Clinical Pharmacology of the New Thromboxane A₂ Receptor Antagonist, S-1452 : A Repeated Administration Study

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The present study was undertaken to examine whether the pharmacokinetic and pharmacodynamic alterations of S-1452, a new thromboxane A₂ (TXA₂) receptor antagonist, occur with a repeated administration. Fifty mg of S-1452 was given orally, twice a day for 8 days in 5 healthy subjects. Blood samples for determination of plasma drug concentration and its effect on platelet aggregation *ex vivo* were taken after the 1st and 15th administrations. S-1452 was rapidly absorbed with a plasma peak at 0.8 hour after the 1st and at 1.1 hour after the 15th administration. Thereafter, the drug was rapidly eliminated (mean elimination half-life : 0.5 hour after the 1st and 15th administrations). No significant difference was observed in any pharmacokinetic parameter between the 1st and 15th administrations. The inhibition of U-46619-induced platelet aggregation (a TXA₂ receptor agonist) persisted up to 9 hours after the 1st and 15th administrations. The effect after the 15th administration was significantly greater than that after the 1st administration. These results suggest that although the pharmacokinetic alteration of S-1452 is negligible, its inhibitory effect on platelet aggregation is enhanced with a repeated treatment.

Key words : S-1452, TXA₂ receptor antagonist, repeated administration, platelet aggregation

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Introduction

Thromboxane A_2 (TXA_2) is involved in the development of myocardial infarction and bronchial asthma¹⁾. S-1452, calcium 5 (Z)-1R, 2S, 3S, 4S, 7-[3-phenylsulfonyl-amiobicyclo[2.2.1]hept-2-yl]-5-heptenoate hydrate, is a highly specific TXA_2 receptor antagonist²⁾ which has been shown to prevent the TXA_2 -induced abnormal changes in electrocardiogram (ECG) and bronchial hyperresponsiveness in animal studies^{3,4)}. We have observed in healthy male subjects that although plasma S-1452 is rapidly eliminated, the inhibitory effects on platelet aggregation persist for a longer period⁵⁾.

The present study was undertaken to examine the pharmacokinetic and pharmacodynamic alterations of S-1452 and its safety during a repeated oral dosing in human subjects.

Materials and Methods

Eight male healthy subjects were studied. The mean age was 31.4 years (range : 27~37 years) and mean body weight was 64.7 kg (range : 56~76 kg). None had a history of cardiovascular, renal or hepatic disease. Physical examination, ECG and laboratory screening results were normal before inclusion. The subjects gave informed consent to participate in this study. The study was approved by the Review Board of Shionogi Institute for Clinical Pharmacology.

A 25 mg tablet of S-1452 and placebo were used in this study. These drugs were provided by Shionogi & Co., Ltd. (Osaka, Japan). The subjects were admitted to a special hospital ward, and were confined to the study area from 12 hours before the study and during the study. Meals were taken between 7 : 30 a. m. and 8 : 00 a. m., 12 : 00 noon to 12 : 30 p. m., and 6 : 00 p. m. to 6 : 30 p. m. Alcoholic beverages, coffee,

tea and smoking were not allowed on the day before and during the study. Subjects were requested not to take any cyclooxygenase inhibitor, such as aspirin or indomethacin, for 10 days before the study and until the end of the trial.

The subjects were randomly divided into two groups. Group I (n=6) received 50 mg of S-1452 orally with 100 ml water at 8 : 00 a. m. and 8 : 00 p. m. for 8 days while Group II (n=2) received a placebo tablet by a single-blind design. The morning dose was given without breakfast on days 1 and 8, and after breakfast on days 2, 3, 4, 5, 6 and 7. The night dose was not given on day 8. Subjects remained in the sitting position for at least 4 hours after the 1st (on day 1) and 15th (on day 8) administrations. Physical examination was done, and blood pressure, ECG and pulmonary functions were monitored at frequent intervals. The subjects were often questioned about adverse symptoms. Laboratory examinations for blood, urine and stool were done before, during and at the end of the study. Bleeding time (Ivy method)⁶⁾ was measured just before and at 1 and 9 hours after the 1st and 15th administrations.

Blood samples for assay of plasma S-1452 were obtained before and at 0.25, 0.5, 1, 2, 3, 4, 6, 9 and 12 hours after the 1st and 15th administrations. Platelet aggregation was determined at 0, 1, 3, 6, and 9 hours after the 1st administration and at 0, 1, 3, 6, 9, and 24 hours after the 15th administration. Urine samples for analysis of S-1452 and its metabolites [bisnor-(+)-S-145 and tetranor-(+)-S-145] were collected between 0 and 4, and 4 and 12 hours after the 1st and 15th administrations.

The plasma concentration of S-1452 (unchanged and total ; unchanged+glucuronide), and urinary concentration of S-1452 (total ; unchanged+glucuronide), bisnor-(+)-S-145

(total ; unchanged+glucuronide) and tetranor-(+)-S-1452 (total ; unchanged+glucuronide) were measured by capillary gas chromatography^{7,8)}. Platelet aggregation *ex vivo* was determined by a method described previously⁹⁾. As an aggregating agent, 4 μ M U-46619, a TXA₂ receptor agonist (Shionogi & Co., Ltd., Osaka, Japan), was used. Maximum platelet aggregation in response to 1, 2 and 4 μ M U-46619 was determined when these volunteers were selected. As the 4 μ M, but not 1 or 2 μ M, U-46619 induced more than 50% aggregation in all subjects, this dose was used in the study.

The maximum plasma concentration ($C_{\max}$) and time to maximum concentration ($t_{\max}$) were determined directly from the plasma concentration data for each subject. The area under the plasma concentration-time curve from 0 to 12 hours (AUC_{0-12}) was determined by the linear trapezoidal rule. The terminal elimination rate constant (K_{10}) was obtained by curve fitting the plasma concentrations to a one-compartment open model. The elimination half-life ($t_{1/2}$) was calculated as ;

$$t_{1/2} = \ln 2 / K_{10}.$$

Data are expressed as mean $\pm$ SD. Statistical analysis was performed by analysis of variance and Student's t-test as appropriate. A p value of less than 0.05 was considered to be significant.

Results

As an ECG abnormality (incomplete right bundle branch block) was observed just before the morning dose of active drug on day 2, one subject was withdrawn from the study. Therefore, the results of this subject were not included. S-1452 was rapidly absorbed and its plasma peak (unchanged form) was observed at 0.8 hour after the 1st administration (Fig. 1 and Table 1). Thereafter, the unchanged form of

Fig. 1 Plasma concentrations of S-1452 after the 1st (○—○) and 15th (●—●) administrations of 50 mg in 5 subjects (Mean $\pm$ SD)

S-1452 was rapidly eliminated and was not detected at 4 hours (Fig. 1). The time course of plasma S-1452 after the 15th administration was similar to that after the 1st administration (Fig. 1). No significant difference was observed in any pharmacokinetic parameter of S-1452 between the 1st and 15th administrations (Table 1). There was no significant difference in the urinary amount of drug (S-1452+the metabolites) between the 1st and 15th administrations [4-hour urine (% of the dose) : 1st $65 \pm 2\%$, 15th $63 \pm 9\%$, 12-hour urine : 1st $76 \pm 3\%$, 15th $80 \pm 4\%$]. The inhibitory effect of S-1452 on the U-46619-induced platelet aggregation was observed at 1 hour and persisted up to 9 hours after the 1st administration (Fig. 2). The inhibition of platelet aggregation was detected just before and at 1, 3, 6 and 9 hours after the 15th administration. The inhibitory effect of S-1452 after the 15th administration was signifi-

Tab. 1 Pharmacokinetic Parameters of S-1452 after the 1st and 15th Administration of 50 mg in 5 Subjects (Mean $\pm$ SD)

Compound	Parameter	Administration	
		1st	15th
S-1452 (Unchanged)	$C_{\max}$ (ng/ml)	46.0 ± 18.9	47.1 ± 22.1
	$t_{\max}$ (hr)	0.8 ± 0.3	1.1 ± 0.8
	$t_{1/2}$ (hr)	0.5 ± 0.1	0.5 ± 0.3
	AUC_{0-12} (ng·hr/ml)	62 ± 21	58 ± 11
S-1452 (Total)	$C_{\max}$ (ng/ml)	174.5 ± 83.0	163.3 ± 88.2
	$t_{\max}$ (hr)	1.0 ± 0.6	1.2 ± 0.8
	AUC_{0-12} (ng·hr/ml)	248 ± 80	213 ± 66

$C_{\max}$: plasma maximum concentration

$t_{\max}$: time to maximum concentration

$t_{1/2}$: elimination half-life

AUC_{0-12} : area under the plasma concentration-time curve from 0 to 12 hours

Fig. 2 Platelet aggregation stimulated by U-46619 ($4 \mu\text{M}$) after the 1st ($\circ-\circ$) and 15th ($\bullet-\bullet$) administration of 50 mg in 5 subjects (Mean $\pm$ SD), and the 1st ($\triangle-\triangle$) and 15th ($\blacktriangle-\blacktriangle$) administration of placebo in 2 subjects (Mean)

* $p < 0.05$, ** $p < 0.01$ compared with the value just before the 1st administration in the active drug group.

cantly greater than that after the 1st administration. The values of maximum platelet aggregation in the two placebo-treated subjects were approximately 80% after the 1st and 15th administrations (Fig. 2).

Bleeding time was prolonged slightly after the 1st and 15th administrations of S-1452 (Table 2). This parameter went over the normal range (2~6 minutes) at 1 hour after the 1st

administration in two subjects (6.5 and 7.0 minutes) and after the 15th administration in one subject (7.5 minutes). However, this prolongation in bleeding time disappeared at 9 hours. Except for this episode, no drug-related abnormalities were observed in physical examination, blood pressure, ECG, pulmonary functions or laboratory screening results. No adverse symptoms were recorded during the study.

Tab. 2 Bleeding Time after the 1st and 15th Administration of 50 mg of S-145 (n=5) and Placebo (n=2)

Administration	Agent	Time after administration (hr)			
		0	1	9	
1st	Placebo	3.0, 4.5	3.0, 4.5	3.0, 3.5	(Individual data)
	S-1452	3.7±0.7	5.9±1.0	3.8±0.8	(Mean±SD)
15th	Placebo	3.5, 4.0	2.0, 4.0	2.5, 3.0	(Individual data)
	S-1452	4.1±1.1	4.9±1.7	3.5±0.5	(Mean±SD)
Unit : min		Normal range=2~6 min			

Discussion

Plasma concentrations of unchanged S-1452 rapidly increased after the 1st administration and the mean $t_{\max}$ was 0.8 hour which is similar to previous data of the single administration study (mean $t_{\max}$: 0.6 hour)⁵⁾. These results suggest that the absorption of S-1452 is fast after a single oral administration. The plasma unchanged drug was rapidly eliminated with a mean $t_{1/2}$ of 0.5 hour in this study. S-1452 is shown to be extensively converted to metabolites and thereafter, excreted in the urine⁸⁾. Such extensive metabolism of S-1452 might produce rapid elimination of the drug.

Pharmacokinetic alterations are reported for many drugs during long-term treatment¹⁰⁾. In this study, the pharmacokinetic parameters of S-1452 after the 15th administration were not significantly different from those after the 1st administration. In addition, the urinary excretion of S-1452 and its metabolites, the main pathway of elimination⁸⁾, of the two administrations did not differ significantly. Based on these findings, we think that the pharmacokinetic alteration of S-1452 during long-term treatment is negligible. However, as only a small number of subjects were included in this study, it remains possible that a type II error might be made. More than 50 subjects are necessary for detecting 20% differences between the 1st and

15th administrations with $\alpha=0.05$ and $1-\beta=0.8$.

According to the results obtained from *in vitro* studies using human platelets, the antiaggregatory activity of S-1452 to collagen- or arachidonic acid-induced aggregation is qualitatively and quantitatively the same as to U-46619-induced aggregation^{11,12)}. ADP-induced aggregation consists of two steps. The first step which is induced through a specific receptor is not inhibited by S-1452, but the second step which is induced by TXA_2 produced during the first step process is completely inhibited by S-1452 with a concentration range similar to that required for other agents^{11,12)}. Thus, U-46619 was chosen as a representative of other aggregating agents in this study.

The inhibition of platelet aggregation persisted more than 9 hours after the 1st administration of S-1452 in this study. However, the plasma concentrations of unchanged S-1452 (active) rapidly decreased and could not be detected at 4 hours after administration. This long-lasting action of S-1452 could be, at least in part, explained by the extremely slow dissociation of the drug from platelet receptors¹³⁾.

The inhibition of platelet aggregation was observed just before the 15th administration in this study. This finding suggests that the blockade of TXA_2 receptor was sufficient to inhibit platelet aggregation at 12 hours after the 14th

administration of S-1452. However, the inhibition disappeared at 24 hours after the final administration of the drug. The inhibitory effect of S-1452 after the 15th administration was significantly greater than that after the 1st administration. This enhanced action of S-1452 during the repeated treatment might be caused by the long-lasting characteristics of the drug. We have no explanation for the elevation in the U-46619-stimulated platelet aggregation at 6 hours after the 1st and 15th administrations.

Drug-induced abnormalities were not observed in physical examination, blood pressure, ECG, pulmonary function or laboratory screening results. The subjects did not complain of any adverse symptoms. Although the bleeding time was slightly prolonged after oral administration of S-1452, no evidence of gingival or gastrointestinal bleeding was obtained in this study. As S-1452 is usually prescribed on the basis of chronic use, careful observations are needed to prevent potential bleeding episodes during repeat treatment with this drug.

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