International Journal of Pharmaceutics, 103 (1994) 147–154

IJP 3376

Pharmacokinetics of FT-ADM after intravenous administration of DA-125, a prodrug of FT-ADM or FT-ADM to rats. A new adriamycin analog containing fluorine

Hyun J. Shim [a], Eung D. Lee [a], Eun J. Yoon [b], Sang D. Lee [a], Won B. Kim [a], Junnick Yang [a] and Myung G. Lee [b]

[a] *Research Lab., Dong-A Pharmaceutical Company Ltd, 47 Sanggal-Ri, Kiheung-Up, Yongin-Gun, Kyunggi-Do 449-900 (South Korea) and* [b] *College of Pharmacy, Seoul National University, San 56–1, Shinlim-Dong, Kwanak-Gu, Seoul 151–742 (South Korea)*

(Received 27 April 1993)
(Modified version received 28 July 1993)
(Accepted 3 August 1993)

Key words: Pharmacokinetics; DA-125; M1; M2; M3; M4; Stability; Blood partitioning

Summary

The pharmacokinetics of DA-125 or its active metabolite, M1 (FT-ADM), an adriamycin analog containing fluorine were compared after intravenous (i.v.) administration of DA-125 or M1 in rats. DA-125, 20 mg kg^{-1} was dissolved in 1 mM lactic acid/0.9% NaCl solution (treatment I) or 100% dimethylsulfoxide (DMSO, treatment II), and M1, 20 mg/kg was dissolved in 100% DMSO (treatment III) due to its poor water solubility. The plasma concentrations of DA-125 and M1, and the pharmacokinetic parameters of DA-125, such as terminal half-life ($t_{1/2}$, 1.64 vs 2.07 min), mean residence time (MRT, 1.52 vs 2.60 min), total body clearance (CL, 165 vs 186 ml min^{-1} kg^{-1}) and apparent volume of distribution at steady state (Vd_{ss}, 254 vs 411 ml kg^{-1}), and of M1 (based on plasma data up to 1 h), such as $t_{1/2}$ (30.2 vs 38.7 min), MRT (19.1 vs 31.6 min), CL (187 vs 189 ml min^{-1} kg^{-1}) and Vd_{ss} (2670 vs 5700 ml kg^{-1}) were similar between treatments I and II, indicating that the effect of 100% DMSO on the pharmacokinetics of DA-125 or M1 seemed to be negligible, if any. The plasma concentrations of M1, and the pharmacokinetic parameters of M1 (based on plasma data up to 8 h when the dose of M1, 20 mg kg^{-1} was normalized to the dose of DA-125, 20 mg kg^{-1}), such as $t_{1/2}$ (255 vs 221 min), MRT (269 vs 235 min), CL (103 vs 112 ml min^{-1} kg^{-1}) and Vd_{ss} (28 500 vs 26 300 ml kg^{-1}) were also similar between treatments II and III. The above results indicate that DA-125 is rapidly hydrolyzed to M1 after i.v. administration of DA-125. Therefore, the estimation of the pharmacokinetic parameters of M1 after i.v. administration of DA-125 appeared not to cause any differences, if any when compared with the values after i.v. dose of M1. The rapid hydrolysis of DA-125 to M1 was demonstrated during an in vitro study; the $t_{1/2}$ values of hydrolysis of DA-125 were 1.97, 1.72, 0.54 and 0.54 min in the plasma from mouse, rat, dog and human, respectively, when the plasma containing DA-125 was incubated in a shaking water bath kept at 37°C and at a rate of 300 rpm.

Correspondence to: M.G. Lee, College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea.

Abbreviations: ME2303, (8*s*,10*s*)-8-(6-carboxyhexanoyloxyacetyl)-10-[(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione; FT-ADM (M1), (8*s*,10*s*)-8-hydroxyacetyl-10-[(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione; DA-125, (8*s*,10*s*)-8-(3-aminopropanoyloxyacetyl)-10-[(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione.

SSDI 0378-5173(93)E0240-Q

Introduction

Adriamycin (ADM) is one of the most widely used cancer chemotherapeutic agents, and is highly active against hemological tumors and a number of human solid tumors (Kitaura et al., 1972; Carter, 1975). However, ADM has severe side effects, such as myelosuppression, alopecia and cardiotoxicity which limit its clinical use. A new fluorine-containing anthracycline, ME-2303, has been developed by Meiji Seika Kaisha Ltd (Tokyo, Japan), and its cytocidal activity and LD_{50} (Omoto et al., 1988), and tissue distribution (Iigo et al., 1990) have been reported. ME-2303, a pimelic acid derivative of FT-ADM (M1), is hydrolyzed to FT-ADM, which also has anticancer activity. The potential disadvantage of M1 is its low water solubility, therefore, it may be difficult to develop M1 as a parenteral preparation.

Recently, Research Lab., Dong-A Pharmaceutical Co. Ltd (Yongin, South Korea) developed an adriamycin analog containing fluorine, DA-125, as a water-soluble prodrug of FT-ADM. It is expected that DA-125, a β-alanine derivative of FT-ADM, should be rapidly hydrolyzed to FT-ADM (M1) after intravenous (i.v.) administration. M1 is metabolized to M2 or M3, and both M2 and M3 are further metabolized to M4 (Fig. 1). DA-125 was found to have higher in vivo and in vitro cytocidal activity (Yang et al., 1993), and lower cardiotoxicity and hematotoxicity than that of ADM (Kim et al., 1993). An investigational new drug application for phase I clinical trials on DA-125 has been completed in Korea and the first clinical study is expected to have commenced in 1993.

The purpose of this study is to compare the pharmacokinetic parameters of DA-125 and/or M1 after i.v. administration of DA-125 or M1 either dissolved in 1 mM of lactic acid/0.9% NaCl solution (DA-125) or in 100% dimethylsulfoxide, DMSO (DA-125 and M1) to rats. The stability of DA-125 in plasma from mouse, rat, dog and human, and blood partitioning of DA-125 or M1 between blood cells and plasma from rat whole blood are also reported.

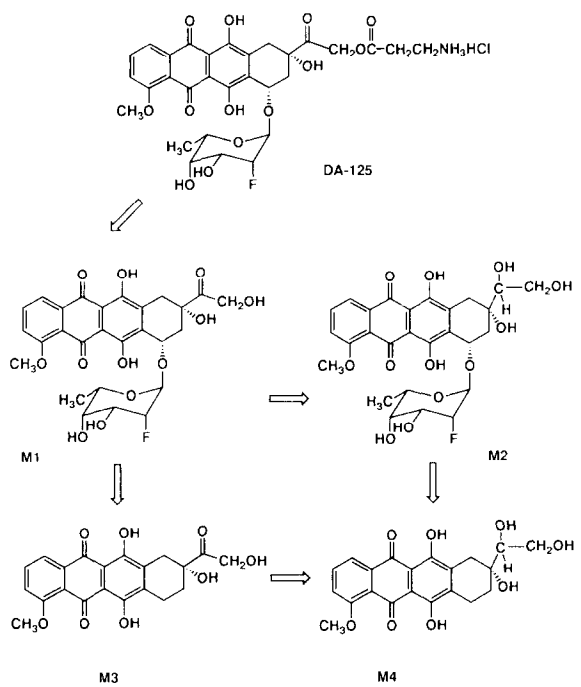

Fig. 1. Metabolic pathways of DA-125.

Materials and Methods

Chemicals

DA-125, M1, M2, M3 and M4 were kindly donated by Research Lab., Dong-A Pharmaceutical Co. Ltd, and fluorescein, an internal standard for HPLC assays, was a product of Sigma Chemical Co. (St. Louis, MO, U.S.A.). The other chemicals were reagent grade or HPLC grade, and were used without further purification.

Stability of DA-125 in plasma from mouse, rat, dog and human

To a test tube containing 4.5 ml of fresh heparinized plasma from mouse, rat, dog or human, 0.5 ml of DA-125 stock solution (DA-125 powder was dissolved in 1 mM of lactic acid/0.9% NaCl solution of pH 4.0 to give a final concentration of 1 mg ml^{-1}) was added. After a vigorous shaking by hand, each tube was incubated in a water bath chamber kept at 37°C and at a rate of 300 rpm using a magnetic stirrer. At designated times (0.33, 0.67, 1, 2, 3, 5 and 7 min), 0.1 ml of plasma

was collected and 0.2 ml of 0.03 N HCl was added immediately in an ice-bath (to stabilize DA-125), and then stored in a freezer prior to the HPLC analysis of DA-125 and M1 (Shim et al., 1992).

Blood partitioning of DA-125 or M1 between blood cells and plasma from rat whole blood

0.03 ml of the DA-125 stock solution was spiked into 0.27 ml of rat whole blood. After brief mixing for a few seconds by hand, the blood was immediately centrifuged. To 0.1 ml of plasma, 0.2 ml of 0.03 N HCl was added immediately in an ice-bath, and stored in the freezer prior to HPLC analysis of DA-125 (Shim et al., 1992). The concentrations of DA-125 in blood cells were also measured using similar procedures to those for the plasma.

10 μl of M1 stock solution (2 mg of M1 was dissolved in 1 ml of 100% DMSO) was spiked into eight test tubes where each tube contained 1 ml of rat whole blood. After brief mixing by hand, each test tube was then placed in a shaking water bath kept at 37°C and at a rate of 50 oscillations per min (opm). At 1, 2, 3, 5, 7, 10, 20 and 30 min, each tube was collected and centrifuged. To 100 μl of each sample of plasma or blood cells, 100 μl of fluorescein (30 μg ml^{-1}, dissolved in distilled water) and 500 μl of acetonitrile were added. After vortex-mixing and centrifugation, HPLC analysis was performed (Shim et al., 1992).

Blood storage effect of DA-125 in rat whole blood

1 ml of the DA-125 stock solution was added to a test tube containing 9 ml of rat whole blood. After brief mixing by hand, the test tube was allowed to stand at room temperature (22°C). Approx. 1 ml of whole blood was collected at 0.5, 1, 2, 3, 5, 6, 10 and 15 min, respectively, after brief mixing by hand to maintain a constant hematocrit value. After immediate centrifugation, 0.1 ml of plasma was collected and 0.2 ml of 0.03 N HCl was added immediately in an ice bath, and then stored in the freezer prior to the HPLC analysis of DA-125 and M1 (Shim et al., 1992).

Animals

Male Sprague-Dawley (SD) rats (8 weeks of age, weighing 240–280 g) were obtained from Charles River Co. (Atsugi, Japan). The animals were housed in a clean room and allowed food (Samyang Co., Seoul, South Korea) and water ad libitum.

Pretreatment of rats

Early in the morning (after overnight fasting with free access to water), the carotid artery and femoral vein were catheterized with polyethylene tubing (Clay Adams, Parsippany, NY, U.S.A.) under light ether anesthesia. Each rat was allowed to recover from anesthesia for 4–5 h before study. They were in the supine position during the study.

Intravenous administration of DA-125 or M1 to rats

DA-125, 20 mg kg^{-1} (dissolved in 1 mM lactic acid/0.9% NaCl solution) was administered by i.v. bolus injection in 10 s via the femoral vein (total injection: volume approx. 0.5 ml kg^{-1}) to five rats. Blood samples (0.25 ml) were collected via the carotid artery at 0 (to serve as a control), 0.5, 1, 2, 3, 5, 7, 10, 20, 30 and 60 min after drug administration. Heparinized normal saline (10 U ml^{-1}), 0.25 ml, was used to flush the cannula after each blood sampling in order to prevent blood from clotting. Blood samples were centrifuged immediately to reduce or minimize the 'blood storage effect', and to prevent the hydrolysis of DA-125 to M1 (Shim et al., 1992). Plasma, 0.1 ml, was collected and 0.2 ml of 0.03 N HCl was added immediately in an ice-bath, and stored in the freezer prior to HPLC analysis (Shim et al., 1992).

DA-125, 20 mg kg^{-1} (dissolved in 100% DMSO) was similarly administered (injection volume: 0.5 ml kg^{-1}) to four other rats. Blood samples were collected at 0, 2, 5, 15, 30, 60, 120, 240, 360 and 480 min. The other procedures were similar to those described above.

M1, 20 mg kg^{-1} (dissolved in 100% DMSO) was also similarly administered (injection volume: 0.5 ml kg^{-1}) to four additional rats. The blood samples were collected at 0, 2, 5, 15, 30, 60, 120, 240, 360 and 480 min. The other procedures were similar to those described above.

Pharmacokinetic analysis

The area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated by extrapolation according to the trapezoidal rule (Choi et al., 1991); this method employed the logarithmic trapezoidal rule (Chiou, 1978) for calculation of the area during the declining plasma-level phase and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

A standard method (Gibaldi and Perrier, 1982) was used to calculate the following pharmacokinetic parameters after i.v. bolus administration; the time-averaged total body clearance (CL), area under the first moment of plasma concentration-time curve (AUMC), mean residence time (MRT), and apparent volume of distribution at steady state (Vd_{ss}).

The mean values of CL, Vd_{ss}, and $t_{1/2}$ were calculated by the harmonic mean method (Chiou, 1979).

Statistical analysis

Levels of statistical significance were assessed using *t*-test between two means for unpaired data. Significant differences were judged as $p < 0.05$. All results are expressed as mean $\pm$ standard deviation.

Results and Discussion

Stability of DA-125 in plasma from mouse, rat, dog and human

The plasma concentrations of DA-125 declined with apparent first-order kinetics in all plasma samples studied (data not shown); the disappearance rate constants of DA-125 were 1.28, 1.28, 0.423 and 0.352 min^{-1} for plasma from human, dog, rat and mouse, respectively, and the corresponding $t_{1/2}$ values were 0.54, 0.54, 1.72 and 1.97 min. The sum of the plasma concentrations of DA-125 and M1 which are expressed in terms of DA-125 were almost constant for up to 3 min of incubation in all the plasma samples studied indicating that further metabolism of M1 in the plasma incubated for up to 3 min is negligible, if any. This was supported by the fact that M2, M3 and M4 were not detected in the present HPLC chromatogram.

Blood partitioning of DA-125 or M1 between blood cells and plasma from rat whole blood

Mean concentrations of DA-125 in plasma and blood cells were 157 ± 7.49 and 43.1 ± 3.34 μg ml^{-1}, respectively, when rat whole blood ($n = 5$, mean hematocrit of approx. 50%) was immediately centrifuged after addition of DA-125 and then briefly mixed manually for a few seconds. The blood cell to plasma concentration ratio of DA-125 was 0.275, indicating that approx. 21.6% of DA-125 is bound (or partitioned) to blood cells as soon as DA-125 is spiked into rat whole blood. Recovery of the amount of DA-125 from plasma and blood cells was approx. 99.94%, suggesting that DA-125 is fairly stable on the addition of 0.03 N HCl.

The concentrations of M1 in both blood cells and plasma decreased with increasing incubation time (due to increased formation of M2 from M1 in rat whole blood) after incubation of M1 with rat whole blood, however, the blood cell to plasma ratio of M1 was almost constant, 0.813–0.934 for up to 30 min of incubation (data not shown). The concentrations of M2 in both blood cells and plasma increased with increasing incubation time of M1, however, the blood cell to plasma ratio of M2 decreased from 3.02 (at 2 min of incubation) to 1.08 (at 10 min of incubation) and remained almost constant thereafter (0.877 at 30 min of incubation). M3 and M4 were not detected in the HPLC chromatogram.

Blood storage effect of DA-125 in rat blood

The plasma concentrations of DA-125 declined rapidly with apparent first order kinetics after rat whole blood spiked with DA-125 had been left to stand (Fig. 2); the disappearance rate constant and the corresponding $t_{1/2}$ of DA-125 were 0.162 min^{-1} and 4.28 min, respectively.

In pharmacokinetic studies, accurately measured plasma drug concentrations are usually assumed to equal their in vivo plasma concentrations. Such an assumption might not be valid for

DA-125 due to a significant blood storage effect of DA-125 if the blood samples of DA-125 is not centrifuged immediately. For example, if the blood sample is centrifuged at 10 min after standing at room temperature, then 80% of the DA-125 in plasma disappears (possibly due to hydrolysis of DA-125 to M1) when compared with that for immediate centrifugation. A significant blood storage effect has also been reported for a number of drugs (Lee et al., 1981a,b, 1984; Chen et al., 1983; Chang et al., 1988; Shin et al., 1992).

Pharmacokinetics of DA-125 and its metabolite, M1 after intravenous administration of DA-125, 20 mg kg^{-1} dissolved in 1 mM of lactic acid / 0.9% NaCl solution in rats

Mean plasma concentration-time profiles of DA-125 and its metabolite, M1 after i.v. administration of DA-125, 20 mg kg^{-1} are shown in Fig. 3, and the relevant pharmacokinetic parameters are listed in Table 1. The data for M2, M3 and M4 are not included in Fig. 3 and Table 1. After i.v. administration of DA-125, the plasma level of DA-125 decayed rapidly; the mean values of $t_{1/2}$, MRT and CL of DA-125 were 1.64 min, 1.52 min and 165 ml min^{-1} kg^{-1}, respectively (Table 1). Plasma concentrations of DA-125 were detected for up to 10 min after i.v. dose due to rapid

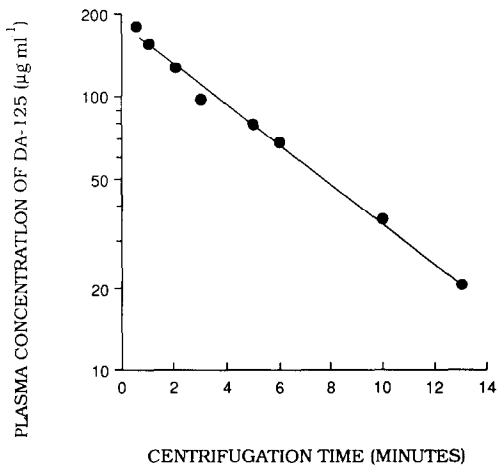

Fig. 2. Plasma concentrations of DA-125 as a function of blood centrifugation time after standing rat whole blood containing 100 μg of DA-125 per ml of whole blood at room temperature.

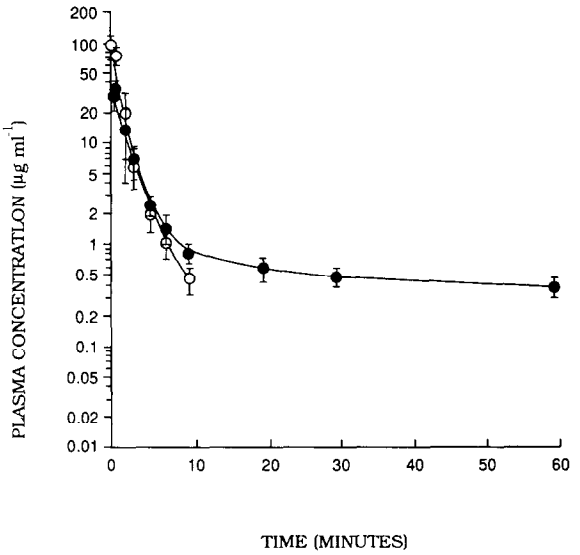

Fig. 3. Plasma concentration-time profiles of DA-125 (○) and M1 (●) after intravenous administration of DA-125, 20 mg kg^{-1} dissolved in 1 mM lactic acid/0.9% NaCl solution to five rats. Bars represent standard deviation.

hydrolysis of DA-125 to M1, and were expected based on in vitro stability testing. The maximum plasma concentration of M1 was reached at 1 min after i.v. administration of DA-125 which also indicated that DA-125 is rapidly hydrolyzed to M1 after i.v. administration of DA-125. Plasma concentrations of M1 declined rapidly for up to 10 min, and declined slowly thereafter with a mean terminal half-life of 30.2 min. Although DA-125 is rapidly hydrolyzed to M1, the apparent terminal half-life of M1 was approx. 20-times

TABLE 1

Mean (± standard deviation) pharmacokinetic parameters of DA-125 and M1 after intravenous administration of DA-125, 20 mg kg^{-1} dissolved in 1 mM lactic acid / 0.9% NaCl solution to rats (n = 5)

Pharmacokinetic parameters	DA-125	M1
$t_{1/2}$ (min)	1.64 ± 0.331	30.2 ± 12.4
AUC (μg min ml^{-1})	129 ± 35.4	99.4 ± 26.2
AUMC (μg min^2 ml^{-1})	196 ± 60.9	1820 ± 915
MRT (min)	1.52 ± 0.222	19.1 ± 11.2
CL (ml min^{-1} kg^{-1})	165 ± 55.4	187 ± 50.9
Vd_{ss} (ml kg^{-1})	254 ± 105	2670 ± 1060

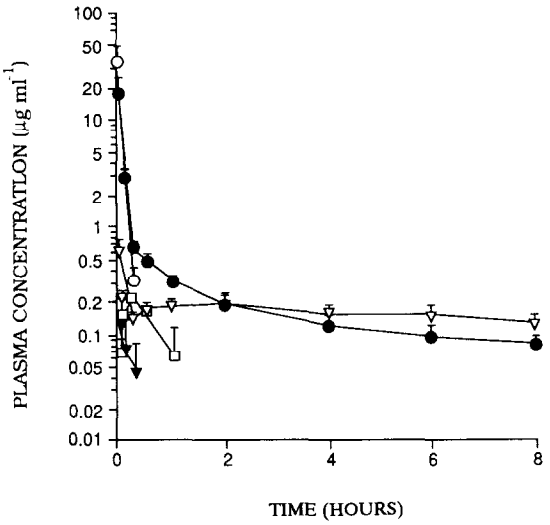

Fig. 4. Plasma concentration-time profiles of DA-125 (○), M1 (●), M2 (▽), M3 (▼) and M4 (□) after intravenous administration of DA-125, 20 mg kg^{-1} dissolved in DMSO to four rats. Bars represent standard deviation.

greater than that of DA-125, and could be due to a significant increase in Vd_{ss} for M1 when compared with that for DA-125 (254 vs 2670 ml kg^{-1}) since the CL values were similar between DA-125 and M1 (Table 1).

Pharmacokinetics of DA-125, M1, M2, M3 and M4 after intravenous administrations of DA-125, 20 mg kg^{-1} dissolved in 100% DMSO in rats

Mean plasma concentration-time profiles of DA-125, M1, M2, M3 and M4 after i.v. administration of DA-125, 20 mg kg^{-1} are shown in Fig. 4, and the pharmacokinetic parameters of DA-125 and M1 are listed in Table 2. The mean plasma concentrations of DA-125 declined rapidly with mean values of $t_{1/2}$, MRT and CL of 2.07 min, 2.60 min, and 186 ml min^{-1} kg^{-1}, respectively. However, the mean plasma concentrations of M1 declined rapidly up to 2 h and declined slowly thereafter with a mean terminal half-life of 255 min (Table 2). The mean plasma concentrations of M2 also declined rapidly up to 15 min and the plasma level, approx. 0.2 μg ml^{-1}, was maintained for up to 8 h after the i.v. dose of DA-125. It is interesting to note that the plasma concentrations of M3 were lower than those of M1, M2 and M4, and were detected only for up to 15 min after i.v. administration of DA-125. Mean plasma concentrations of M4 declined rapidly and were detected for up to 1 h after i.v. administration of DA-125.

Since the pharmacokinetic parameters of M1 in Table 1 were estimated based on the plasma data up to 1 h, the parameters of M1 in Table 2 were also estimated based on the plasma data up to 1 h for comparison. It should be noted that the plasma concentrations of DA-125 and M1 (Figs 3 and 4), and the pharmacokinetic parameters of DA-125, and M1 were very close after the i.v. dose of DA-125 either dissolved in 1 mM lactic acid/0.9% NaCl solution (Table 1) or in 100% DMSO (Table 2 based on plasma data up to 1 h). Therefore, it could be concluded that the effect, if any of DMSO on the pharmacokinetics of DA-125 and M1 appeared to be negligible. The extent of hemolysis due to injection of DMSO

TABLE 2

Mean (±standard deviation) pharmacokinetic parameters of DA-125 and M1 after intravenous administration of DA-125, 20 mg kg^{-1} dissolved in 100% dimethylsulfoxide to four rats

Pharmacokinetic parameters	DA-125	M1	
		Based on plasma data up to 1 h	Based on plasma data up to 8 h
$t_{1/2}$ (min)	2.07 ± 0.290	38.7 ± 17.4	255 ± 74.3
AUC (μg min ml^{-1})	124 ± 56.0	94.0 ± 25.0	163 ± 23.5
AUMC (μg min^2 ml^{-1})	315 ± 109	3160 ± 2290	42800 ± 14000
MRT (min)	2.60 ± 0.294	31.6 ± 29.7	269 ± 106
CL (ml min^{-1} kg^{-1})	186 ± 77.1	189 ± 62.9	103 ± 14.6
Vd_{ss} (ml kg^{-1})	411 ± 194	5700 ± 2960	28500 ± 14800

was determined by injection of 0.5 ml kg^{-1} of 100% DMSO via the rat femoral vein and arterial blood was collected at 5 min after injection. After immediate centrifugation of the blood, plasma was collected and filtered through a 0.45 μm membrane filter. The percentages of hemolysis were only 2.1% as determined by measuring the absorbance at 415 nm using a spectrophotometer.

Pharmacokinetics of M1, M2, M3 and M4 after intravenous administration of M1, 20 mg kg^{-1} dissolved in 100% DMSO in rats

Mean plasma concentration-time profiles of M1, M2, M3 and M4 after i.v. administration of M1, 20 mg kg^{-1} are shown in Fig. 5, and the pharmacokinetic parameters of M1 are listed in Table 3. The decay patterns of plasma concentrations of M1, M2, M3 and M4 were comparable to those after i.v. administration of DA-125 dissolved in 100% DMSO (Fig. 4). It is interesting to note that the plasma concentrations of M1 and M2 after the i.v. dose of DA-125, 20 mg kg^{-1} (Fig. 4) and M1, 20 mg kg^{-1} (Fig. 5) dissolved in both 100% DMSO were very similar when the dose of M1, 20 mg kg^{-1} was normalized to that of DA-125, 20 mg kg^{-1}. Moreover, the pharmacokinetic parameters of M1 given in Tables 2 and 3 (based on plasma data up to 8 h) were very similar. This suggested that the intravenously administered DA-125 is essentially completely hydrolyzed to M1 after injection to rats, therefore, the estimation of the pharmacokinetic parameters of M1 after the i.v. dose of DA-125 did not appear to result in any differences, if any when compared with the values after the i.v. dose of M1.

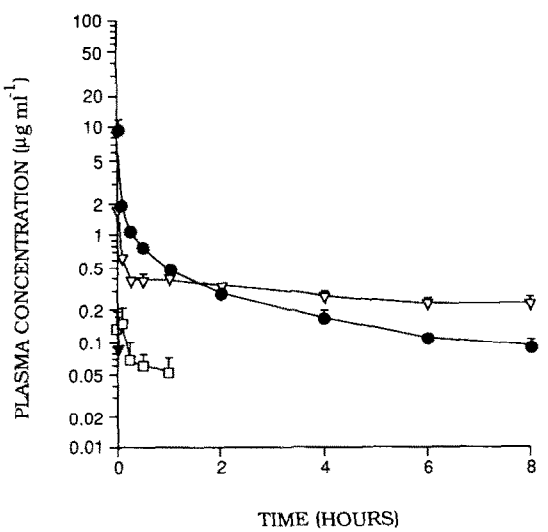

Fig. 5. Plasma concentration-time profiles of M1 (●), M2 (▽), M3 (▼) and M4 (□) after intravenous administration of M1, 20 mg kg^{-1} dissolved in DMSO to four rats. Bars represent standard deviation.

TABLE 3

Mean (± standard deviation) pharmacokinetic parameters of M1 after intravenous administration of M1 dissolved in 100% DMSO, 20 mg kg^{-1} to four rats

Pharmacokinetic parameters	M1
$t_{1/2}$ (min)	221 ± 21.7
AUC (μg min ml^{-1})	178 ± 15.7
AUMC (μg min^2 ml^{-1})	42100 ± 8860
MRT (min)	235 ± 29.6
CL (ml min^{-1} kg^{-1})	112 ± 10.1
Vd_{ss} (ml kg^{-1})	26300 ± 2140

Acknowledgement

This work was supported by a contract from Research Lab., Dong-A Pharmaceutical Co. Ltd, Yongin, South Korea, 1992.

References

Carter, S.K., Adriamycin – a review. *J. Natl. Cancer Inst.*, 55 (1975) 1265–1274.

Chang, H.W., Yoon, E.J., Lee, M.G. and Kim, N.D., Pharmacokinetics of drugs in blood: VI. Unusual distribution and blood storage effect of bumetanide. *Seoul Univ. J. Pharm. Sci.*, 13 (1988) 1–7.

Chen, M.-L., Lee, M.G. and Chiou, W.L., Pharmacokinetics of drugs in blood: III. Metabolism of procainamide and storage effect of blood samples. *J. Pharm. Sci.*, 72 (1983) 572–574.

Chiou, W.L., Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma concentration-time curve. *J. Pharmacokinet. Biopharm.*, 6 (1978) 539–546.

Chiou, W.L., New calculation method for mean apparent drug volume of distribution and application to rationale dosage regimens. *J. Pharm. Sci.*, 68 (1979) 1067–1069.

Choi, Y.M., Kim, S.H. and Lee, M.G., Effects of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetics and pharmacodynamics of furosemide in rats. *J. Pharm. Sci.,* 80 (1991) 638–642.

Gibaldi, M. and Perrier D., *Pharmacokinetics,* 2nd Edn, Dekker, New York, 1982.

Iigo, M., Nishikata, K.-i., Nakajima, Y., Hoshi, A., Kadosawa, H. and Nakajima, S., Therapeutic activity and tissue distribution of ME2303, a new anthracycline containing fluorine, and its metabolites in mice bearing hepatic metastases of Lewis lung carcinoma. *Intr. Cancer Drugs,* 1 (1990) 77–82.

Kim, M.S., Kim, J.H., Park, J.W., Chung, M.H. and Baik, N.G., Studies on the side-effects and the cardiotoxicity on DA-125, a new anthracycline antineoplastic agent. *Second Congress of New Drug Development,* Abstr., p. 38, April 15, 1993, Seoul, South Korea.

Kitaura, K., Watanabe, Y., Ishihara, Y. and Takahira, H., Antitumor activity of adriamycin. *Jap. J. Antibiot.,* 25 (1972) 65–71.

Lee, M.G., Chen, M.-L. and Chiou, W.L., Pharmacokinetics of drugs in blood: II. Unusual distribution and storage effect of furosemide. *Res. Commun. Chem. Pathol. Pharmacol.,* 34 (1981a) 17–23.

Lee, M.G., Chen, M.-L., Huang, S.M. and Chiou, W.L., Pharmacokinetics of drug in blood: I. Unusual distribution of gentamicin. *Biopharm. Drug Dispos.,* 2 (1981b) 89–97.

Lee, M.G., Lui, C.Y., Chen, M.-L. and Chiou, W.L., Pharmacokinetics of drugs in blood: IV. Unusual distribution, storage effect and metabolism of methotrexate. *Int. J. Clin. Pharmacol. Ther. Toxicol.,* 22 (1984) 530–537.

Omoto, S., Kondo, S., Fukastu, S., Takeuchi, T. and Umezawa, K., Biological properties of ME2303 (FAD-104), a new anthracycline. *Drugs Exp. Clin. Res.,* 14 (1988) 435–439.

Shim, H.J., Lee, E.D., Lee, S.D., Kim, W.B. and Lee, M.G., HPLC assay and stability test of DA-125, a water soluble prodrug of FT-ADM. *41st Annual Convention of the Pharmaceutical Society of Korea,* Abstr., p. 167, Oct. 23–24, 1992, Dae-Jeon, South Korea.

Shin, W.G., Lee, M.G., Lee, M.H. and Kim, N.D., Pharmacokinetics of drugs in blood: VII. Unusual distribution and blood storage effects of vancomycin. *Biopharm. Drug Dispos.,* 13 (1992) 305–310.

Yang, J., Kim, W.B., Kim, K.S. and Rhu, H.M., Chemical, physicochemical and pharmacological studies on DA-125, a new anticancer drug. *Second Congress of New Drug Development,* Abstr., p. 36, April 15, 1993, Seoul, South Korea.