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Short Communication

Radioimmunoassay for DS-4574, an anti-allergic agent: development, evaluation and application to human plasma samples*

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Introduction

DS-4574 is a newly synthesized anti-allergic drug [1–5]. An HPLC method had been previously developed for determination of DS-4574. However, it was not sensitive enough to detect nanogram levels of drug in human plasma.

A 100-fold more sensitive method using radioimmunoassay (RIA) was developed, and by applying this method to clinical study, pharmacokinetic properties in human after a single oral administration of DS-4574 were elucidated.

Experimental

Preparation of anti-DS-4574 antisera

The immunogen was prepared by a modified N-succinimide method [6]. Anti-DS-4574 antisera was obtained from rabbits immunized with the immunogen emulsified in Freund's complete adjuvant.

Assay procedure

A mixture of 0.1 ml of 2000-times diluted anti-DS-4574 antisera, 0.1 ml of diluted plasma sample and 0.4 ml of 0.1% of BSA/0.05 M phosphate buffer (pH 7.4) was incubated with 0.1 ml of [^{3}H]DS-4574 (50 000 cpm ml^{-1}, 1.66 ng ml^{-1}) in a plastic test tube at 4°C for 16 h. B/F separation was carried out by the dextran-coated charcoal method and radioactivity of the bound fraction was measured.

Clinical study

A total of 36 volunteers participated. They were divided into six groups and ingested either a 10, 20, 50, 100, 200 and 400-mg dose of DS-4574 with 150 ml of water after overnight fasting. Blood samples were obtained from a forearm vein at 0 (predose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h after administration. The concentration of the drug in plasma was determined by the RIA method described, and various pharmacokinetic parameters, C_{max}, T_{max}, AUC, MRT and $T_{½}$ were calculated from the individual data of plasma concentration. T_{max} and C_{max} values were obtained from the raw data. AUC and MRT values were calculated by the non-compartment analysis. $T_{½}$ values were obtained by regression analysis of linear portions of transformed mean plasma concentration into logarithm-time curve.

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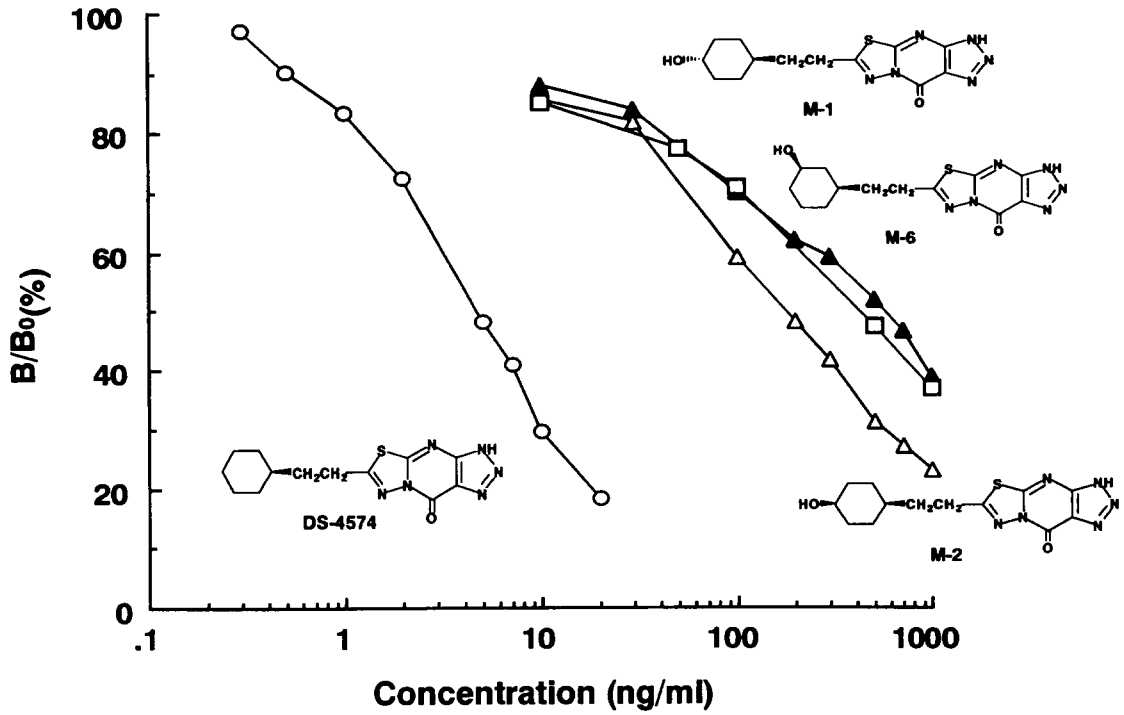

Figure 1
Cross reactivity with metabolites of DS-4574.

Results

RIA method

Useful dose-response curves could be constructed in the range of 1–10 ng ml^{-1} plasma. The quantitation limit of DS-4574 was approximately 1 ng ml^{-1} plasma. The percentage cross-reactions with M-1, M-2 and M-6 were 0.9, 2.9 and 1.4%, respectively (Fig. 1). Several validation studies were performed to confirm the reliability of antisera [6].

Clinical study

Mean plasma concentration — time profiles of unchanged DS-4574 are shown in Fig. 2. Plasma concentration reached C_{max} at 0.5–2 h and decreased with a half-life of 0.7–1.4 h from the plasma. Several pharmacokinetic data are shown in Table 1. T_{max}, MRt and $T_{1/2}$ were almost independent of the dose. The correlation analysis between dose and AUC gave a straight line (r = 0.999) through the origin.

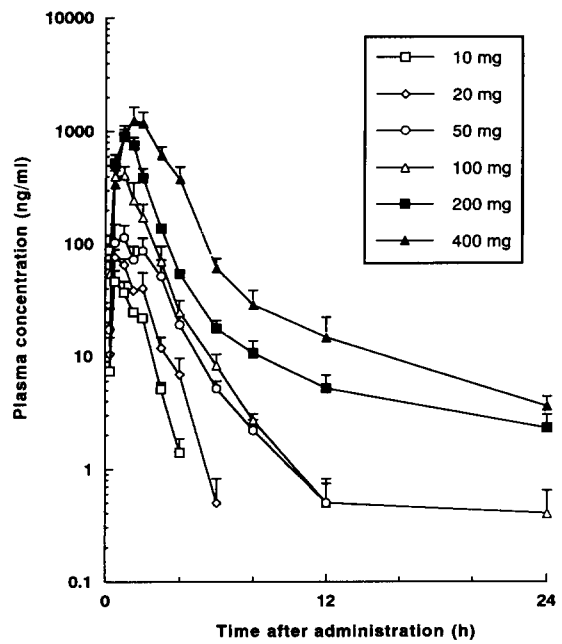

Figure 2
Mean plasma concentration of DS-4574. Plasma samples were obtained from overnight-fasting Japanese male volunteers after a single oral administration of 10–400 mg of DS-4574.

Discussion

Based on animal data, the plasma concentration of unchanged DS-4574 in human was expected to be low. The available HPLC

Table 1
Pharmacokinetic parameters* of DS-4574

	Dose (mg)	T_{max} (h)	C_{max} (ng ml^{-1})	AUC_{0-inf} (ng h ml^{-1})	MRT (h)	$T_{1/2}$ (α)† (h)	$T_{1/2}$ (β)† (h)
Step 1	10	0.92	59	72	1.31	0.98	—
Step 2	20	0.83	93	134	1.48	0.66	—
Step 3	50	1.58	180	298	2.22	1.03	—
Step 5	100	1.17	522	747	1.82	0.73	—
Step 6	200	1.17	1022	1696	2.61	0.823	6.14
Step 7	400	1.42	1517	3670	2.99	1.36	4.92

*Values are mean (n = 6).
† $T_{1/2}$ values were calculated by regression analysis of linear portions of mean plasma concentration (transformed into logarithm)–time curve.

method did not have adequate sensitivity. Therefore, a sensitive method had to be developed for clinical evaluation of the pharmacokinetic properties of DS-4574. The developed RIA method demonstrated superior sensitivity and permitted detection of 1 ng ml^{-1} of DS-4574 in plasma, which was 100 fold more sensitive than the HPLC method developed previously. In the development of RIA methods, the cross-reactivity of the employed antisera with drug metabolites is the most important determinant of the specificity. As the three major metabolites have a hydroxyl group on the cyclohexyl ring, the triazole ring in the terminal part of molecule was attached to ovalbumin to prepare an immunogen. As a result, the cross-reactivity of the present RIA method with these three metabolites was negligible.

In the clinical study, plasma concentration increased rapidly and decreased with a half-life of 0.7–1.4 h from the plasma. It revealed that DS-4574 was rapidly absorbed from the gastrointestinal tract. From the regression analysis between AUC and dose, it is estimated that plasma pharmacokinetics of DS-4574 were linear up to 400 mg of dose, and absorption or metabolic saturation were negligible in human.

Conclusion

The RIA method described herein has been shown to have suitable sensitivity, and pharmacokinetic properties of DS-4574 in human have been revealed for the first time.

References

[1] S. Aibara, M. Mori, T. Iwamoto, M. Tsubokawa, H. Takamori and W. Tsukada, *Japan J. Pharmacol.* **61**, 267–276 (1993).
[2] S. Aibara, M. Mori, T. Iwamoto, T. Chiba and W. Tsukada, *Arch. Int. Pharmacodyn. Ther.* **314**, 147–159 (1991).
[3] S. Aibara, M. Mori, M. Tsubokura, T. Iwamoto and W. Tsukada, *Int. Arch. Allergy Immunol.* **98**, 146–152 (1992).
[4] S. Aibara, M. Mori and W. Tsukada, *Int. Arch. Allergy Immunol.* **100**, 268–273 (1993).
[5] Y. Tabuchi and Y. Kurebayashi, *Japan J. Pharmacol.* **60**, 335–340 (1992).
[6] N. Murayama, M. Nakaoka, H. Nomura and H. Hakusui, *J. Pharm. Sci.* (to appear).

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