (38)	271 (39)	654 (41)	660 (51)	595 (32)	848 (41)	860 (47)
AUC (ng h/mL)	418 (58)	915 (45)	906 (48)	899 (46)	2108 (40)	1881 (32)	1753 (32)	5448 (37)	5268 (41)	—	10 233 (38)	10 446 (45)

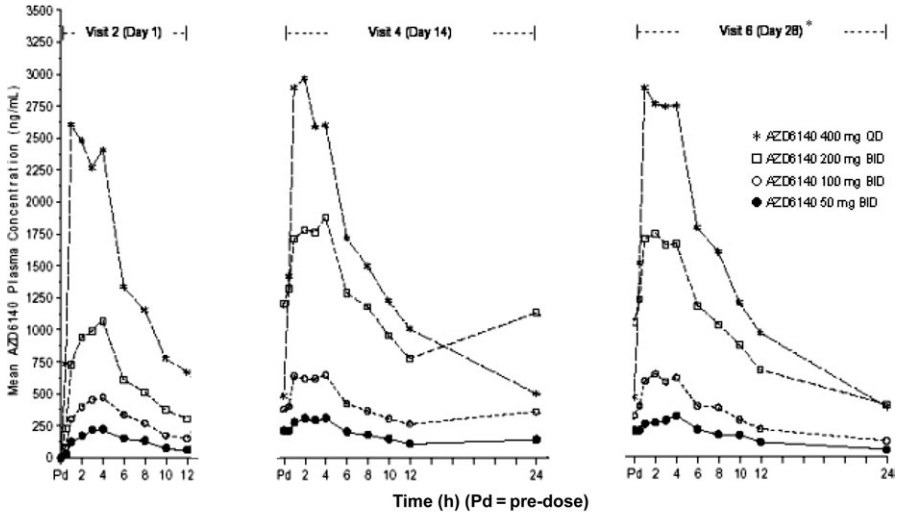


Figure 5 Mean plasma concentrations of AZD6140 vs. time in patients with atherosclerosis treated with AZD6140 50 mg bid, 100 mg bid, 200 mg bid, or 400 mg qd for 28 days. (Data are from all patients who received at least one dose of study medication and had valuable pharmacokinetic data.)

Table 5 Number (percentage) of patients with adverse events

	AZD6140				Clopidogrel 75 mg qd
	50 mg bid	100 mg bid	200 mg bid	400 mg qd	
Minor bleeding events[a]	12 (29)	17 (44)	19 (51)	22 (48)	12 (32)
Venipuncture site bruise	1 (2)	0 (0)	1 (3)	2 (4)	4 (11)
Epistaxis	1 (2)	4 (10)	4 (11)	8 (17)	2 (5)
Contusion	5 (12)	9 (23)	9 (24)	12 (26)	8 (22)
Red blood cells in urine	3 (7)	0 (0)	4 (11)	0 (0)	1 (3)
Dyspnoea	4 (10)	4 (10)	6 (16)	9 (20)	0 (0)
Dizziness	4 (10)	2 (5)	1 (3)	4 (9)	1 (3)
Headache	0 (0)	5 (13)	1 (3)	1 (2)	3 (8)

Adverse events reported in at least 10% of patients in any treatment group are listed.
[a]Number (percentage) of patients with at least one minor bleeding event. A given patient could have experienced more than one minor bleeding event.

Discussion

In this randomized, double-blind study, the first to investigate AZD6140 in patients with atherosclerosis, AZD6140 100 mg bid, 200 mg bid, and 400 mg qd rapidly and nearly completely inhibited $P2Y_{12}$-mediated platelet aggregation as measured by optical aggregometry after initial dosing and at steady state. These three doses of AZD6140 were associated with greater steady-state IPA than AZD6140 50 mg bid or clopidogrel 75 mg qd. The pattern of results was similar regardless of whether platelet aggregation inhibition was assessed from the final extent of aggregation (i.e. that observed at the end of the platelet aggregation response) or from the maximal extent of aggregation. However, as expected, maximal-extent measures were associated with smaller absolute responses than final-extent measures. Maximal-extent platelet aggregation inhibition is only partly modifiable by a $P2Y_{12}$ receptor antagonist because it depends on both $P2Y_1$ and $P2Y_{12}$ receptors.[18] The final-extent response is more sensitive to modification by a $P2Y_{12}$ receptor antagonist because it is mediated primarily by the $P2Y_{12}$ receptor. The pattern of results for the inhibition of collagen-induced platelet aggregation was comparable to that for the inhibition of maximal ADP-induced platelet aggregation (*Figure 4*), a finding that confirms that at least part of the aggregation response is mediated by interaction with $P2Y_{12}$ receptors.[19]

The early onset of peak IPA, occurring by 2 h post-dose on the first day of dosing with AZD6140, distinguishes it from clopidogrel, which minimally inhibited platelet aggregation on day 1. The latter finding is consistent with the previous observation that, with the maintenance dose of 75 mg, clopidogrel does not achieve full antiplatelet activity for 4–8 days.[3] Although loading doses have been used in an attempt to circumvent this problem, neither the best timing nor the best dose for a prompt antiplatelet effect of clopidogrel have been firmly established, particularly for acute applications.[9] Loading doses of AZD6140 and clopidogrel were not administered in this study because study medication was not initiated during the acute disease state. Additional studies will compare loading doses of the drugs.

In addition to having an earlier onset of effect than clopidogrel, AZD6140 more robustly inhibited platelet aggregation in this study. All three of the higher doses of AZD6140 (100 mg bid, 200 mg bid, and 400 mg qd) showed higher levels of platelet inhibition than clopidogrel 75 mg qd both at the initiation of therapy (day 1) and at steady state (day 14 and 28). IPA with AZD6140 was reversible as indicated by the declining levels of platelet inhibition at 24 h after the last dose. Reversibility may be an important characteristic in some clinical settings, such as surgery, in which recovery of platelet function is needed sooner than the 5–7 days required for clopidogrel. IPA with the three highest doses of AZD6140 remained higher than that with clopidogrel at 24 h on day 28, when only a single dose was administered. This finding shows that platelet inhibition remains adequate if a dose is missed.

The level of IPA achieved with clopidogrel in this study is consistent with that in previous studies of the drug.[20,21] Clopidogrel only moderately inhibits the *ex vivo* platelet aggregation response to ADP. The magnitude of IPA by clopidogrel is highly variable between patients, and resistance to the antiplatelet effects of clopidogrel has been observed.[10–13] The degree of resistance to clopidogrel inhibition of ADP-induced platelet aggregation was directly related to degree of risk for a recurrent cardiovascular event in a study of 60 patients who had suffered acute myocardial infarction.[11]

Both AZD6140 and clopidogrel were given with aspirin in this study, a practice consistent with present use of the currently available $P2Y_{12}$ antagonists in clinical practice. Clopidogrel 75 mg qd with aspirin 75–325 mg per day, a regimen comparable to that assessed in this study, reduced the risk of a cardiovascular event of death, myocardial infarction, or stroke by 20% relative to the reduction in risk by aspirin alone in the CURE (Clopidogrel in Unstable Angina to Prevent Recurrent Events) study, which had a mean treatment duration of 9 months.[5] The aspirin dosage of 75–100 mg chosen for use in this study was supported by the *post hoc* analysis of the CURE study, which shows this dose range to be optimal when aspirin is combined with clopidogrel.[22]

Pharmacokinetic data show that plasma concentrations of AZD6140 and its active metabolite AR-C124910XX were stable and predictable. Pharmacokinetics were linear after initial dosing on day 1 and slightly greater than dose-proportional at the 200 mg bid and 400 mg qd doses at steady state. Pharmacokinetic parameters were not affected by sex or age.

The onset and magnitude of effect of AZD6140 on IPA appear to be related to plasma concentrations of AZD6140 and its metabolite given that the mean time to peak IPA (IPA_{max}) (2–4 h across AZD6140 doses) mirrored the mean t_{max} of 2–3 h for both AZD6140 and its metabolite. Results for the active metabolite in this study in patients with atherosclerosis are similar to previous findings in healthy volunteers (AstraZeneca data on file, Phase I studies SC-532-5169, SC-532-5171). The parent compound accounts for the majority of the antiplatelet effect.

AZD6140 was generally well tolerated across the dose range evaluated in this study. All bleeding events except one in a patient receiving 400 mg qd were considered to be minor. The observed events were those expected with antiplatelet therapy (i.e. skin and mucous-membrane bleeds). The only two adverse events that appeared to increase in incidence with dose of AZD6140 were minor bleeding events and dyspnoea, all instances of which were mild or moderate. Dyspnoea, a non-specific symptom that has previously been reported with clopidogrel, was not reported with clopidogrel in this study and has not previously been reported with AZD6140. The frequency of dyspnoea after administration of AZD6140 and its aetiology will be investigated further in future studies.

In conclusion, AZD6140 100 mg and 200 mg bid appeared to have a more beneficial safety and tolerability profile than AZD6140 400 mg qd and were superior to AZD6140 50 mg bid and clopidogrel 75 mg qd with regard to antiplatelet efficacy. For these reasons, these two doses have been carried forward for further clinical evaluation.

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