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Determination of plasma concentrations of SCH 44643 by a GC method and correlation of plasma concentration and anti-PAF activity in cynomolgus monkeys

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Abstract

Inhibiting the action of platelet-activating factor (PAF) is a new therapeutic approach for the treatment of allergic disorders. SCH 44643 is a new orally-active antagonist of response to both PAF and histamine. This study was done to determine plasma drug concentrations using a GC method and to compare them to ex-vivo anti-PAF activity in the plasma of cynomolgus monkeys following a single oral (12.5 mg kg $^{-1}$) administration. The GC method involved organic solvent extraction of monkey plasma followed by a GC analysis in a RTX-1 capillary column with a nitrogen/phosphorus detector. The method showed good precision (RSD < 8%) and accuracy (bias < 9%) with a limit of quantitation of 20 ng ml^{-1}.

The plasma profiles of SCH 44643 concentration and anti-PAF activity were very similar in each of the six monkeys. There was an excellent correlation ($r = 0.9003$) between anti-PAF activity and plasma concentration of SCH 44643, suggesting that the anti-PAF activity in cynomolgus monkeys was primarily due to unchanged SCH 44643, rather than its potential metabolite(s).

Keywords: Anti-PAF activity; Cynomolgus monkeys; GC method; Plasma levels

1. Introduction

Platelet-activating factor (PAF) is synthesized and secreted by a variety of cells involved in inflammation [1,2]. It activates platelets, causing aggregation and degranulation, and stimulates other inflammatory cells including neutrophils, macrophages and eosinophils. Both in-vitro and in-vivo, PAF shares with histamine the ability to induce bronchospasm and vasopermeability. PAF exerts it biological action through a specific receptor [3–6] and diverse classes of chemically distinct PAF receptor antagonists have been reported [2].

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Attention has been focused recently on inhibiting the action of PAF as a new therapeutic approach for treating allergic disorders. SCH 44643 (Fig. 1) is an analog of loratadine and has been identified as a potent, orally-active antagonist of reponse to both PAF and histamine.

The present study was carried out to determine drug concentrations by a GC method and ex-vivo anti-PAF activity in the plasma of cynomolgus monkeys following oral administration of SCH 44643. The relationship between the plasma drug concentrations and the ex-vivo anti-PAF activity in plasma was also investigated.

2. Materials and methods

2.1. Materials

SCH 44643 (Batch No. 23135-95, micronized) was prepared by the Medicinal Chemistry Department at the Schering-Plough Research Institute (Kenilworth, NJ). PAF was obtained from Avanti Polar Lipids Inc. (Alabaster, AL).

For in-vitro experiments, a stock solution of SCH 44643 was prepared in DMSO and then diluted with the appropriate physiological buffer to the final prescribed concentration. Control samples contained an equal amount of DMSO that did not exceed a final concentration of 0.5%. For in-vivo experiments, SCH 44643 was prepared in 0.4% methylcellulose to yield a suitable suspension for oral administration.

2.2. Drug administration

Male cynomolgus monkeys (5.7–8.5 kg) were fasted overnight, although they had free access to water. On the following morning, the monkeys were dosed orally by gastric tube with 12.5 mg SCH 44643 kg^{-1} or vehicle (0.4% methylcellulose in isotonic saline). For each blood collection, an -imals were anesthetized lightly with an intramuscular administration of ketamine (10 mg kg^{-1}). Venous blood (5.4 ml) was withdrawn using a sterilized syringe and needle into tubes containing 3.8% sodium citrate and 2% dextrose immediately before (0 h) and at 1, 2, 4, 8, 12, 24, and 48 h after dosing. Blood samples were centrifuged immediately at 12 000 *g* for 20 min, and the plasma was transferred to capped vials and stored at −20°C pending analyses.

Table 1
Reproducibility of anti-PAF activity measurement

Monkey No.	Time (min)	% Inhibition of aggregation		Difference (%)
		Assay 1	Assay 2	
2	10	67	62	8.06
3	10	83	83	0
	25	46	48	4.34
4	10	77	72	6.94
	25	74	73	1.36
	50	42	43	2.33
			Mean	3.84

2.3. Determination of SCH 44643 by GC

To a 0.2 ml aliquot of plasma sample, 30 μl of internal standard solution (1 μg ml^{-1} of SCH 44986, Fig. 1) and 0.5 ml of water were added. The sample was mixed vigorously on a vortex action mixer for 20 s, 6.0 ml of a mixture containing hexane, methylene chloride and methyl-*t*-butyl ether (3:1:1, v/v/v) was added, the samples were mixed for 10 min and then centrifuged for 5 min. A 5.5 ml aliquot of the organic layer was removed and transferred to a second tube (pre-rinsed with methanol) and then evaporated to dryness under nitrogen. The residue was redissolved in 0.3 ml of

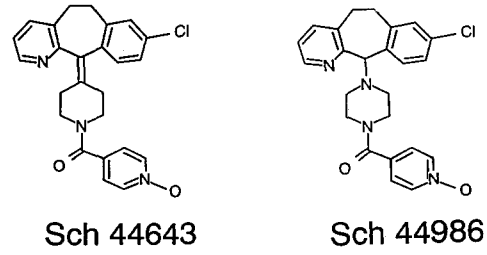

Fig. 1. Chemical Structures of SCH 44643 and Internal Standard (SCH 44986).

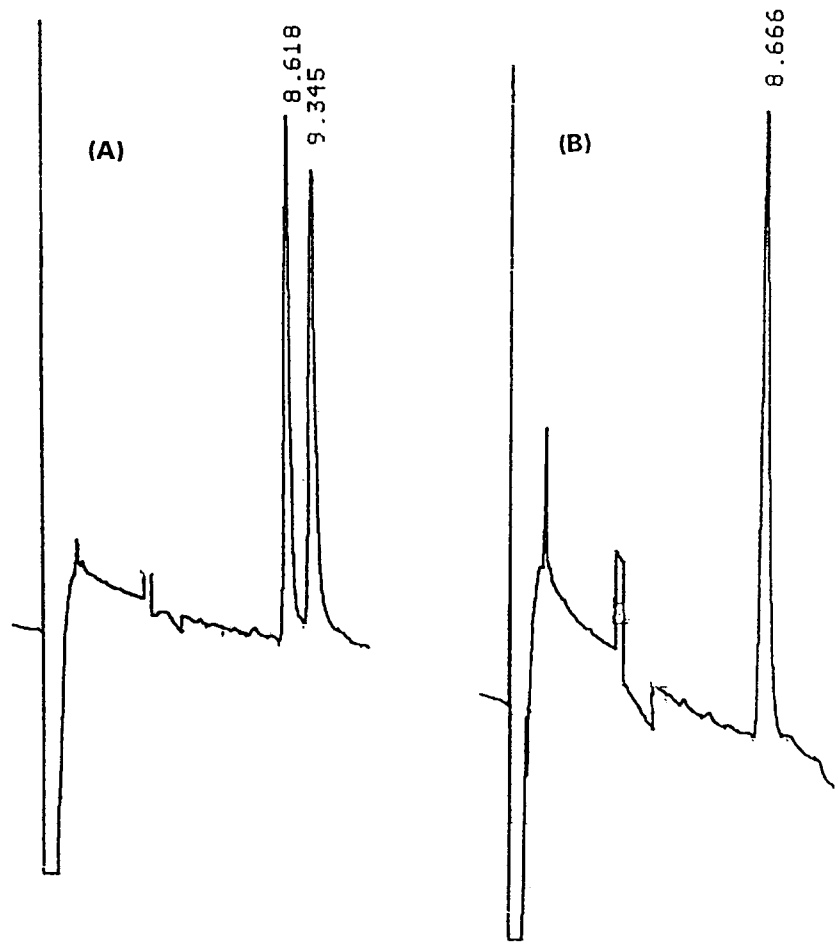

Fig. 2. Chromatograms of (A) monkey plasma containing 250 ng of SCH 44643 and 250 ng of internal standard and (B) monkey plasma containing only internal standard (250 ng). The y axis represents the detector response in counts (each count equivalent to 0.125 μV) and the x axis represents the retention time.

1.5% isoamyl alcohol in toluene and then transferred to a 0.6 ml amber injection vial to which 10 μl of 1-octanol was added. The vial was then placed in a Speed Vac and evaporated to about 10 μl. A 40 μl aliquot of toluene was added, the sample was mixed vigorously and a 1 μl aliquot was injected into the GC system.

The GC system consisted of a Hewlett Packard model 5890 Series II GC system equipped with a nitrogen/phosphorus detector, a Hewlett Packard Model 7673 auto-injector, and a RTX-1 fused silica capillary (30 m $\times$ 0.53 mm i.d.) column (cross-bonded 100% dimethylpolysiloxane with 0.25 μm film thickness). The operating temperatures for the injector, column and detector were 315°C, 290°C and 315°C respectively. Helium was used as the carrier gas (5.5 ml min^{-1}) and also as the make-up gas (25 ml min^{-1}). The data system consisted of a Hewlett Packard laboratory automation system with a Hewlett Packard model 3396A integrator.

2.4. Determination of ex-vivo anti-PAF activity

The anti-PAF activity in cynomolgus monkey was evaluated by determining the ability to inhibit PAF-induced aggregation of human platelets in platelet-rich plasma. Human blood (50 ml) was

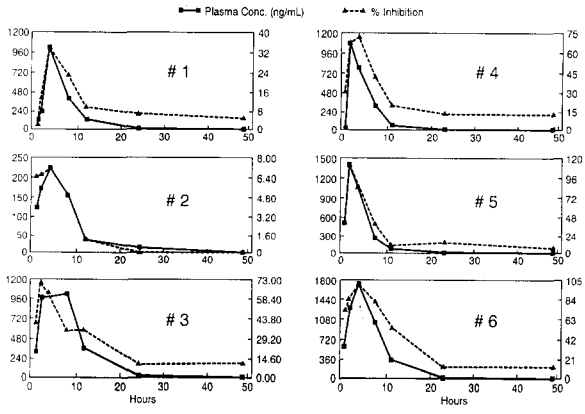

Fig. 3. Plasma drug concentrations and anti-PAF activity profiles in cynomolgus monkeys following a single oral dose (12.5 mg kg^{-1}) of SCH 44643.

collected from male healthy volunteers in an anticoagulant solution (5 ml) containing sodium citrate (3.8%) and dextrose (2%). Blood was centrifuged at 110 *g* for 15 min to obtain platelet-rich plasma.

Aggregation assays were performed using a dual-channel aggregometer (model 440, Chrono-Log Corp., Havertown, PA). Aliquots of cynomolgus monkey plasma were added to the platelet-rich plasma and, after incubation for 2 min, PAF solution was added to achieve a final concentration of 2×10^{-8} M. The final assay volume was 250 μl, of which 200 μl was human platelet-rich plamsa. Incubations were continued until the increase in light transmission reached a maximum (usually 2 min). Values for inhibition

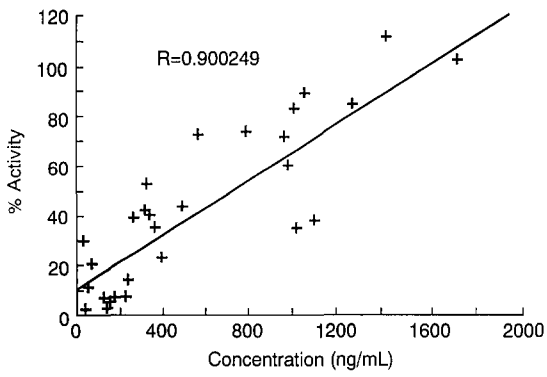

Fig. 4. Correlation between plasma anti-PAF activity and plasma concentrations of SCH 44643.

Table 2
Reproducibility and Accuracy of the GC Assay for SCH 44643

Concentration (ng ml^{-1})		RSD (%)	Bias (%)
Added	Measured		
20	20.5	4.23	2.50
40	39.8	6.20	0.60
60	65.4	4.17	8.90
100	100.0	3.37	0
200	193.6	7.33	3.20
500	509.3	1.86	1.90
1000	1061	3.63	6.10
2000	2000	7.65	0

were calculated by comparing the rates of aggregation obtained in the absence and presence of the compound. The measurement of inhibition (%) was quite reproducible, since the mean difference between the two assays was less then 4% (Table 1).

The plasma samples from each monkey were analyzed to find the plasma sample with the highest anti-PAF activity. A volume of this sample inhibiting platelet aggregation by 60–70% was determined (inhibition curve was linear up to 70%). Inhibition of platelet aggregation at this plasma volume was then determined for plasma samples obtained at various time points, after oral administration.

3. Results and discussion

3.1. Plasma concentrations of SCH 44643

Representative GC chromatograms of a drug-free monkey plasma sample and a plasma sample spiked with SCH 44643 are shown in Fig. 2. There was clear resolution between the SCH 44643 and the internal standard. The linearity of the assay was demonstrated by multiple analyses ($n = 40$) of plasma samples containing 20–2000 ng SCH 44643 ml^{-1}. Linear regression analysis of the observed concentration (y) versus added concentrations (x) gave the equation $y = 0.9901x + 1.2861$ with a correlation (r^2) of 0.9990. The

standard errors were 0.017402 and 3.205165 for the slope and intercept respectively ($n = 40$ with eight repeats for each of five concentrations). The slope approached unity, indicating negligible proportional error in the assay, and the intercept was relatively small, indicating negligible interference.

The reproducibility and accuracy of the method at eight different concentrations of SCH 44643 in monkey plasma were demonstrated by the consistently low relative standard deviation (% RSD, an index of precision), ranging from 1.9–7.7%, and the low percentage bias, ranging from 0–8.9% (Table 2). The limit of quantitation (LOQ) was defined as the lowest concentration on the standard curve which could be measured with acceptable precision (RSD < 20%) and accuracy (bias < 20%). At the LOQ of 20 ng ml $^{-1}$, RSD and bias were 4.2 and 2.5% respectively ($n = 4$). Plasma concentrations of SCH 44643 in the six cynomolgus monkeys following oral administration (12.5 mg kg $^{-1}$) as determined by the GC method are illustrated in Fig. 3.

3.2. Ex-vivo anti-PAF activity in plasma

Anti-PAF activity in the plasma of cynomolgus monkeys following oral administration (12.5 mg kg $^{-1}$) of SCH 44643 was determined (Fig. 3). The anti-PAF activity in monkey plasma was evaluated by determining its ability to inhibit PAF-induced aggregation of human platelets in platelet-rich plasma. Values for inhibition were calculated by comparing the rates of aggregation obtained in the absence and presence of SCH 44643. Since the inhibition curve was linear up to 70% inhibition, the volume of monkey plasma added varied with anti-PAF activity so as to ensure that the percentage of inhibition was within the linear range of the standard curve. For effective comparison, the anti-PAF activities of all plasma samples were then normalized to the value using the same volume of plasma.

The adjusted plasma anti-PAF activity and plasma SCH 44643 concentrations are compared for individual monkeys (Fig. 3). It is apparent that the plasma profiles for SCH 44643 and anti-PAF activities were very similar for all monkeys. The correlation between anti-PAF activity in plasma and the plasma concentration of SCH 44643 were illustrated in Fig. 4.. Regression analysis yielded a correlation coefficient of 0.9003. These results clearly indicate excellent correlation between anti-PAF activity and plasma concentration of SCH 44643 and suggest that the anti-PAF activity in the cynomolgus monkey following oral administration was primarily due to unchanged SCH 44643 rather than to its potential metabolites.

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