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Determination of a novel anti-psychotic agent AD-5423 and its metabolites in plasma by high-performance liquid chromatography with fluorescence detection[1]

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Abstract

AD-5423, 2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta[*b*]pyridine, is a novel anti-psychotic agent. In order to investigate the pharmacokinetics of AD-5423, a simple and sensitive high-performance liquid chromatographic (HPLC) method has been developed for the simultaneous determination of AD-5423 and its two *N*-oxidized metabolites (*N*-desethyl AD-5423 and AD-5423 *N*-oxide) in plasma.

After pretreatment of a plasma sample by solid-phase extraction, AD-5423 and its metabolites were analyzed on a HPLC with fluorescence detection (335/410 nm). Chromatography was performed on two C_{18} reversed-phase columns connected by a switching system, with a mobile phase of acetonitrile–methanol–25 mM sodium phosphate buffer (pH 2.5) containing 25 mM sodium 1-heptanesulfonate (36:26:38, v/v/v).

The method gives satisfactory accuracy and precision for the determination of AD-5423 and its metabolites. In human plasma, accurate determinations are possible over the concentration ranges of 0.04–5 ng ml^{-1} for AD-5423 and 0.1–5 ng ml^{-1} for *N*-oxidized metabolites. The intra- and inter-day assay precision (R.S.D.) of AD-5423 (0.5 ng ml^{-1}) were 3.6 and 7.2%, respectively. In plasma of experimental animals, the validated quantitative range are 0.1–100 ng ml^{-1} for both AD-5423 and its metabolites. © 1997 Elsevier Science B.V.

Keywords: AD-5423; AD-5423 metabolite; Anti-psychotic agent; High-performance liquid chromatography; Plasma; Simultaneous determination

1. Introduction

AD-5423 (2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl) - 5,6,7,8,9,10 - hexahydrocycloocta[*b*]pyridine; Fig. 1), is a novel anti-psychotic agent [1–4]. It is a potent antagonist of dopamine D_2^- and serotonin 5-HT_2-receptors, like risperidone [5] and SM-9018 [6], but hardly blocks dopamine D_1^- and adrenaline α_1-receptors.

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After oral administration of AD-5423 in rats, more than 10 metabolites were present in plasma. The metabolic pathway of AD-5423 involved oxidation of the cyclooctane moiety and *N*-oxidation of the *N*-ethyl piperazine ring, the latter yielding *N*-desethyl AD-5423 and AD-5423 *N*-oxide [7]. The anti-psychotic activities of two *N*-oxidized metabolites were lower than those of AD-5423. In order to investigate the pharmacokinetics of AD-5423, simple and sensitive assay methods for the unchanged drug and its two *N*-oxidized metabolites (*N*-desethyl AD-5423 and AD-5423 *N*-oxide) are necessary.

This paper describes a simple and sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous determination of AD-5423 and its two *N*-oxidized metabolites in plasma.

2. Experimental

2.1. Chemicals and reagents

AD-5423 and its two *N*-oxidized metabolites, *N*-desethyl AD-5423 and AD-5423 *N*-oxide, were synthesized in the authors' laboratories [1] (Fig. 1). AD-5332 and AD-5392 (Fig. 1) were used as internal standard substances for HPLC in human and animal samples, respectively. Methanol for residual PCB grade was purchased from Katayama Kagaku (Osaka, Japan). All other reagents and chemicals were commercially available and of analytical-reagent grade.

The concentration of each metabolite was expressed as the equivalent of the unchanged drug, AD-5423.

2.2. Chromatography

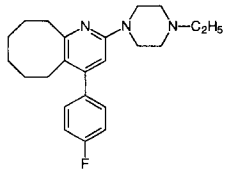
HPLC was carried out using a Model 800 series high-performance liquid chromatograph (Japan Spectroscopic, Tokyo, Japan), consisting of two Model 880-PU pumps, a Model 850-AS autosampler, a Model 892-01 automatic switching valve and a Model 821-FPS fluorescence detector (335/410 nm). The signals were recorded and integrated with a chromatography workstation Maxima 820 (Waters, MA, USA).

Two stainless-steel columns packed with STR ODS-H (Shimadzu Techno-Research, Kyoto, Japan) were used: a pre-column (5 μm, 10 × 4.0 mm i.d.) and an analytical column (5 μm, 150 × 4.6 mm i.d.). For a coupled-column switching technique, the pre-column was connected to the analytical column via the automatic six-port switching valve. For human samples after the analytes had flowed into the analytical column (2.0 min. after injection), the valve was switched; the pre-column was then washed out to remove undesirable substances remaining in the column while the analytes in the analytical column were detected. The analytical column was kept at 40°C, but the pre-column was left at room temperature.

The mobile phase consisted of acetonitrile–methanol–25 mM sodium phosphate buffer (pH 2.5) containing 25 mM sodium 1-heptanesulfonate (36:26:38, v/v/v). The solution was filtered through a 0.45-μm filter and degassed under reduced pressure. The flow-rate was 1.0 ml min^{-1}.

2.3. Stock solutions

Stock solutions of AD-5423 and its metabolites were prepared in methanol (20 μg ml^{-1}) and stored at 4°C. To prepare the samples for construction of calibration curves and assessment of validation, the stock solutions were further diluted with 50 mM phosphate buffer (pH 2.5). Internal standard (AD-5332 and AD-5392) stock



	R_1	R_2
AD-5423	C_2H_5	–
N-desethyl AD-5423	H	–
AD-5423 N-oxide	C_2H_5	O
AD-5332	n-C_4H_9	–
AD-5392		–

Fig. 1. Chemical structures of AD-5423 (upper), its two *N*-oxidized metabolites (*N*-desethyl AD-5423 and AD-5423 *N*-oxide) and internal standards (AD-5332 and AD-5392) (lower).

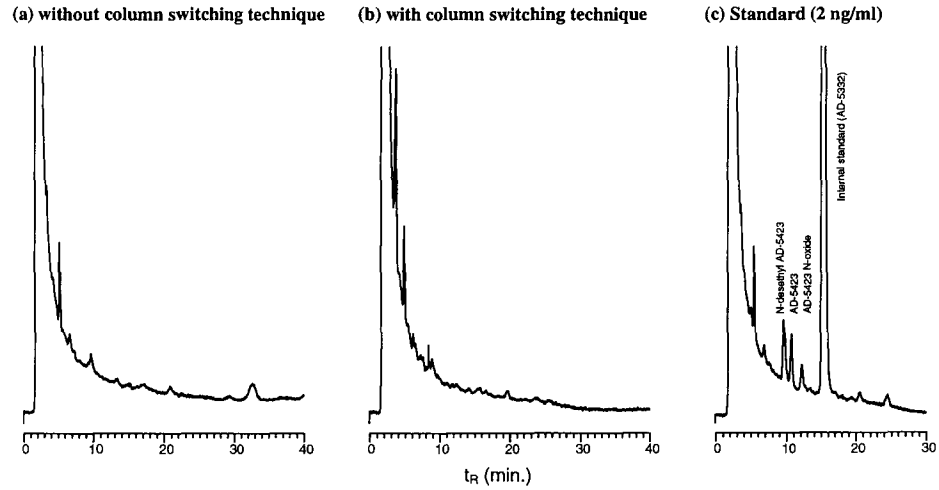


Fig. 2. Typical chromatograms of human plasma.

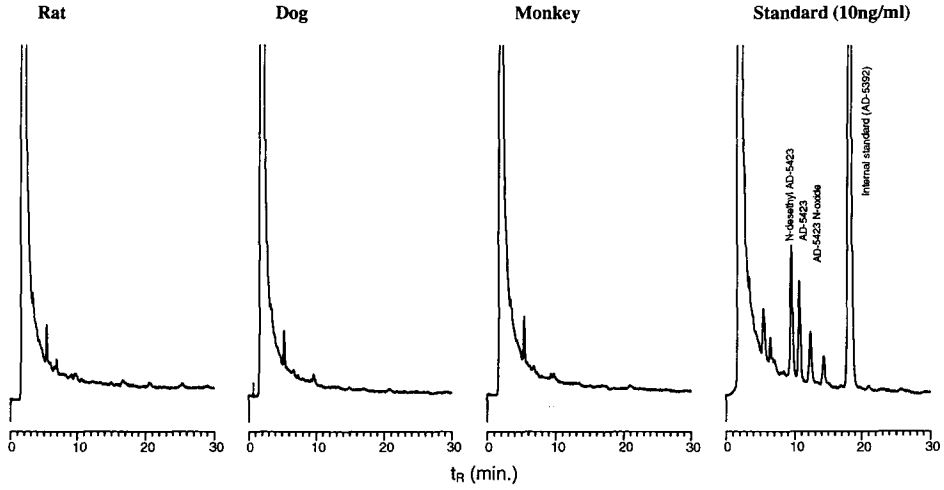


Fig. 3. Typical chromatograms of rat, dog and monkey plasma.

solutions were also prepared in methanol (40 μg ml^{-1}) and stored at 4°C. The stock solutions of AD-5332 and AD-5392 were diluted with 50 mM phosphate buffer (pH 2.5) to final concentrations of 400 and 300 ng ml^{-1}, respectively.

2.4. Sample preparation

To 1 ml of plasma were added 0.1 ml of 50 mM phosphate buffer (pH 2.5) and 0.4 ml of working internal standard solution (AD-5332 and AD-5392 for human and animal samples, respectively). The mixture was applied to a Bond Elut C_{18} column (200 mg, Varian Sample Preparation Products, CA, USA). The column was washed twice with 3 ml of distilled water and then twice with 3 ml of 55% (v/v) methanol. AD-5423, its metabolites and the internal standard were eluted with 2 ml of methanol–50 mM phosphate buffer (pH 2.5) (9:1, v/v). The eluate was evaporated to dryness in vacuo at 55°C. The residue was dissolved in 0.1 ml of the mobile phase and an aliquot of the solution was injected into the HPLC. The injection volumes were 70 and 40 μl for human and animal samples, respectively.

Table 1
Recovery of AD-5423, its metabolites and internal standards extracted from plasma on C_{18} solid-phase extraction column

	Concentration (ng ml^{-1})	Recovery (mean ± S.D. (%))[a]			
		AD-5423	*N*-desethyl AD-5423	AD-5423 *N*-oxide	Internal standard[b]
Human	0.04	80.0 ± 5.9		—	
	0.1	73.7 ± 3.4	96.0 ± 3.4	75.6 ± 3.6	87.5 ± 6.7
	0.5	78.3 ± 5.7	100.4 ± 9.4	104.0 ± 5.9	
	5	72.1 ± 3.3	111.5 ± 7.1	75.0 ± 1.4	
Rat	0.1	95.3 ± 2.1	78.2 ± 7.4	79.6 ± 3.6	
	2	81.0 ± 3.3	92.2 ± 7.1	91.6 ± 1.4	77.9 ± 2.5
	20	77.7 ± 1.8	90.6 ± 1.3	82.8 ± 0.4	
	100	78.3 ± 0.4	85.5 ± 0.9	82.7 ± 0.1	

[a] Recoveries of AD-5423 and its metabolites were obtained from three samples and those of internal standards were obtained from 12 samples.
[b] AD-5332 and AD-5392 were used as internal standard substances in human and rat samples, respectively.

2.5. Recovery, calibration curve, accuracy and precision

Drug-free human and rat plasma were used in the construction of calibration curves. The stock solutions of AD-5423 and its metabolites were diluted every day with 50 mM phosphate buffer (pH 2.5) to prepare the working standard solutions at different concentrations. To each 1 ml of plasma was added 0.1 ml of the working standard solution instead of 0.1 ml of 50 mM phosphate buffer (pH 2.5), and the mixture was treated according to the procedure described in the previous section.

The absolute extraction recovery of AD-5423, its metabolites and internal standards from plasma samples was determined by comparing the peak areas of the respective peaks from extracted standards in human and rat plasma with those of unextracted standards.

The calibration curve was obtained by plotting the peak–area ratio of AD-5423 or its metabolites to the internal standard against the amount of each compound added, using least-square regression and a weighting factor of $1/y$, because of the wide concentration range (0.1–100 ng ml^{-1}) for the construction of the calibration curve in rat plasma.

Assay accuracy was estimated based on the mean percentage error [(observed concentration/nominal concentration) × 100]. The precision, expressed as a percentage, was evaluated by calculating the relative standard deviation (R.S.D.).

2.6. Plasma levels of AD-5423 and its metabolites

The plasma samples from a healthy male volunteer (24 years old, 58.0 kg) were obtained from Dr M. Murasaki (Kitasato University School of Medicine, Kanagawa, Japan). The volunteer who had given written informed consent, was given orally 2 mg of AD-5423.

Male SD rats (230–245 g), a male beagle dog (12.0 kg) and a male cynomolgus monkey (6.2 kg) were used. Animals were fasted overnight before experiments with water ad libitum. AD-5423 was orally administered to the rats and monkey at 3 mg kg^{-1} and to the dog at 1 mg kg^{-1}.

Blood samples were obtained at a fixed time after administration. Plasma samples were immediately separated by centrifuging the blood samples and were stored at −20°C until analysis.

3. Results and discussion

3.1. Assay specificity

For the determination of AD-5423 and its two *N*-oxidized metabolites (*N*-desethyl AD-5423 and

Table 2
Accuracy and precision data[a] for AD-5423 and its metabolites in human plasma

Compound	Nominal conc. (ng ml^{-1})	Observed conc. (mean ± S.D.) (ng ml^{-1})	Precision (R.S.D.) (%)	Accuracy (%)
AD-5423	0.0436	0.0460 ± 0.0018	3.9	105.4
	0.109	0.117 ± 0.004	3.4	107.7
	0.218	0.215 ± 0.013	6.0	98.8
	0.545	0.540 ± 0.008	1.5	99.1
	1.09	1.00 ± 0.04	4.0	91.9
	2.18	2.00 ± 0.02	1.0	91.8
	5.45	5.76 ± 0.13	2.3	105.6
N-desethyl AD-5423	0.0870	0.0759 ± 0.0039	5.1	87.2
	0.174	0.190 ± 0.006	3.2	109.4
	0.435	0.443 ± 0.051	11.5	101.8
	0.870	0.822 ± 0.037	4.5	94.5
	1.74	1.85 ± 0.06	3.2	106.1
	4.35	4.30 ± 0.16	3.7	98.8
AD-5423 *N*-oxide	0.0914	0.0990 ± 0.0095	9.6	108.3
	0.183	0.193 ± 0.012	6.2	105.6
	0.457	0.464 ± 0.015	3.2	101.6
	0.914	0.836 ± 0.034	4.1	91.4
	1.83	1.76 ± 0.09	5.1	96.0
	4.57	4.72 ± 0.10	2.1	103.2

[a] Results were obtained from three experiments.

AD-5423 *N*-oxide) in plasma, the sample is usually pretreated by a solvent extraction or by a solid-phase extraction. Extraction of two metabolites with ethyl acetate, chloroform or hexane gave a low recovery. Therefore, a solid-phase extraction method was chosen for the simultaneous determination of AD-5423 and its two metabolites in plasma.

Typical chromatograms of blank and spiked human plasma containing AD-5423, its two *N*-oxidized metabolites and the internal standard (AD-5332) are shown in Fig. 2. In Fig. 3, typical chromatograms of drug-free rat, dog and monkey plasma and rat plasma spiked with AD-5423, its metabolites and the internal standard (AD-5392) are presented. With the assay procedure, the peaks of AD-5423, its metabolites and internal standards were well separated from each other and from the peaks derived from control plasma and solvent. Using the column switching technique for human samples, the blank peak which was eluted at 32.7 min. was not detected (Fig. 2(a) and (b)), leading to reduction of the total run time.

3.2. Recovery of AD-5423, its metabolites and internal standards from plasma

The absolute extraction recovery of AD-5423, its *N*-oxidized metabolites and internal standards from human and rat plasma samples is shown in Table 1. The extraction efficiencies were found to be 72.1–95.3% for AD-5423, 78.2–111.5% for *N*-desethyl AD-5423, 75.0–104.0% for AD-5423 *N*-oxide, 87.5% for AD-5332 (internal standard for human samples) and 77.9% for AD-5392 (internal standard for experimental animal samples). There was not much difference between human and rat samples.

Table 3
Intra- and inter-day assay precision of AD-5423 in human plasma

Concentration (ng ml^{-1})	R.S.D. (%)			
	Intra-day ($n = 4$)			Inter-day ($n = 12$)
	Day 1	Day 2	Day 3	
0.5	3.6	5.9	7.6	7.2
2	6.4	6.2	4.2	6.5

Table 4
Accuracy and precision data[a] for AD-5423 and its metabolites in rat plasma

Compound	Nominal conc. (ng ml^{-1})	Observed conc. (mean ± S.D.) (ng ml^{-1})	Precision (R.S.D.) (%)	Accuracy (%)
AD-5423	0.109	0.116 ± 0.004	3.4	106.4
	0.545	0.465 ± 0.033	7.1	85.3
	2.18	2.27 ± 0.09	4.0	104.1
	5.45	5.54 ± 0.19	3.4	101.6
	21.8	22.6 ± 02	0.9	103.5
	54.5	55.2 ± 0.3	0.5	101.3
	109	107 ± 2	1.9	98.5
N-desthyl AD-5423	0.0870	0.094 ± 0.009	9.6	107.7
	0.435	0.424 ± 0.017	4.0	97.5
	1.74	1.65 ± 0.13	7.9	94.8
	4.35	4.34 ± 0.23	5.3	99.7
	17.4	18.1 ± 0.1	0.6	103.8
	43.5	43.4 ± 0.5	1.2	99.8
	87.0	86.6 ± 1.6	1.8	99.5
AD-5423 *N*-oxide	0.0914	0.0863 ± 0.005	5.8	94.5
	0.457	0.419 ± 0.008	1.9	91.7
	1.83	1.91 ± 0.03	1.6	104.5
	4.57	4.76 ± 0.10	2.1	104.1
	18.3	19.1 ± 0.4	2.1	104.6
	45.7	46.4 ± 0.1	0.2	101.5
	91.4	89.8 ± 0.6	0.7	98.2

[a] Results were obtained from three experiments.

3.3. Determination of AD-5423 and its metabolites in human plasma

In human plasma, accurate determinations were possible over the concentration ranges of 0.04–5 ng ml^{-1} for AD-5423 and 0.1–5 ng ml^{-1} for two metabolites. The calibration curves were linear and passed through the origin. The correlation coefficients (r) ranged from 0.997 to 0.998. The relative standard deviations (R.S.D.s) in the concentration ranges examined were 1.0–6.0% for AD-5423, 3.2–11.5% for *N*-desethyl AD-5423 and 2.1–9.6% for AD-5423 *N*-oxide, respectively (Table 2). For plasma levels of AD-5423 above 0.04 ng ml^{-1}, the assay accuracy ranged from 91.8 to 107.7% of the nominal values.

Table 3 shows the intra- and inter-day assay precision of AD-5423 in human plasma. The val-

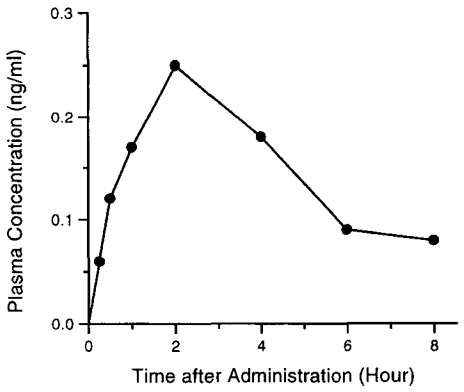


Fig. 4. Plasma levels of AD-5423 in a human subject following single oral administration at a dose of 2 mg of AD-5423.

ues of the intra- and inter-day precision (R.S.D.) of AD-5423 were less than 8% at concentrations of 0.5 and 2 ng ml^{-1}.

AD-5423 and its metabolites in human plasma were stable for at least 24 h at 37 or 4°C and 4 weeks at −20°C.

3.4. Determination of AD-5423 and its metabolites in rat plasma

Using rat plasma, similar studies were conducted for the determination of AD-5423 and its metabolites. The calibration curves showed excellent linearity in the concentration range of 0.1–100 ng ml^{-1} and passed through the origin. The correlation coefficients (r) were 0.999 for all substances. The R.S.D.s at concentrations examined were 0.5–7.1% for AD-5423, 0.6–9.6% for N-desethyl AD-5423 and 0.2–5.8% for AD-5423 N-oxide, respectively (Table 4).

3.5. Application of the assay to human and animal studies

The plasma concentration–time curve of AD-5423 in a human subject after single oral administration of AD-5423 (2 mg) is shown in Fig. 4. Plasma levels of AD-5423 reached a maximum plasma concentration of 0.25 ng ml^{-1} at 2 h after administration, followed by a monophasic decrease with an apparent plasma elimination half-life of 3.4 h. Plasma levels of two N-oxidized metabolites, N-desethyl AD-5423 and AD-5423 N-oxide, were less than the determination limit (0.1 ng ml^{-1}) at all time points.

Fig. 5 presents plasma concentration–time curves of AD-5423 and its metabolites in rats, dog and monkey after single oral administration of AD-5423. Plasma levels of AD-5423 reached a peak 0.5–4 h after administration, followed by a biphasic decrease in the three animal species examined. The two metabolites were also detected in experimental animal plasma.

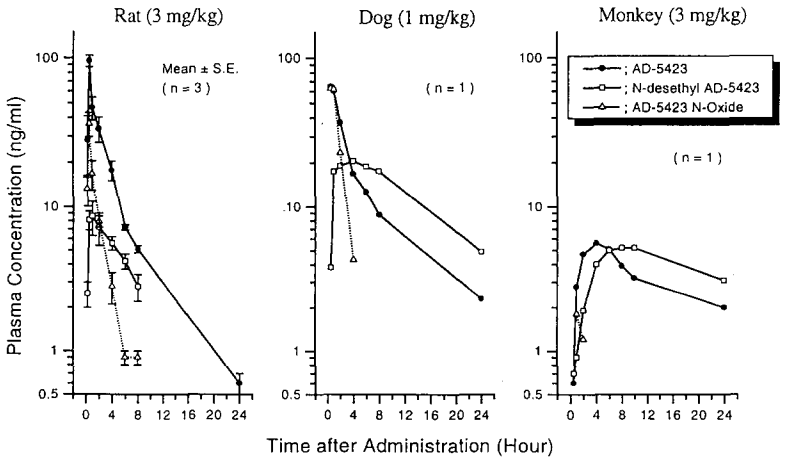


Fig. 5. Plasma levels of AD-5423 and its N-oxidized metabolites in experimental animals following single oral administration of Ad-5423.

A further pharmacokinetic profile of AD-5423 will be published elsewhere.

4. Conclusions

High-performance liquid chromatographic methods have been developed for the determination of AD-5423 and its metabolites in human and experimental animal plasma. The results demonstrated that both AD-5423 and its metabolites in plasma can be quantified with high precision and sensitivity by these methods. Therefore, the developed methods are considered to be effectively applied to the pharmacokinetic study on AD-5423 in animals and human subjects.

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