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Note

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

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Abstract

The pharmacokinetic parameters, tissue distribution and biliary excretion of M1–M4 were estimated after intravenous (i.v.) administration of DA-125, 2.5 mg per kg to beagle dogs. The mean values of the terminal half-life, mean residence time, total body clearance and apparent volume of distribution at steady state of M1 were 266 min, 170 min, 61.3 ml min^{-1} kg^{-1} and 9500 ml kg^{-1}, respectively (four male and four female dogs). M1 was highly concentrated in lung (five male and five female dogs), and this probably means that lung tumors are subjected to greater exposure to M1, an active metabolite of DA-125. The 8 h biliary excretion of M2 was significantly greater than that of M1, 53.6 vs 6860 μg (three male and two female dogs). The amount of glucuronide and/or sulfate conjugates of M1–M4 in the bile sample was negligible.

Key words: Pharmacokinetics; Tissue distribution; Biliary excretion; DA-125; M1; M2; M3; M4

In the preceding paper (Shim et al., 1994a), the stability, blood partitioning and pharmacokinetics of DA-125, a soluble prodrug of FT-ADM (M1), in rats were reported. DA-125 was rapidly hydrolyzed to M1 in plasma from humans, dogs, rats and mice with 'apparent first-order', and the blood cell to plasma concentration ratio of DA-125 was found to be 0.275 (Shim et al., 1994a). The pharmacokinetic parameters of M1, an active component of DA-125 estimated after intravenous (i.v.) administration of DA-125 (dissolved in 100% dimethyl sulfoxide, DMSO) were very similar (not significantly different at a p value of less than 0.05 using unpaired t-test) when com-

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Abbreviations: 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-aminopropanoxyloxyacetyl)-10-[(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione.

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pared with the values after i.v. administration of M1 (dissolved in 100% DMSO) to rats (Shim et al., 1994a). Therefore, in the present dog studies, the pharmacokinetic parameters of M1 (not DA-125) were estimated after i.v. administration of DA-125. M1 was metabolized to M2 and M3, and M2 or M3 was further metabolized to M4 in rats (Shim et al., 1994a). The phase I clinical study of DA-125 was initiated on December 1993 in South Korea.

The purpose of this paper is to report the pharmacokinetic parameters, tissue distribution and biliary excretion of M1–M4 after i.v. administration of DA-125, 2.5 mg per kg to beagle dogs.

16 conditioned beagle dogs (eight males and eight females), weighing 7.0–13.0 kg were purchased from Marshall Farms (New York, NY, U.S.A.). They were restrained by means of a dog sling (Alice King Catham Medical Arts, Los Angeles, CA, U.S.A.) during the study. An i.v. cannula (2 inch, 22 gauge, Sovereign, St. Louis, MO, U.S.A.) was inserted into the cephalic vein of each foreleg for drug administration and blood sampling, respectively, and an indwelling polypropylene urinary catheter (5 Fr., 22 inch, Sovereign) was inserted into the urinary bladder for urine collection (Lui et al., 1984). The washout period between pharmacokinetic analyses and tissue distribution or biliary excretion studies was longer than 1 week.

DA-125 (dissolved in 1 mM lactic acid/0.9% NaCl injectable solution to make a final concentration of 2.5 mg per ml), 2.5 mg per kg was infused in 1 min via the cephalic vein of four male dogs (dogs 1–4) and four female dogs (dogs 5–8). Approx. 2.5 ml of blood was collected via the other cephalic vein at 0 (to serve as a control), 1 (at the end of infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, and 480 min after commencing drug infusion. Following immediate centrifugation of blood samples (Shim et al., 1994a), 1 ml of plasma was stored in a freezer prior to HPLC analysis (Shim et al., 1994b). Urine samples were collected for 8 h (0–8 h). Blood and urine (0–8 h) collection methods were similar to those reported previously (Lui et al., 1984).

DA-125, 2.5 mg per kg was similarly infused via the cephalic vein of five male dogs (dogs 1, 3, 11, 13 and 15) and five female dogs (dogs 6, 7, 12, 14 and 16). Each male and female dog was exsanguinated at 0.5, 1, 2, 4, and 8 h, respectively, after initiating infusion. Approx. 1–2 g of liver, lung, kidney, spleen, stomach, small intestine, large intestine, heart, muscle, testis (for male dog), uterus (for female dog), urinary bladder, mesentery, brain and fat was cut into small pieces with scissors after either perfusion or washing with cold 0.9% NaCl injectable solution. Each tissue was homogenized (Ultra-Turrax, T25, Janke & Kunkel, IKA-Labortechnik, Germany) with 4 volumes of 0.1 N HCl (to prevent further hydrolysis of DA-125 to M1; Shim et al., 1994a) and centrifuged for 30 min at 3000 rpm. After discarding the floating fat layer, two 1.0 ml portions of the supernatant were stored in the freezer prior to HPLC analysis (Shim et al., 1994b). Approx. 1–2 ml of plasma, bone marrow, cerebrospinal fluid (CSF) and bile juice were also diluted with 4 volumes of 0.1 N HCl, vortex-mixed and two 1.0 ml samples were stored in the freezer prior to HPLC analysis (Shim et al., 1994b).

Three male dogs (dogs 2, 4 and 9) and three female dogs (dogs 5, 8 and 10) were anesthetized by intramuscular injection with 0.02–0.03 ml per kg of acepromazine (10 mg per ml, Sam Woo Chemical Co., Seoul, South Korea), 0.15 ml per kg of rompun (Xylazine, 20 mg per ml, Korea Bayer Chemical Co., Seoul, South Korea) and 0.5–1.0 ml per kg of ketamine (50 mg per ml, kindly supplied by the Yuhan Research Center, Kunpo, South Korea). After opening the abdomen, polyethylene tubing (PE100, Clay Adams, Parsippany, NJ, U.S.A.) was cannulated into the bile duct, and the abdomen was closed using surgical suture. After recovery from anesthesia, each dog was restrained in a dog sling and DA-125, 2.5 mg per kg was similarly infused via the cephalic vein. Bile samples were collected at 0–0.5, 0.5–1, 1–2, 2–4, and 4–8 h. At 8 h after i.v. infusion of DA-125, the gall bladder was excised and the bile juice left in the gall bladder was combined with the 4–8 h bile sample. After measuring the exact volume of each bile sample, 1.0 ml of bile sample was stored in the freezer prior to HPLC analysis (Shim et al., 1994b). A portion (0.1 ml) of each bile sample was added to 0.9 ml

of 0.2 M acetate buffer (pH 5.0) containing 0.1 ml of β-glucuronidase (*Helix pomatia,* Sigma Chemical Co., St. Louis, MO, U.S.A.; β-glucuronidase activity of 132000 U per ml and sulfatase activity of 5000 U per ml), and the mixture was incubated for 2 h in a water-bath shaker kept at 37°C to measure the amount of both glucuronide and sulfate conjugates of M1–M4 in bile samples. After 2 h of incubation, 0.5 ml of the mixture was sampled and 0.5 ml of 0.25 N HCl was added to terminate the enzyme reaction. 0.5 ml of methanol solution containing an internal standard and 5 ml of ethyl acetate were added. After vortex-centrifugation, the ethyl acetate layer was collected and processed for HPLC assay (Shim et al., 1994b). In the preliminary study, 1 h incubation of the bile sample with β-glucuronidase was sufficient to terminate the enzyme reaction.

The pharmacokinetic parameters, such as the total area under the plasma concentration-time curve from time zero to time infinity (AUC), terminal half-life ($t_{1/2}$), area under the first moment of the plasma concentration-time curve (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and time-averaged total body (CL), renal (CL_R) and nonrenal (CL_{NR}) clearances were estimated according to reported methods (Gibaldi and Perrier, 1982; Kim et al., 1993). In the calculation of CL_R, plasma and urine data at 8 h were used. The harmonic mean was employed for calculation of the mean values of $t_{1/2}$, CL, CL_R, CL_{NR} and V_{ss} (Chiou, 1979).

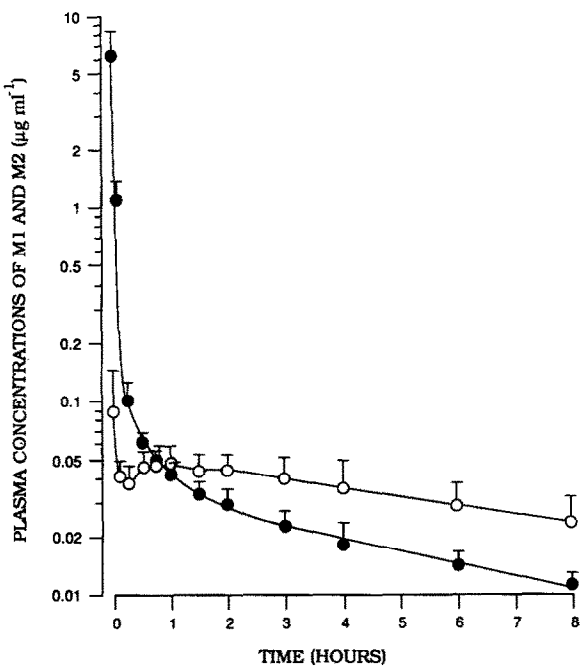

Fig. 1. Mean plasma concentration-time profiles of M1 (●) and M2 (○) after 1 min intravenous administration of DA-125, 2.5 mg per kg to four male and four female beagle dogs. Bars represent standard deviation.

Table 1
Mean (±standard deviation) pharmacokinetic parameters of M1 and M2 after 1 min intravenous infusion of DA-125, 2.5 mg per kg to four male and four female beagle dogs

Pharmacokinetic parameters	M1	M2
$t_{1/2}$ (min)	266 ± 76.2	410 ±150
AUC (μg min ml^{-1})	33.8 ± 6.22	34.0 ± 10.4
AUMC (μg min^2 ml^{-1})	5730 ±2010	
MRT (min)	170 ± 57.2	
CL (ml min^{-1} kg^{-1})	61.3 ± 15.6	
CL_R (ml min^{-1} kg^{-1})	0.106± 0.0538	2.79± 0.446
CL_{NR} (ml min^{-1} kg^{-1})	61.1 ± 15.6	
V_{SS} (ml kg^{-1})	9500 ±3970	
$Xu_{0-8\,h}$ (μg)	47.8 ± 25.1	595 ±167

The mean plasma concentration-time profiles of M1 and M2 after i.v. administration of DA-125, 2.5 mg per kg to four male and four female dogs are shown in Fig. 1, and the relevant pharmacokinetic parameters are listed in Table 1. After i.v. administration, the plasma concentrations of M1 decayed polyexponentially with a mean terminal half-life of 266 min. The mean AUC, MRT, CL, CL_R, CL_{NR}, V_{ss} and the total amount of M1 excreted in the 8 h urine sample ($Xu_{0-8\,h}$) were 33.8 μg min ml^{-1}, 170 min, 61.3 ml min^{-1} kg^{-1}, 0.106 ml min^{-1} kg^{-1}, 61.1 ml min^{-1} kg^{-1}, 9500 ml kg^{-1} and 47.8 μg, respectively (Table 1). The considerably higher value of V_{ss} indicates that M1 has a strong affinity to tissues (or organs) and this was proved in tissue distribution studies (Fig.

2). The CL_{NR} contributed considerably to CL; the contribution of CL_{NR} to the CL of M1 was 99.67%, indicating that almost all of the M1 could be metabolized to M2–M4, and their conjugates, and/or other unknown metabolites as reflected in the negligible $Xu_{0-8\,h}$ value of M1 (47.8 μg, 0.194% of the administered i.v. dose when expressed in terms of DA-125).

The plasma concentrations of M2 also declined polyexponentially with a mean terminal half-life of 410 min. The mean plasma level of M2 was highest at 1 min after i.v. administration of DA-125, indicating that M1 may be rapidly biotransformed to M2 in dog. The mean AUC, CL_R and $Xu_{0-8\,h}$ of M2 were 34.0 μg min ml^{-1}, 2.79 ml min^{-1} kg^{-1} and 595 μg, respectively (Table 1). The mean values of $t_{1/2}$ (266 ± 76.2 vs 410 ± 150 min) and AUC (33.8 vs 34.0 μg min ml^{-1}) of M2 were comparable to those of M1, however, the values of CL_R (0.106 vs 2.79 ml min^{-1} kg^{-1}) and $Xu_{0-8\,h}$ (47.8 vs 595 μg; 0.194 vs 2.43% of the administered i.v. dose of DA-125) were significantly greater than that of M1. It should be noted that the plasma concentrations and pharmacokinetic parameters of M1 or M2 were similar (not significantly different at a p

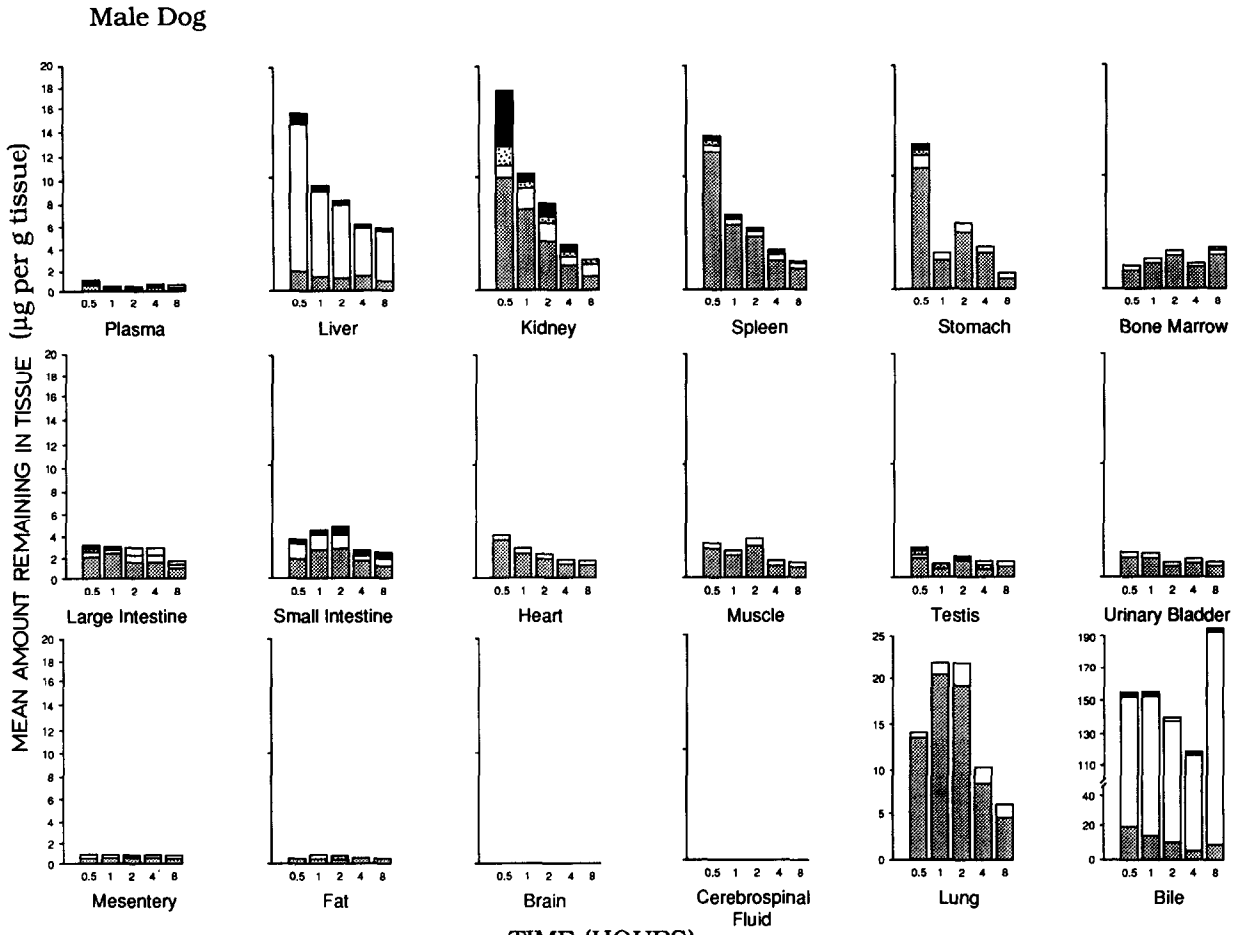

Fig. 2. Amount (μg per g tissue) of M1 (stippled bars), M2 (empty bars), M3 (dotted bars) and M4 (filled bars) remaining in each tissue at 0.5, 1, 2, 4 and 8 h after 1 min intravenous administration of DA-125, 2.5 mg per kg to five male and five female beagle dogs.

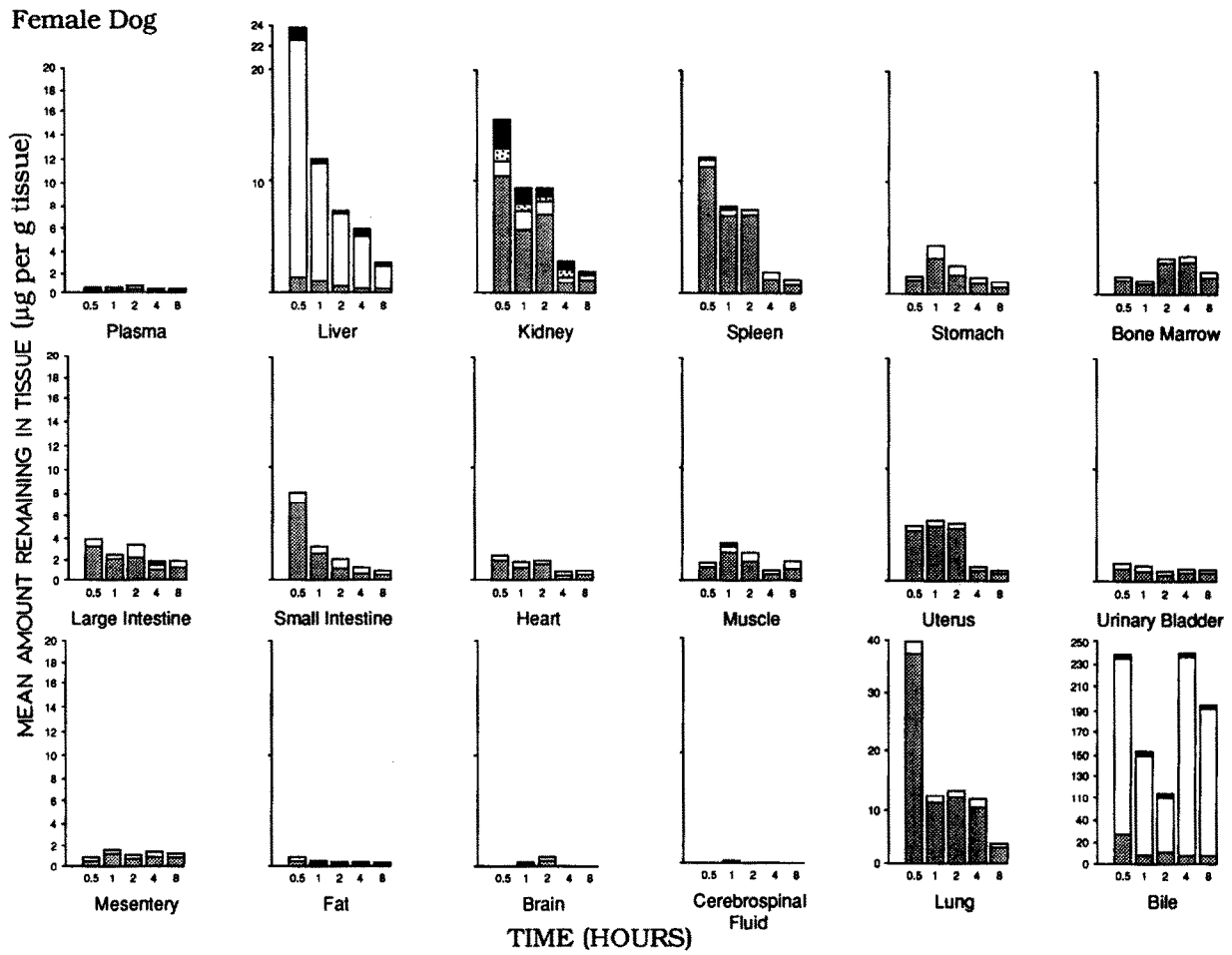

Fig. 2 (continued).

value of less than 0.05 using unpaired t-test) between four male and four female dogs. Therefore, the mean pharmacokinetic parameters of M1 or M2 which are listed in Table 1 are the mean values of eight dogs. In the present dog study, M3 was detected in neither plasma nor urine samples and might at least be partly due to our HPLC assay sensitivity. M4 was only detected in plasma samples at 1, 5, and 15 min after i.v. administration of DA-125 and was not detected in urine.

The amounts (μg per g tissue) of M1–M4 remaining per g tissue (or organ) at 0.5, 1, 2, 4 and 8 h, respectively after i.v. administration of DA-125, 2.5 mg per kg to five male and five female dogs are shown in Fig. 2. Generally, M1 was highly concentrated in lung, spleen, stomach, kidney, bone marrow, small intestine, large intestine, heart, muscle, testis (or uterus), liver, bile and urinary bladder as reflected in the tissue to plasma ratio of greater than unity. M1, an active antineoplastic moiety of DA-125, had a specific affinity to lung, which probably indicates that lung tumors are subjected to greater exposure to M1. Similar results could be obtained from kidney, spleen and gastrointestinal tract. The high affinity of M1 to tissues (or organs) could also support the considerably higher value of V_{ss} of M1 (Table 1). However, M1 did not appear to be detectable in brain and cerebrospinal fluid, which

Table 2
Mean (±standard deviation) amount (μg) of M1–M4 excreted in bile samples after 1 min intravenous administration of DA-125, 2.5 mg per kg to three male and two female beagle dogs

		M1	M2	M3	M4
0–0.5 h	A [a]	8.78 ± 14.8	212 ± 365	0.0866 ± 0.130	0.0572 ± 0.128
	B [b]	8.66 ± 16.5	192 ± 342	0 ± 0	0.185 ± 0.301
0.5–1	A	11.2 ± 7.56	437 ± 447	0 ± 0	0.186 ± 0.311
	B	11.3 ± 8.10	663 ± 580	0.0700 ± 0.157	1.46 ± 1.79
1–2	A	6.28 ± 4.76	1220 ± 802	0.0656 ± 0.147	0.737 ± 1.50
	B	6.96 ± 5.80	1150 ± 768	0.253 ± 0.266	3.14 ± 2.42
2–4	A	5.11 ± 9.23	1290 ± 803	0.105 ± 0.234	0.270 ± 0.434
	B	5.01 ± 9.59	1210 ± 773	0.834 ± 0.684	4.34 ± 2.69
4–8	A	22.2 ± 31.8	3400 ± 2280	0.188 ± 0.420	0 ± 0
	B	27.5 ± 38.3	3340 ± 1830	7.10 ± 2.38	14.4 ± 12.4
0–8	A	53.6 ± 22.3	6860 ± 2750	0.445 ± 0.762	1.25 ± 1.43
		(0.264 ± 0.146) [c]	(30.2 ± 5.36)	(0.00818 ± 0.0142)	(0.00498 ± 0.00499)
	B	60.5 ± 25.1	6540 ± 2510	8.19 ± 2.88	23.5 ± 14.4
		(0.304 ± 0.186)	(28.8 ± 4.69)	(0.0369 ± 0.00722)	(0.114 ± 0.0893)

[a] Amount before incubation with β-glucuronidase.
[b] Amount after incubation with β-glucuronidase.
[c] Values in parentheses represent percentages of intravenous dose of DA-125 excreted in 8 h bile juice as expressed in terms of DA-125.

indicates that M1 does not penetrate well through the blood-brain barrier. M1 was less concentrated in the mesentery and fat than in plasma. Similar trends were also observed for M2 (Fig. 2). M3 and/or M4 were either not detected or detected only in very small amounts in all the tissues (or organs) studied except liver and kidney.

The mean amounts (μg) of M1–M4 excreted in 8 h bile samples after i.v. administration of DA-125, 2.5 mg per kg to three male and three female dogs are listed in Table 2 (data for dog 10 are not included due to the failure of cannulation into the bile duct). Negligible amounts of M1, M3 and M4 were excreted in 8 h bile samples after i.v. administration of DA-125, 2.5 mg per kg to five dogs; the mean percentages of intravenously administered dose of DA-125 excreted in 8 h bile samples as M1, M3 and M4 were 0.264, 0.00818 and 0.00498%, respectively, when expressed in terms of DA-125. However, the corresponding value for M2 was considerable, the mean value being 30.2% (Table 2). The mean amount of M1 (47.8 vs 53.6 μg), M2 (595 vs 6860 μg), M3 (below detection limit vs 0.445 μg) and M4 (below detection limit vs 1.25 μg) excreted in 8 h bile samples appeared to be greater than that of 8 h urine samples (Tables 1 and 2). The amount of glucuronide and/or sulfate conjugates of M1–M4 seemed to be negligible after incubation of bile samples with β-glucuronidase; the mean percentages of intravenously administered DA-125 excreted in 8 h bile samples as M1–M4 after incubation with β-glucuronidase were 0.304, 28.8, 0.0369 and 0.114%, respectively (Table 2).

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