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Determination of MKT-077, a novel antineoplastic agent, in plasma samples by high-performance liquid chromatography and its application to pharmacokinetics in rats

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Abstract

A simple high-performance liquid chromatographic method was developed for determination of a novel antineoplastic agent MKT-077 in plasma. MKT-077 was extracted from 50 μl of plasma with acetonitrile containing 1 ml trifluoroacetic acid per liter. Chromatographic separation was achieved within 13.5 min using a reverse-phase Puresil C_{18} analytical column. A visible detector operated at 490 nm was used. The linearity of the calibration curve was obtained ($r^2 = 0.99986$) over the analytical range of 10–500 ng ml^{-1}. The intra- and inter-assay precision was in the range of 0.9–11.1 and 0.3–4.4%, respectively. The intra- and inter-assay bias ranged from −7.3 to 11.1% and from 0.4 to 11.6%, respectively. The utility of this assay was demonstrated after the administration of a single dose of MKT-077 to rats. The plasma elimination half-life of MKT-077 was 1.8–4 h.

Keywords: MKT-077; Delocalized lipophilic cation; Reverse-phase liquid chromatography; Rat plasma; Pharmacokinetics; Antineoplastic

1. Introduction

Delocalized lipophilic cationic compounds (DLCs) accumulate and remain in the mitochondria in response to high negative mitochondria membrane electrical potentials [1–3]. Cytotoxic DLCs selectively kill carcinoma cells compared to normal cells grown in vitro because the mitochondrial membrane potential of carcinoma cells is higher than that of normal cells. Therefore, DLCs may provide a new approach to cancer therapy.

A DLC compound, 1-ethyl-2-{[3-ethyl-5-(3-methylbenzothiazolin-2-yliden)]-4-oxothiazolidin-

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2-ylidenemethyl} pyridinium chloride (MKT-077, Fig. 1), which is categorized in rhodacyanine dyes, was developed as a novel antineoplastic compound against carcinoma [4,5]. In vitro, MKT-077 inhibits the growth of five human cancer cell lines (colon carcinoma CX-1, breast carcinoma MCF-7, pancreatic carcinoma CRL1420, bladder transitional cell carcinoma EJ, and melanoma LOX). In nude mice, MKT-077 inhibits the growth of subcutaneously implanted A498 human renal carcinoma cells and DU145 human prostate carcinoma cells and prolongs the survival of mice bearing intraperitoneal implanted [6–8]. Phase I/II clinical trials are in progress.

In the pharmacokinetic study using radiolabeled MKT-077, MKT-077 declined very rapidly to several ng ml^{-1} in a short period after intravenous dosing (submitting data). Establishment of the analytical method for determination of MKT-077 such as the column-switching method and the EIA method was attempted; however a reliable and simple high-performance liquid chromatographic (HPLC) method was needed. In this paper, we describe a simple HPLC method with visible detection for the quantitative determination of MKT-077 in plasma and this method has been applied to evaluate the pharmacokinetics of MKT-077 after an intravenous bolus administration to rats.

2. Experiment

2.1. Instrumentation

The HPLC system consisted of the Hewlett Packard Model 1050 series (HP LC1050 quad pump, HP 1050 Auto sampler (ALS), HP 1050 variable wavelength detector (VWD), and HP LC3D Chemstation (DOS version)). MKT-077 was detected at 490 nm. The HPLC analysis was performed using a gradient mobile phase consisting of acetonitrile–water containing 1 ml trifluoroacetic acid per liter of mobile phase (pH 2.1) (30:70 to 70:30, v/v; start: 0 min, end time: 8 min, hold: 2 min and then recondition, running time: 13.5 min). Mobile phases were degassed and filtered through a solvent filtration apparatus and pumped at a constant rate of 1.0 ml min^{-1}. The separation was performed on a Puresil C_{18}, 4.6 × 150 mm i.d. (Waters, USA) with a precolumn filter (Puresil C_{18}: Waters). The data were collected using Hewlett Packard 1050 series interface, HPLC Chemstation software and Hewlett Packard workstation (DOS version). The column was maintained at 30°C.

2.2. Reagents and standards

MKT-077 was supplied by Fuji Photo Film. The HPLC-grade acetonitrile and water were purchased from EM Science (Gibbstown, NJ). Trifluoroacetic acid was purchased from Aldrich (USA).

MKT-077 was dissolved in DMSO to make 1 mg ml^{-1} of stock solution and stored in the dark at −20°C. Quality control (QC) samples (10, 100 and 1000 ng ml^{-1}) and calibration standards (10, 50, 100, 500 and 1000 ng ml^{-1}) were prepared by mixing a control plasma with solutions of MKT-077. The calibration standards were prepared prior to each assay run. The QC samples were stored and frozen (−20°C) until required.

2.3. Sample preparation

The MKT-077 was added to the plasma at the final concentrations of 5, 10, 50, 100, 500 and 1000 ng $ml^{-1}$and these were used for the calibration. A total of 50 μl working plasma standard was transferred to a 1.5-ml centrifuge tube. A total of 200 μl of acetonitrile containing 1 ml trifluoroacetic acid per liter was added and the sample mixture was vortex-mixed well and centrifuged for 2 min at 10000 rpm. After centrifugation, the supernatant was filtered with 0.5 μm

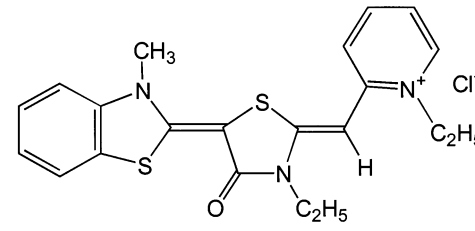

Fig. 1. Chemical Structure of MKT-077.

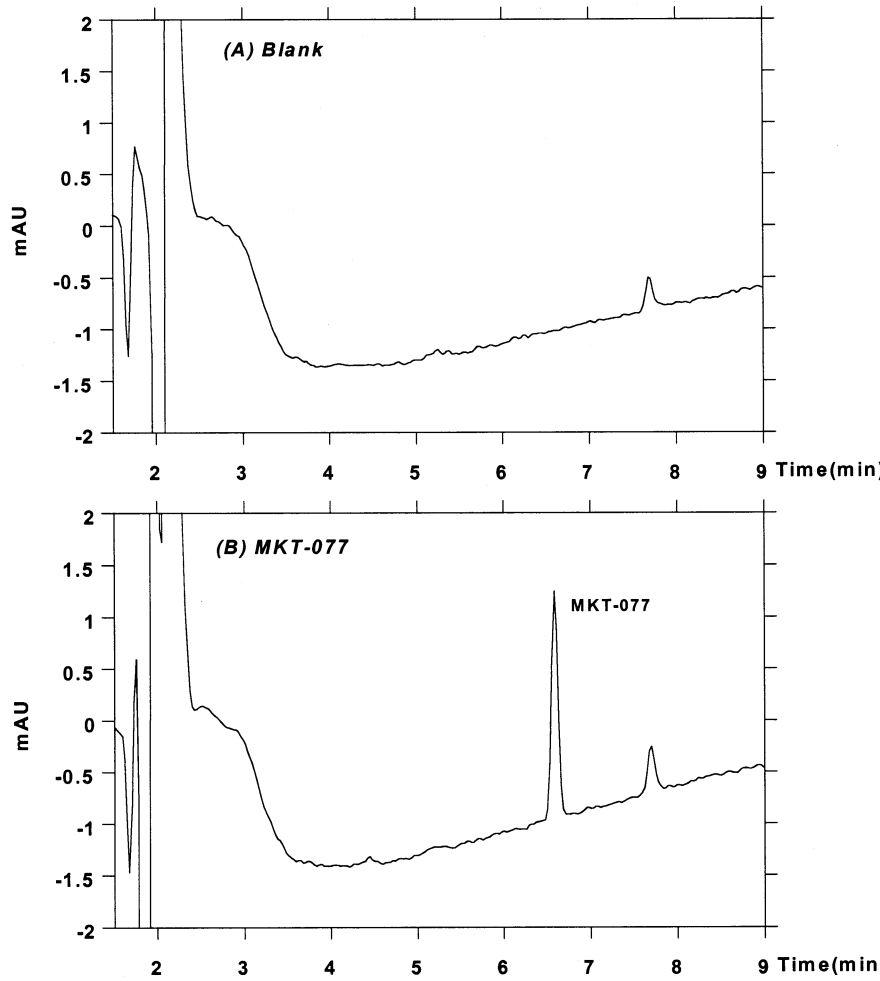

Fig. 2. Chromatograms of extracts from (A) blank rat plasma and (B) plasma containing 0.1 μg ml^{-1} of MKT-077.

pore size filter (Millipore) and transferred into another centrifuge tube. A total of 150 μl of the filtrate was diluted with 150 μl of water containing 1 ml trifluoroacetic acid per liter and mixed well. Then 100 μl of the solution was loaded onto the HPLC system.

2.4. Assay validation and calculations

The intra-assay and inter-assay validation was performed with three aliquots of the QC samples and the accuracy and precision were determined as the RSD (%) from the peak-area of MKT-077. The inter-day coefficient of variation for the slope of the calibration curve was also calculated with calibration standards, to validate the inter-assay reproducibility. The recovery from the plasma samples was determined based on the comparison of the peak-area of extracted samples with those of unextracted standard solutions.

2.5. Animal experiment

Two female Sprague–Dawley rats weighing 242 and 244 g were used for the experiment. The MKT-077 in saline (5 mg ml^{-1}) was injected into the tail vein (10 mg kg^{-1}), and the blood samples were obtained from the orbital sinus at 10, 30

Table 1
Accuracy and precision for MKT-077 assay in rat plasma

Nominal concentration (ng ml^{-1})	Observed concentration (mean ± S.D. ng ml^{-1})	RSD (%)	Bias (%)
Intra-day ($n=3$)			
1000	932 ± 11.2	1.1	−7.3
100	100.1 ± 0.9	0.9	0.1
10	11.1 ± 0.1	1.1	11.1
Inter-day ($n=4$)			
1000	1004 ± 3.4	0.3	0.4
100	101.9 ± 2.5	2.5	1.9
10	11.6 ± 0.5	4.4	11.6

Table 2
Inter-day variation of calibration curve for MKT-077 assay

Concentration range	Equation	r^2	n
10–500 ng ml^{-1}	$y=0.0700\ (\pm 0.0014)x+(0.4581\pm 0.0061)$	0.99986	3

Where x is the concentration of MKT-077 in 10–500 ng ml^{-1} and y is the peak-area unit.

min, 1, 2, 4 and 6 h after injection. The samples were immediately centrifuged to obtain 100 μl of plasma and the plasma was stored at −20°C until analysis.

Each plasma sample (50 μl) was transferred to a 1.5-ml centrifuge tube. A total of 200 μl of acetonitrile containing 1 ml of trifluoroacetic acid per liter was added and the sample mixture was vortex-mixed well and centrifuged for 2 min at 10000 rpm. After centrifugation, the supernatant was filtered and transferred into another centrifuge tube. A total of 150 μl of the filtrate was diluted with 150 μl of water containing 1 ml of trifluoroacetic acid per liter and mixed well. Then 100 μl of the solution was loaded onto the HPLC system.

Table 3
Recovery of MKT-077

Concentration (ng ml^{-1})	Recovery (%) (mean ± SD)	RSD (%)
1000	102.4 ± 4.8	4.7
100	94.4 ± 3.6	3.8
10	73.4 ± 5.8	7.8

3. Result and discussion

3.1. Method evaluation

Fig. 2 shows a typical chromatogram of blank rat plasma (A) and plasma spiked with 0.1 μg ml^{-1} of MKT-077 (B). A sharp peak was obtained for MKT-077. The retention time of MKT-077 was 6.6 min. The lower limit of detection (LLOD) was 5 ng ml^{-1} with a S/N = 4. The lower limit of quantitation (LLOQ) is considered to be 10 ng ml^{-1} from 50 μl of plasma.

The assay precision and accuracy are listed in Table 1. The precision (RSD) from MKT-077 at the three concentrations in the intra-assay study varied between 0.9 and 1.1% and the inter-assay study varied between 0.3 and 4.4%. The measured concentrations of QC samples were found to be in agreement with actual concentrations. The inter-assay precision (RSD) calculated from the slope of the calibration line was less than 0.5% (Table 2). Table 3 lists the recovery results at three concentrations. The recovery at the LLOQ (10 ng ml^{-1}) concentration was lower than that of 100 and 1000 ng ml^{-1} samples. In addition, the recovery of 50 ng ml^{-1} sample was checked and found

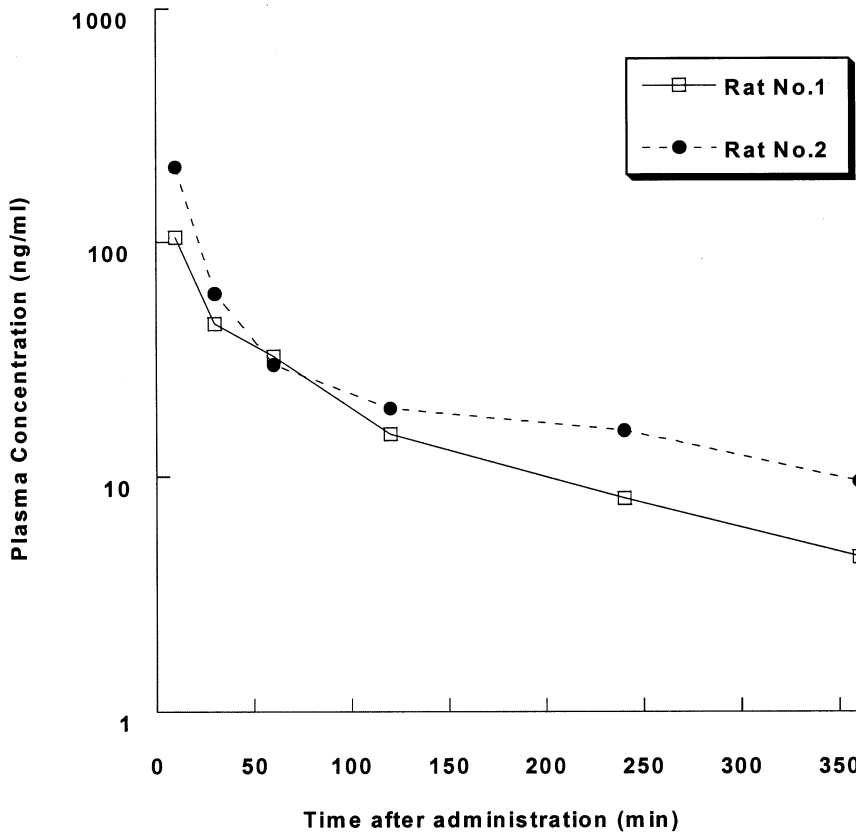

Fig. 3. Time course of MKT-077 concentration in plasma after the intravenous administration of 10 mg kg^{-1} of MKT-077 to rats

to be $90.9 \pm 3.0\%$. In lower concentrations of MKT-077, the absorption of a certain amount of MKT-077 to plasma protein may be due to low recovery.

3.2. Pharmacokinetic results

The present HPLC method was applied to determine the concentration of MKT-077 in plasma after intravenous bolus administration to rats. Fig. 3 shows the plasma concentration time profile of MKT-077 after 10 mg kg^{-1} intravenous administration. The plasma concentration declined in a biexponetial manner. After administration, MKT-077 declined rapidly with $T_{1/2}$ of ~ 1 min. In slower phase, $T_{1/2}$ was 1.8–4 h. These results demonstrated the application of the HPLC method for pharmacokinetic studies of MKT-077.

4. Conclusion

We have demonstrated that a sensitive, accurate and reproducible HPLC method for the analysis of MKT-077, which is expected to be a novel antineoplastic agent, in plasma sample was developed. The work for the pharmacokinetic study in clinical trials is in progress using a similar method such as extraction procedure and detection wavelength with minor modifications. Sensitivity was improved by means of increased plasma sample volume. The results of the clinical pharmacokinetic study will be presented elsewhere.

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References

[1] L.B. Chen, Annu. Rev. Cell Biol. 4 (1988) 155–181.
[2] J.R. Wong, L.B. Chen, Adv. Cell Biol. 2 (1988) 263–290.
[3] L.B. Chen, Anticancer Drugs 191 (1989) 21–28.
[4] M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, A. Ikegawa, K. Ogawa, T. Shishido, L.B. Chen, J. Med. Chem. 40 (1997) 3151–3160.
[5] M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, T. Shishido, L.B. Chen, J. Med. Chem. J. Med. Chem. 41 (1997) 130–142.
[6] K. Koya, Y. Li, H. Wang, T. Ukai, N. Tatsuta, M. Kawakami, T. Shishido, L.B. Chen, Cancer Res. 56 (1996) 538–543.
[7] J.S. Modica-Napolitano, K. Koya, E. Weisberg, B.T. Brunelli, Y. Li, L.B. Chen, Cancer Res. 56 (1996) 544–550.
[8] E.L. Weisberg, K. Koya, J. Modica-Napolitano, Y. Li, L.B. Chen, Cancer Res. 56 (1996) 551–555.