BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **19**: 79–89 (1998)

Pharmacokinetic Changes of M1, M2, M3 and M4 after Intravenous Administration of a New Anthracycline, DA-125, to Rats Pretreated with Phenobarbital, 3-Methylcholanthrene, Chloramphenicol, or SKF-525A

Yun J. Choi[a], Hee J. Lee[a], Jong W. Kwon[b], Won B. Kim[b], Junnick Yang[b] and Myung G. Lee[a,*]

[a] *College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Republic of Korea*

[b] *Research Laboratories, Dong-A Pharmaceutical Company Ltd., 47 Sanggal-Ri, Kiheung-Up, Yongin-Gun, Kyunggi-Do 449-900, Republic of Korea*

ABSTRACT: The pharmacokinetics of M1–M4, the metabolites of a new anthracycline antineoplastic agent, DA-125, were compared after intravenous (IV) administration of DA-125, 15 mg kg^{-1}, to rats pretreated with enzyme inducers, such as phenobarbital (PBT, $n = 14$) and 3-methylcholanthrene (MCT, $n = 15$), or enzyme inhibitors, such as SKF-525A (SKT, $n = 11$) and chloramphenicol (CMT, $n = 15$), and to their control rats ($n = 15$ for PBC, CMC or SKC, and $n = 11$ for MCC). After IV administration of DA-125, the plasma concentrations of both M1 and M2 declined slowly from 1 to 2 h onwards to 8 h in all groups of rats due to the continuous formation of M2 from M1. The $AUC_{0-8\,h}$ of M1 (47.1 versus 7.85 μg min mL^{-1}) and M2 (20.7 versus 44.3 μg min mL^{-1}) decreased significantly in the PBT group compared to those in the PBC group. However, the corresponding value of only M1 (74.6 versus 89.9 μg min mL^{-1}) decreased significantly in the MCT group. The above data indicate that metabolism of M1 is increased by pretreatment with both PB and 3-MC, and that of M2 with PB, but not with 3-MC. The $AUC_{0-8\,h}$ of both M1 (126 versus 78.5 μg min mL^{-1}) and M2 (69.2 versus 44.3 μg min mL^{-1}) increased significantly in the SKT group compared to the SKC group. However, the corresponding values were not significantly different between CMC and CMT groups. The above data indicate that the metabolism of both M1 and M2 is inhibited by pretreatment with SKF-525A, but not with CM. © 1998 John Wiley & Sons, Ltd.

Key words: pharmacokinetics; DA-125; M1, M2, M3, and M4; phenobarbital; 3-methylcholanthrene; chloramphenicol; SKF-525A

Introduction

New anthracycline antineoplastic agents containing fluorine have been synthesized to decrease cardiotoxicity and increase antitumour activity of adriamycin. For this purpose, the Research Laboratories of Dong-A Pharmaceutical (Yongin, Republic of Korea) have recently developed DA-125, a β-alanine derivative of M1 (FT-ADM). Having completed phase I clinical trials as an antineoplastic agent, DA-125, a water-soluble prodrug of M1, is currently being evaluated in phase II clinical trials. DA-125 is hydrolysed to its active component, M1, and M1 is further metabolized to M2 and M3, which then are further metabolized to M4 (Figure 1). Among M1–M4, only M1 has antineoplastic activity.

In the preceding papers, the simultaneous HPLC analysis of DA-125, and M1–M4 in plasma and urine [1], stability, blood partition, and pharmacokinetics of DA-125 in rats [2], pharmacokinetics of DA-125 in dogs [3], and in uranyl-nitrate-induced acute renal failure rats or protein-calorie malnutrition rats [4], pharmacokinetics and antitumour activities of DA-125 against subcutaneously implanted Lewis-lung-carcinoma-bearing BDF_1 mice [5], nonlinear pharmacokinetics of DA-125 in rats and rabbits [6], pharmacokinetics of DA-125 after multiple dosing to rats [7], and the pharmacokinetics of DA-125 in spontaneously hypertensive rats and deoxycorticosterone-acetate-salt-induced hypertensive rats [8] have been reported from our laboratory. The exact metabolizing organs for M1 were not thoroughly studied. However, it has been reported [6] that essentially almost all of the M1 was metabolized in the rat liver and kidney based on tissue homogenate study; 1.42 and 2.57%—expressed in terms of DA-125—of M1 remained after 30 min incubation in the rat liver and kidney homogenate, respectively. The role of kidney for the metabolism of M1 was also proved by the fact that the areas under the plasma concentration–time curves of M1,

* Correspondence to: College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Republic of Korea.

Contract grant sponsor: Korea Ministry of Science and Technology; Contract grant number: HAN 4-1-3

CCC 0142–2782/98/020079–11$17.50

Received 21 August 1996
Accepted 28 January 1997

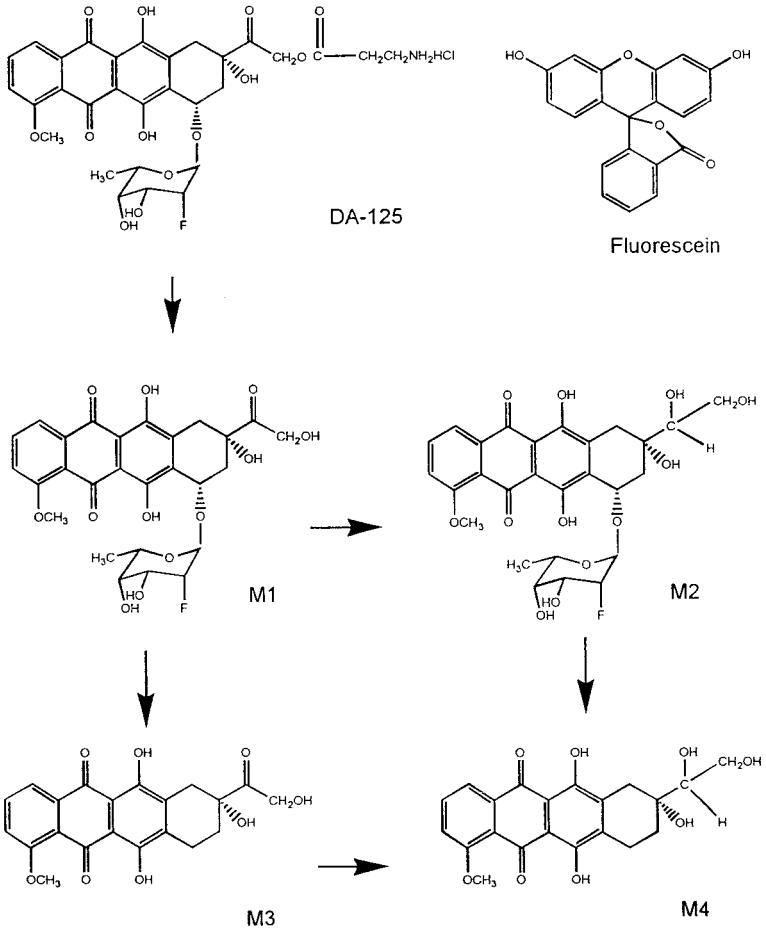

Figure 1. Structures of DA-125, M1–M4, and fluorescein (internal standard), and metabolic pathways of DA-125

M2 and M4 from time zero to the last measured time were significantly higher after intravenous administration of DA-125 to uranyl-nitrate-induced acute renal failure rats than those for the control rats.

The effects of enzyme inducers, such as phenobarbital (PB) and 3-methylcholanthrene (3-MC), and/or enzyme inhibitor, such as chloramphenicol (CM), on the pharmacokinetics and pharmacodynamics of furosemide [9], bumetanide [10], and azosemide [11] have been reported from our laboratory. The purpose of this paper is to report the pharmacokinetic changes of M1–M4 after intravenous (IV) administration of DA-125 to rats pretreated with enzyme inducers, such as PB and 3-MC, or enzyme inhibitors, such as SKF-525A and CM.

Materials and Methods

Chemicals

DA-125, and M1–M4 were kindly donated by the Research Laboratories of Dong-A Pharmaceutical. Fluorescein, the internal standard used in HPLC analysis, and 3-MC were products of Sigma (St. Louis, MO). CM (as a sodium succinate salt, IV powder) and SKF-525A were gifts from Chong-Kun-Dang Corporation (Seoul, Republic of Korea) and SKF (Smith Kline and French Laboratories, Philadelphia, PA, USA), respectively. Sodium PB IV solution was a product from Jeil Pharmaceutical (Taegu, Republic of Korea). Heparin IV solution was obtained from Choong-Wae Pharmaceutical (Seoul, Republic of Korea). Other chemicals were of reagent grade or HPLC grade, and thus were used without further purification.

Pretreatment with Enzyme Inducers, PB and 3-MC, or Enzyme Inhibitors, SKF-525A and CM, in Rats

Male Sprague–Dawley rats of 9 weeks of age, weighing 285–370 g, were purchased from Charles River (Atsugi, Japan). The animals were housed in a clean room and given food (Samyang, Seoul, Republic of Korea) and water *ad libitum*.

Rats received four daily intraperitoneal (IP) injections of 80 mg kg^{-1} sodium PB diluted with normal saline injectable solution (PBT group) [9,10,12], 20 mg kg^{-1} 3-MC dissolved in corn oil (MCT group) [9,10,12], five daily IP injections of 30 mg kg^{-1} CM sodium succinate reconstituted with normal saline

injectable solution (CMT group) [13], four daily IP injections of normal saline injectable solution (PBC, CMC, or SKC group) or corn oil (MCC group), or a single IP injection of 50 mg kg^{-1} SKF-525A 1 h before the experiment began (SKT group) [14]. During pretreatment, rats had free access to food and water. The experiment was performed in the morning at 1 h following pretreatment for the SKT group, on the fifth day for PBT, MCT, PBC, SKC, and MCC groups, or on the sixth day for CMT and CMC groups after the commencement of their respective pretreatment.

Intravenous Infusion Studies

The carotid artery and the jugular vein of each rat were cannulated individually with polyethylene tubing (Clay Adams, Parsippany, NJ) under light ether anaesthesia. Both cannulae were exteriorized to the dorsal side of the neck and then each polyethylene tubing was connected individually to a long Silastic tubing (Dow Corning, Midland, MI). Both Silastic tubings were covered with a wire to allow free movement of the rat. The exposed areas were surgically sutured. Heparinized normal saline injectable solution (15 units mL^{-1}), 0.25 mL, was used to flush each cannula to prevent blood clotting. Each rat was housed individually in a metabolic cage (Daejong Scientific, Seoul, Republic of Korea) and allowed to recover from anaesthesia for 4–5 h before the experiment started. They were not restrained at any time during the study.

DA-125 powder (dissolved in 1 mM lactic acid of pH 4.0/normal saline injectable solution), 15 mg kg^{-1}, was injected via the jugular vein of the control ($n = 15$ for PBC, CMC, or SKC, and $n = 11$ for MCC), PBT ($n = 14$), MCT ($n = 15$), SKT ($n = 11$), and CMT ($n = 15$) rats. The total injection volume was approximately 1 mL. Blood samples (0.12–0.25 mL) were collected via the carotid artery at 0 (to serve as a control), 1 (at the end of infusion), 5, 15, 30, 45, 60, 120, 180, 240, 360, 480, and 600 min following drug administration. Heparinized normal saline injectable solution, 0.25 mL, was also used to flush the cannula immediately after each blood sampling. Blood samples were centrifuged immediately to reduce any possible 'blood storage effect' of the plasma concentration of M1 [2], and 50–100 μl of each plasma were stored in the freezer until the HPLC analysis of M1–M4 [1]. Twenty-four hours after IV injection, each metabolic cage was rinsed with 20 mL distilled water, which was then combined with 24 h urine where 10 mL 0.1 N HCl was previously added (before the commencement of the experiment) to stabilize M1. The urinary bladder was cut and washed into the combined urine, two 0.1 mL samples were frozen until the HPLC analysis of M1–M4 [1]. At the same time, the whole GI tract (including its contents and faeces) of each rat was removed, transferred to a beaker containing 50 mL 0.1 N HCl (to stabilize M1), and cut into small pieces using a pair of scissors. After shaking vigorously for 10 min, two 0.1 mL aliquots of the supernatant were collected from each beaker and stored in the freezer until the HPLC analysis of M1–M4 [1].

Pharmacokinetic Analysis

The area under the plasma concentration–time curves of M1, M2, and M4 from time zero to the last measured time (AUC_{0-t}) was calculated by the trapezoidal rule method [15]; this method employed the logarithmic trapezoidal rule recommended by Chiou [16] for the calculation of area during the declining plasma level phase, and the linear trapezoidal rule for the rising level phase. The total body clearance (Cl) of M1 was measured by dividing the dose of M1 (calculated from DA-125) by $AUC_{0-8\,h}$ of M1. The renal clearances (Cl_r) of M1, M2, and M4 were measured by dividing the total amount (A_e) of M1, M2, and M4 excreted in 24 h (this was assumed to be equal to the total amount of M1, M2, and M4 excreted in urine, since only negligible amounts of them could be found in the urine collected thereafter) by AUC_{0-t} of M1, M2, and M4, respectively. Therefore, the Cl value of M1 and the Cl_r values of M1, M2, and M4 could have been somewhat overestimated. The mean values of Cl and Cl_r were estimated by the harmonic mean method [17].

Statistical Analysis

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

Results and Discussion

In our preceding paper [2], the pharmacokinetic parameters of M1 after IV administration of DA-125 were comparable to those after IV administration of M1, and the *in vitro* degradation half-life of DA-125 to M1 in rat plasma was 1.72 min, both suggesting that DA-125 rapidly transformed to M1 following its IV administration to rats. Therefore, the pharmacokinetic parameters of M1 were estimated after IV dose of DA-125 in the present rat study. Since DA-125 is a water-soluble prodrug of M1—the only metabolite among M1–M4 that has antineoplastic activity—and the plasma concentrations of DA-125 were detected only for a very short period of time after IV administration of DA-125 to rats [2], the concentrations of DA-125 were not measured in the present rat study.

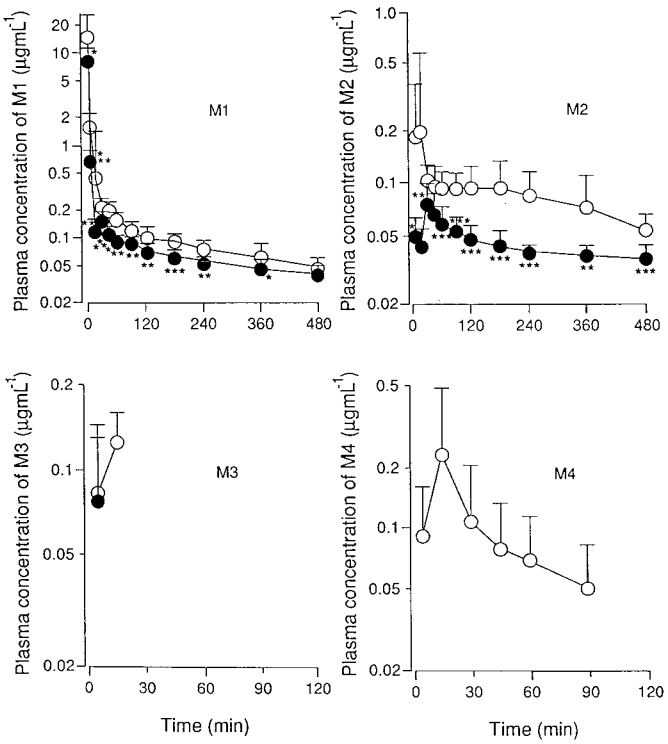

Figure 2. Mean arterial plasma concentration–time profiles of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (○, $n = 15$) and the PB-pretreated (●, $n = 14$) rats. Bars represent standard deviation. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

The mean arterial plasma concentration–time profiles of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the PBT, MCT, SKT, and CMT groups with their corresponding control groups are shown in Figures 2–5, respectively, and relevant pharmacokinetic parameters are listed in Tables 1–4, respectively. After IV administration of DA-125, the plasma concentrations of both M1 and M2 declined very slowly from 1–2 h onwards to 8 h in all groups of rats (Figures 2–5). This could be due to the continuous formation of M2 from M1 [6]. The plasma concentrations of M3 were the lowest among M1–M4 in all groups of rats and were detected only for a few minutes after IV administration of DA-125 (Figures 2–5). Therefore, the AUC_{0-t} of M3 was not calculated. Similar results were also obtained from rats [2,6], rabbits [6], and dogs [3]. This could be due to the fast and essentially complete conversion of M3 to M4 as soon as M3 was formed from M1 [6]. The plasma concentrations of M4 were also generally low.

PB is a hepatic cytochrome P-450 enzyme inducer typically inducing isozyme 2B (as well as 3A), and 3-MC is an oil-soluble hepatic cytochrome P-450 enzyme inducer typically inducing isozyme 1A. The mean arterial plasma concentrations of M1 in the PBT group were significantly lower than those of the PBC group (Figure 2), resulting in a significant decrease in the $AUC_{0-8\ h}$ of M1 in the PBT group (the $AUC_{0-8\ h}$ of M1 in the PBT group was 60% of that in the PBC group) (Table 1). The plasma concentrations of M1 in the MCT group were also significantly lower than those in the MCC group, especially from 90 min after IV administration of DA-125 (Figure 3), and hence the $AUC_{0-8\ h}$ decreased significantly in the MCT group (the $AUC_{0-8\ h}$ of M1 in the MCT group was 83% of that in the MCC group) (Table 2). This indicated that metabolism of M1 increases on pretreatment with both PB and 3-MC. The plasma concentrations of M2 in the PBT group were also significantly lower than those of the PBC group (Figure 2), resulting in a significant decreases in $AUC_{0-8\ h}$ of M2 in the PBT group (the $AUC_{0-8\ h}$ value of M2 in the PBT group was 47% of that in the PC group) (Table 1). However, the plasma concentrations of M2 (Figure 3) and hence the $AUC_{0-8\ h}$ of M2 were not significantly different between MCC and MCT groups (Table 2). The above data indicate that metabolism of M2 increases on pretreatment with PB, but not with 3-MC. It has been reported [8] in rats that a negligible amount of M3 is formed from M2, therefore, in the present PBT group, M2 was metabolized to other metabolite(s). Neither M3 nor M4 was detected in plasma in both PB- (Figure 2) and 3-MC- (Figure 3) pretreated groups except for the detection of M3 at 1 min in the PBT group (Figure 2). It has been found from the previous rat studies [6] that M3 is formed from M1, and M3 is rapidly and essentially completely metabolized to M4 as soon as M3 is formed from M1, and essentially all M4 is formed from M3, not from M2. Therefore, the undetectability of M3 in plasma in both PBT (Figure 2) and MCT (Figure 3) groups could be due to the

Table 1. Mean (± standard deviation) pharmacokinetic parameters of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (PBC, $n = 15$) and the PB-pretreated (PBT, $n = 14$) rats

	PBC group ($n = 15$)				PBT group ($n = 14$)			
	M1	M2	M3	M4	M1	M2	M3	M4
AUC_{0-t} (μg min mL^{-1})	78.5 ± 22.8***	44.3 ± 14.5**	—	8.79 ± 6.88	47.1 ± 14.4	20.7 ± 5.49	—	—
Cl (mL min^{-1} kg^{-1})	192 ± 63.0***				318 ± 66.6			
Cl_r (mL min^{-1} kg^{-1})	0.334 ± 0.188**	14.6 ± 5.66***	U.D.	U.D.	0.783 ± 0.459	5.43 ± 3.60	U.D.	U.D.
Excreted in 24 h urine								
$Ae_{0-24\ h}$ (μg)	9.94 ± 2.50*	229 ± 42.2***	U.D.	1.31 ± 1.16*	12.9 ± 3.82	171 ± 17.0	U.D.	5.21 ± 5.46
% of IV dose[a]	(0.237 ± 0.0595**)	(5.46 ± 1.01***)		(0.0455 ± 0.0379***)	(0.308 ± 0.0877)	(4.08 ± 0.402)		(0.181 ± 0.0189)
Recovered from whole GI tract at 24 h								
Amount (μg)	2.00 ± 2.05	14.0 ± 19.5	U.D.	48.4 ± 85.5	7.02 ± 4.86	11.2 ± 4.86	U.D.	39.4 ± 18.4
% of IV dose[a]	(0.0477 ± 0.0488)	(0.334 ± 0.485)		(1.68 ± 2.98)	(0.167 ± 0.0154)	(0.267 ± 0.133)		(1.37 ± 0.654)

U.D., under the detection limit.
[a] Expressed in terms of DA-125.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. Mean (± standard deviation) pharmacokinetic parameters of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (MCC, $n = 11$) and the 3-MC-pretreated (MCT, $n = 15$) rats

	MCC group ($n = 11$)				MCT group ($n = 15$)			
	M1	M2	M3	M4	M1	M2	M3	M4
AUC_{0-t} (μg min mL^{-1})	89.9 ± 15.6*	34.4 ± 6.78	—	9.14 ± 3.79	74.6 ± 17.5	30.8 ± 6.22	—	—
Cl (mL min^{-1} kg^{-1})	203 ± 38.9				201 ± 40.5			
Cl_r (mL min^{-1} kg^{-1})	0.234 ± 0.171	15.4 ± 5.92	U.D.	U.D.	0.267 ± 0.157	13.8 ± 3.86	U.D.	U.D.
Excreted in 24 h urine								
$Ae_{0-24\ h}$ (μg)	6.77 ± 2.52	150 ± 33.2	U.D.	0.959 ± 1.48	7.66 ± 2.87	142 ± 28.2	U.D.	0.981 ± 0.00686
% of IV dose[a]	(0.161 ± 0.0610)	(3.58 ± 0.762)		(0.0333 ± 0.0526)	(0.183 ± 0.0700)	(3.39 ± 0.690)		(0.034 ± 1.98)
Recovered from whole GI tract at 24 h								
Amount (μg)	2.90 ± 2.05**	11.8 ± 3.76***	U.D.	25.1 ± 6.35	12.1 ± 8.57	58.1 ± 35.8	U.D.	37.4 ± 35.0
% of IV dose[a]	(0.0691 ± 0.0276***)	(0.281 ± 0.0876***)		(0.872 ± 0.229)	(0.289 ± 0.0700)	(1.39 ± 0.849)		(1.30 ± 1.05)

U.D., under the detection limit.

[a] Expressed in terms of DA-125.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3. Mean (± standard deviation) pharmacokinetic parameters of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (SKC, $n = 15$) and SKF-525A-pretreated (SKT, $n = 7$) rats

	SKC group ($n = 15$)				SKT group ($n = 7$)			
	M1	M2	M3	M4	M1	M2	M3	M4
AUC_{0-t} (μg min mL^{-1})	78.5 ± 22.8***	44.3 ± 14.5**	—	8.79 ± 6.88***	126 ± 22.5	69.2 ± 19.0	—	20.2 ± 7.09
Cl (mL min^{-1} kg^{-1})	192 ± 63.0**				115 ± 31.2			
Cl_r (mL min^{-1} kg^{-1})	0.334 ± 0.188	14.6 ± 5.66**	U.D.	U.D.	0.00158 ± 5.32	5.32 ± 5.92	U.D.	U.D.
Excreted in 24 h urine								
$Ae_{0-24\ h}$ (μg)	9.94 ± 2.50	229 ± 42.2	U.D.	1.31 ± 1.16*	7.72 ± 2.89	206 ± 79.5	U.D.	0.307 ± 0.404
% of IV dose[a]	(0.237 ± 0.0595*)	(5.46 ± 1.01)		(0.0455 ± 0.0379*)	(0.184 ± 0.0647)	(4.91 ± 1.82)		(0.0107 ± 0.0124)
Recovered from whole GI tract at 24 h								
Amount (μg)	2.00 ± 2.05**	14.0 ± 19.5	U.D.	48.4 ± 85.5	5.28 ± 3.29	26.2 ± 19.5	U.D.	128 ± 127
% of IV dose[a]	(0.0477 ± 0.0488*)	(0.344 ± 0.485)		(1.68 ± 2.98)	(0.125 ± 0.072)	(0.625 ± 0.433)		(4.45 ± 3.91)

U.D., under the detection limit.

[a] Expressed in terms of DA-125.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 4. Mean ($\pm$ standard deviation) pharmacokinetic parameters of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (CMC, $n = 15$) and CM-pretreated (CMT, $n = 15$) rats

	CMC group ($n = 15$)				CMT group ($n = 15$)			
	M1	M2	M3	M4	M1	M2	M3	M4
AUC_{0-t} (μg min mL^{-1})	78.5 ± 22.8	44.3 ± 14.5	—	8.79 ± 6.88	83.1 ± 27.7	46.5 ± 13.8	—	—
Cl (mL min^{-1} kg^{-1})	192 ± 63.0				178 ± 47.0			
Cl_r (mL min^{-1} kg^{-1})	0.334 ± 0.188	14.6 ± 5.66**	U.D.	U.D.	0.286 ± 0.205	7.35 ± 0.36	U.D.	U.D.
Excreted in 24 h urine								
$Ae_{0-24\ h}$ (μg)	9.94 ± 2.50	229 ± 42.2	U.D.	1.31 ± 1.16	9.92 ± 4.31	228 ± 60.7	U.D.	1.33 ± 3.05
% of IV dose[a]	(0.237 ± 0.0595)	(5.46 ± 1.01)		(0.0455 ± 0.0379)	(0.237 ± 0.104)	(5.44 ± 1.40)		(0.0462 ± 0.106)
Recovered from whole GI tract at 24 h								
Amount (μg)	2.00 ± 2.05	14.0 ± 19.5	U.D.	48.4 ± 85.5	3.31 ± 2.66	17.6 ± 13.5	U.D.	52.9 ± 42.8
% of IV dose[a]	(0.0477 ± 0.0488)	(0.334 ± 0.485)		(1.68 ± 2.98)	(0.0789 ± 0.0657)	(0.420 ± 0.329)		(1.84 ± 1.38)

U.D., under the detection limit.
[a] Expressed in terms of DA-125.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

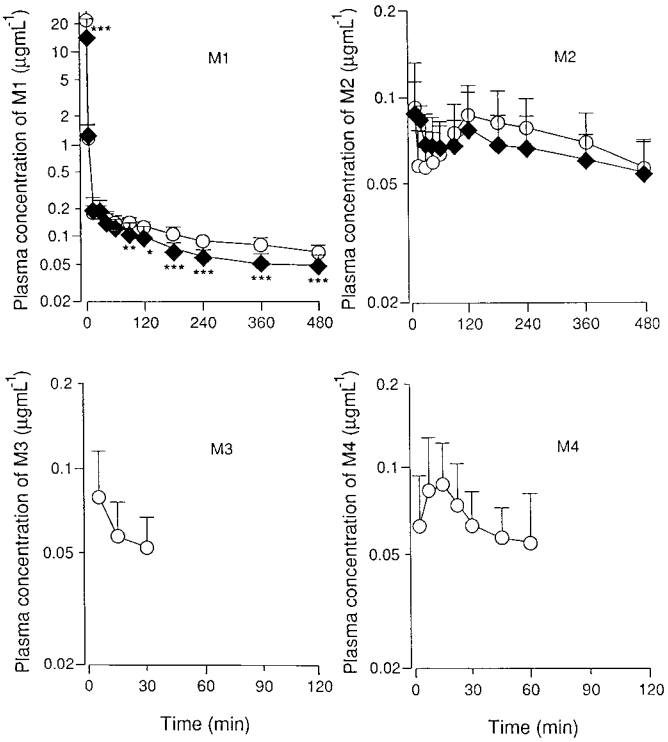

Figure 3. Mean arterial plasma concentration–time profiles of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (○, $n = 11$) and the 3-MC-pretreated (◆, $n = 15$) rats. Bars represent standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

increased formation rate of M4 from M3. The undetectability of M4 in plasma in both PBT and MCT groups could be due to the increased metabolism of M4 to other metabolite(s). It has been reported [8] that after IV administration of DA-125, 20 mg kg^{-1}, to Sprague–Dawley rats, the total amount of unchanged M1–M4 plus their glucuronide and/or sulphate conjugates excreted in both 8 h urine and 8 h bile (after bile duct cannulation) is approximately 50% of IV dose of DA-125. The above data suggests that more than 50% of IV dose of DA-125 was metabolized in rats except M1–M4, and their glucuronide and/or sulphate conjugation.

The Cl_r of M1 was significantly faster (0.783 versus 0.334 mL min^{-1} kg^{-1}) but that of M2 was significantly slower (5.43 versus 14.6 mL min^{-1} kg^{-1}) in the PBT group than in the PBC group (Table 1). The percentages of IV dose of DA-125 excreted in 24 h urine as M1 (0.308 versus 0.237%) and M4 (0.181 versus 0.0455%) were significantly greater, but the values of M2 (4.08 versus 5.46%) were significantly smaller in the PBT group than in the PBC group (Table 1). However, the Cl_r values and the percentages of IV dose of DA-125 excreted in 24 h urine as M1–M4 in the MCT group were all not significantly different from those of the MCC group (Table 2). M2 was the main metabolite among M1–M4 that was excreted in the 24 h urine in PBC, PBT, MCT, and MCC groups. The percentages of the IV dose of DA-125 recovered from the GI tract at 24 h as M1, M2, and M4 were all not significantly different between the PBC and PBT groups, with the highest values being those of M4 in both groups of rats (Table 1). However, the corresponding values of M1 (0.289 versus 0.0691%) and M2 (1.39 versus 0.281%) were significantly greater in the MCT group than in the MCC group (Table 2).

Both SKF-525A and CM are nonspecific inhibitors of cytochrome P-450 isozyme in rats. As mentioned earlier, the plasma concentrations and the resultant $AUC_{0-8\,h}$ of both M1 and M2 decreased significantly in the PBT group (Table 1 and Figure 2); therefore, the plasma concentrations and the resultant $AUC_{0-8\,h}$ of both M1 and M2 could be expected to be increased by pretreatment with both SKF-525A and CM. As expected, the mean arterial plasma concentrations of both M1 and M2 in the SKT group were significantly higher than those of the SKC group throughout the experimental period (Figure 4), resulting in a significant increase in the $AUC_{0-8\,h}$ values of both M1 (the $AUC_{0-8\,h}$ value of M1 in the SKT group was 161% of that in the SKC group) and M2 (the $AUC_{0-8\,h}$ value of M2 in the SKT group was 156% of that in the SKC group) in the SKT group (Table 3). However, different results were obtained from CM-pretreated rats; the plasma concentrations (Figure 5) and hence the $AUC_{0-8\,h}$ value of both M1 and M2 were not significantly different between CMC and CMT groups (Table 4). The above data indicate that the metabolism of both M1 and M2 is inhibited by pretreatment with SKF-525A but not with CM. This could be due to the differences in the enzyme inhibitory activity between CM and 3-MC. M4 was detected in plasma for up to 3 h in the SKT

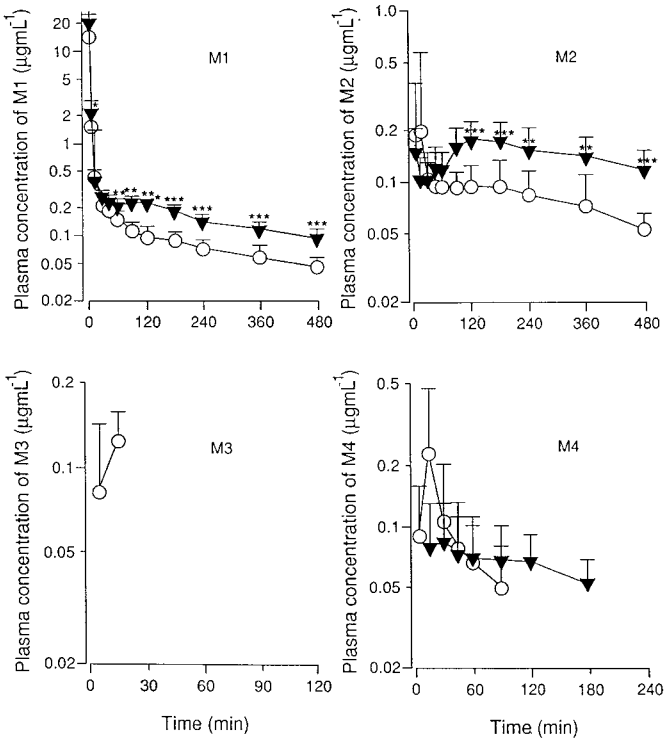

Figure 4. Mean arterial plasma concentration–time profiles of M1–M4 after IV administration of DA-125, 15 mg kg^{-1}, to the control (○, $n = 15$) and the SKF-525A-pretreated (▼, $n = 7$) rats. Bars represent standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

group; however, it was only 90 min in the SKC group (Figure 4), thus the AUC_{0-t} value of M4 was significantly increased in the SKT group (the $AUC_{0-8\,h}$ value of M4 in the SKT group was 230% of that in the SKC group) (Table 3). This could be due to the inhibited metabolism of M4 to other metabolite(s) in the SKT group. More studies are required to find out the reasons for the undetectability of M3

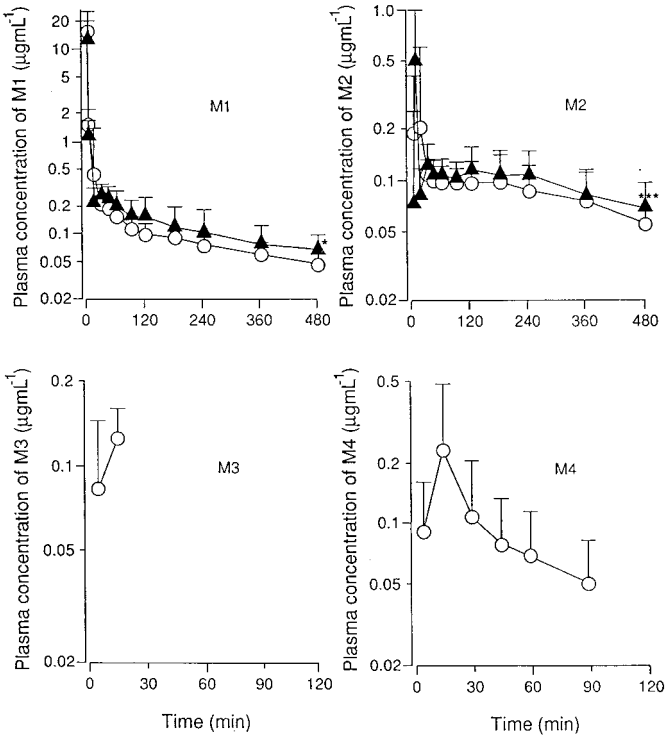

Figure 5. Mean arterial plasma concentration–time profiles of M1–M4 after intravenous administration of DA-125, 15 mg kg^{-1}, to the control (○, $n = 15$) and the CM-pretreated (▲, $n = 15$) rats. Bars represent standard deviation. * $p < 0.05$, *** $p < 0.001$

in plasma in both SKT and CMT groups and of M4 in the CMT group. It is to be noted that the mortality rate of rats pretreated with SKF-525A was notably higher (approximately three times) than that of the control rats.

The Cl_r of M2 was significantly slower in both the SKT (5.32 versus 14.6 mL min^{-1} kg^{-1}, Table 3) and CMT (7.35 versus 14.6 mL min^{-1} kg^{-1}, Table 4) groups than in their respective control groups. M2 was again the main metabolite among M1–M4 that were excreted in the 24 h urine in SKC, SKT, CMC, and CMT groups. The percentages of IV dose of DA-125 excreted in 24 h urine as M2 were respectively 5.46, 4.91, 5.46, and 5.44% in SKC, SKT, CMC, and CMT groups (Tables 3 and 4). The percentages of IV dose of DA-125 recovered from GI tract at 24 h as M1 (0.125 versus 0.0477%) were significantly greater in the SKT group than in the SKC group (Table 3).

In conclusion, the metabolism of the active compound of DA-125, M1, was induced by pretreatment with both PB and 3-MC, and inhibited by pretreatment with SKF-525A, but not with CM.

Acknowledgements

This work was supported in part by the Korea Ministry of Science and Technology (HAN project 4-1-3), 1993–1995. The authors would like to thank Dr. J.H. Schlossen of Smith Kline and French Laboratories for his kind donation of SKF-525A.

References

1. H.J. Shim, E.D. Lee, E.J. Yoon, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, Simultaneous determination of a new anthracycline, DA-125, and its metabolites M1, M2, M3 and M4 in plasma and urine by high-performance liquid chromatography. *J. Chromatogr.*, **656,** 407–414 (1994).
2. H.J. Shim, E.D. Lee, E.J. Yoon, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, 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. *Int. J. Pharm.*, **103,** 147–154 (1994).
3. E.J. Yoon, E.D. Lee, W.H. Yoon, H.J. Shim, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, Pharmacokinetics, tissue distribution and biliary excretion of FT-ADM after intravenous administration of DA-125, a prodrug of FT-ADM to dogs. A new adriamycin analog containing fluorine. *Int. J. Pharm.*, **109,** 181–187 (1994).
4. Y.G. Kim, E.J. Yoon, W.H. Yoon, H.J. Shim, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, Pharmacokinetics of DA-125, a new anthracycline, after intravenous administration to uranyl nitrate-induced acute renal failure rats or protein-calorie malnutrition rats. *Biopharm. Drug Dispos.*, **17,** 183–195 (1996).
5. S.D. Lee, J.B. Park, W.I. Lee, H.J. Shim, E.D. Lee, J.J. Lee, W.B. Kim, J. Yang, C.-K. Kim and M.G. Lee, Pharmacokinetics and antitumor activity of a new anthracycline, DA-125, after intravenous administration to subcutaneously implanted Lewis-lung-carcinoma-bearing mice. *Biopharm. Drug Dispos.*, **16,** 489–505 (1995).
6. E.J. Yoon, W.I. Lee, H.J. Shim, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, Comparison of pharmacokinetics of M1, M2, M3, and M4 after intravenous administration of DA-125 or ME2303 to mice and rats. New adriamycin analogues containing fluorine. *Biopharm. Drug Dispos.*, **17,** 373–420 (1996).
7. S.D. Lee, W.I. Lee, H.J. Shim, E.D. Lee, W.B. Kim, J. Yang, C.-K. Kim and M.G. Lee, Pharmacokinetics of four metabolites of DA-125, a new anthracycline antineoplastic agent after single and multiple intravenous administration to rats. *J. Clin. Pharm. Ther.*, **21,** 201–213 (1996).
8. E.J. Yoon, H.J. Shim, J.J. Lee, S.D. Lee, W.B. Kim, J. Yang and M.G. Lee, Pharmacokinetics of DA-125, a new anthracycline, after intravenous administration to spontaneously hypertensive rats and DOCA-salt-induced hypertensive rats. *Drug Metab. Dispos.*, **25,** 66–74 (1997).
9. Y.M. Choi, S.H. Lee and M.G. Lee, Effects of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetics and pharmacodynamics of furosemide in rats. *J. Pharm. Sci.*, **80,** 638–642 (1991).
10. Y.M. Choi, S.H. Lee, S.H. Jang and M.G. Lee, Effects of phenobarbital and 3-methylcholanthrene pretreatment on the pharmacokinetics and pharmacodynamics of bumetanide in rats. *Biopharm. Drug Dispos.*, **12,** 311–324 (1991).
11. S.H. Lee and M.G. Lee, Effect of phenobarbital, 3-methylcholanthrene, and chloramphenicol pretreatment on the pharmacokinetics and pharmacodynamics of azosemide in rats. *Biopharm. Drug Disposit.*, **18,** 371–386 (1997).
12. J.F. Williams, S. Lowitt and A. Szentivanyi, Effect of phenobarbital and 3-methylcholanthrene pretreatment on the plasma half-life and urinary excretion profile of theophylline and its metabolites in rats. *Biochem. Pharmacol.*, **28,** 2935–2940 (1979).
13. C. Lambert, G. Caille and P. du Souich, Nonrenal clearance of furosemide as a course of diuretic response variability in rat. *J. Pharmacol. Exp. Ther.*, **222,** 232–236 (1982).
14. B.N. La Du, H.G. Mandel and E.L. Way, *Fundamentals of Drug Metabolism and Drug Disposition*, Williams and Wilkins, Baltimore, MD, 1971, p. 269.
15. S.H. Kim, Y.M. Choi and M.G. Lee, Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *J. Pharmacokinet. Biopharm.*, **21,** 1–17 (1993).
16. W.L. Chiou, Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J. Pharm. Sci.*, **67,** 539–546 (1978).
17. W.L. Chiou, New calculation method for mean total body clearance of drugs and its application to dosage regimens. *J. Pharm. Sci.*, **69,** 90–91 (1980).