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The Disposition of Dapsone and Monoacetyldapsone in the Dog[1] (38516)

JOHN H. PETERS, G. ROSS GORDON, JOHN T. BIGGS, JR., AND LOUIS LEVY

Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025; and the Leprosy Research Unit, Public Health Service Hospital, San Francisco, California 94118

In recent years, much has been learned about the disposition in man of dapsone (4,4′-diaminodiphenyl sulfone, DDS), the drug most widely used in the treatment of leprosy (1). Monoacetyl DDS (4-amino-4′-acetamidodiphenyl sulfone, MADDS) has been shown to be the major circulating metabolite (2, 3). Human subjects acetylate DDS polymorphically and readily deacetylate administered MADDS (2, 3). Both DDS and MADDS are cleared from the plasma at a relatively slow rate in man; the half-times of disappearance ($T_{1/2}$) from plasma range from 14 to 53 hr, with means of 28 to 30 hr in various population groups (3, 4). No relationship between $T_{1/2}$ and acetylation capacity was discerned (2–4). DDS was found to be moderately bound to plasma proteins; MADDS was more strongly bound (5, 6). No difference in the degree of protein-binding was found in the two acetylator phenotypes (5).

In contrast to this information on man, knowledge of the disposition of DDS and MADDS in animal species, which might serve as models for man, is relatively limited. Mice exhibit minimal acetylation of DDS and nearly complete deacetylation of MADDS (7); $T_{1/2}$ values for DDS ranged from 2 to 4 hr (7, 8), and values for MADDS could not be measured because of rapid and extensive deacetylation (7). Binding of both DDS and MADDS to plasma proteins of mice was similar to that in man (7). Rats acetylate DDS and deacetylate MADDS, but they also exhibit relatively short $T_{1/2}$ values of about 4 and 6 hr for MADDS and DDS, respectively. They also display extensive protein-binding of both compounds (9, 10). Rabbits acetylate DDS polymorphically, as does man, deacetylate MADDS to a small extent, and exhibit extensive protein-binding of both compounds (9, 11). However, they also exhibit short $T_{1/2}$ values of 1 and 2 hr for DDS and MADDS, respectively (11). Finally, limited studies have also been performed in two species of non-human primates, rhesus and squirrel monkeys (12). The former acetylated DDS very extensively but deacetylated MADDS poorly; and the latter exhibited opposite characteristics, limited acetylation of DDS and extensive deacetylation of MADDS. Mean $T_{1/2}$ values for DDS and MADDS were 2 and 4 hr, respectively, in the rhesus monkey, and 7 hr for both drugs in the squirrel monkey. No studies of protein-binding in these monkeys were performed.

We have extended these studies to the dog for two reasons. First, drugs such as isoniazid and certain sulfonamides that are acetylated polymorphically by man (13, 14) and by rabbits (11) are not acetylated by the dog (14–16). If the dog is also incapable of acetylating DDS, a study of the disposition of DDS would be simpler in this species because concurrent acetylation of DDS and deacetylation of MADDS have made difficult the interpretation of the pharmacokinetics of DDS in man (2). Second, it is easier to perform certain investigative procedures, e.g., studies of renal clearance or biliary excretion, in the dog than in man or smaller mammals. In the studies to be reported, we examined the capability of dogs to acetylate DDS and to deacetylate MADDS, determined $T_{1/2}$ values following iv administration of both drugs, and measured the extent of

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binding of these drugs by plasma proteins of the dog.

Methods. The DDS and MADDS used were described previously (2). Adult female mongrel dogs ranging in weight from 20–23 kg were employed. At least a 2-wk interval separated the study periods of the same or different drugs in the same dogs; during and between the study periods, food and water were provided *ad libitum*.

For studies of the disposition of DDS and MADDS, we administered iv to four dogs solutions of DDS or MADDS (1 mg/ml) in equal parts of isotonic saline and polyethylene glycol (mol wt 190–210) to obtain doses of 1.0 mg DDS or 1.2 mg MADDS/kg. Heparinized blood samples (10 ml) were taken by venipuncture 2, 4, 6, 8, and 24 hr after injection. Plasma was obtained by centrifugation and stored at $-20°$. All plasma samples were analyzed within 2 wk of collection. DDS, MADDS (17), and protein (18) concentrations were determined by published methods. In selected plasma samples that yielded MADDS levels near the limit of sensitivity (0.01 μg/ml) of the standard method (17), we repeated the analyses using a chromatographic–fluorometric procedure (19) sensitive to 0.002 μg MADDS/ml of plasma.

An ultrafiltration technique (5) was employed to measure the extent of binding of DDS and MADDS to plasma proteins. Studies *in vivo* were performed in 2-hr plasma samples obtained following iv injection of 1.0 mg DDS or 1.2 mg MADDS/kg to four dogs. Binding *in vitro* was measured in pooled plasma obtained from these dogs before treatment, to which DDS and MADDS were added. These mixtures were incubated for 30 min at 25° before ultrafiltration. Repeated tests of the acetylation of DDS to MADDS or the deacetylation of MADDS to DDS by plasma from untreated dogs were negative.

$T_{1/2}$ values of DDS and MADDS were calculated from regression lines representing the logarithmic decay of concentration with time. Differences between individual $T_{1/2}$ values in dogs receiving the same drug were determined by comparing the slopes of the regression lines.

Results. Table I lists the plasma levels of DDS and MADDS found in dogs receiving DDS. To determine the reproducibility of these observations, we subjected dogs A and B to a second test with DDS. The levels found in the two tests were nearly identical at all time periods. MADDS levels found in 27 of these 30 plasma samples were below the limit of detection ($<$ 0.01 μg/ml) of the assay procedure. As shown, three plasma samples from dog B suggested the presence of barely detectable levels of MADDS. However, when the samples were reanalyzed by the more sensitive chromatographic–fluorometric procedure, we found $<$ 0.002 μg MADDS/ml.

Similar tests of the disposition of an equimolar dose of MADDS to these four dogs yielded the plasma levels of DDS and MADDS listed in Table II. From the easily detectable levels of DDS, it was apparent that MADDS was consistently deacetylated. But this metabolic hydrolysis was a relatively slow process—at 2 hr the mean deacetylation by the four dogs was 6.4%, and it increased gradually to a mean of 63.6% at 24 hr. These findings clearly show that the dog was incapable of acetylating DDS.

From the data in Tables I and II, we calculated the regression lines and $T_{1/2}$ values for each administration to each dog. Table III lists the $T_{1/2}$ values and the 95% confidence limits for these values. As shown, the two tests of DDS in dogs A and B gave essentially the same results, attesting to the reproducibility of the disposition of DDS in individual dogs. On the other hand, the higher $T_{1/2}$ values for DDS in dog B emphasize the variability among the dogs studied. Comparison of the $T_{1/2}$ values for MADDS with those for DDS in these dogs reveals that MADDS was cleared more rapidly from the circulation than DDS. This resulted partially from the deacetylation of MADDS to DDS.

Table IV presents the results of the limited studies of binding of DDS and MADDS to plasma proteins. Binding of both compounds was extensive *in vivo*, and the binding of MADDS was significantly greater ($P <$ 0.005) than that of DDS. This observation was confirmed by the studies *in vitro*, espe-

TABLE I. PLASMA LEVELS OF DDS AND MADDS IN DOGS RECEIVING 1.0 MG DDS/KG INTRAVENOUSLY.

Dog	Drug measured	Plasma level (μg/ml)				
		2 hr	4 hr	6 hr	8 hr	24 hr
A	DDS	0.74	0.54	0.47	0.42	0.13
	MADDS	<0.01	<0.01	<0.01	<0.01	<0.01
A[a]	DDS	0.70	0.55	0.48	0.39	0.12
	MADDS	<0.01	<0.01	<0.01	<0.01	<0.01
B	DDS	0.83	0.76	0.72	0.62	0.32
	MADDS	0.01[b]	<0.01	0.02[b]	0.01[b]	<0.01
B[a]	DDS	0.75	0.66	0.70	0.60	0.30
	MADDS	<0.01	<0.01	<0.01	<0.01	<0.01
C	DDS	0.70	0.72	0.56	0.52	0.17
	MADDS	<0.01	<0.01	<0.01	<0.01	<0.01
D	DDS	0.78	0.70	0.60	0.43	0.15
	MADDS	<0.01	<0.01	<0.01	<0.01	<0.01

[a] Second trial in the same dog.
[b] <0.002 μg/ml found by chromatographic–fluorometric assay.

TABLE II. PLASMA LEVELS OF DDS AND MADDS IN DOGS RECEIVING 1.2 MG MADDS/KG INTRAVENOUSLY.

Dog	Drug measured	Plasma level (μg/ml)				
		2 hr	4 hr	6 hr	8 hr	24 hr
A	DDS	0.01	0.13	0.17	0.18	0.11
	MADDS	1.10	0.71	0.67	0.46	0.07
B	DDS	0.05	0.10	0.15	0.16	0.18
	MADDS	1.00	0.75	0.57	0.45	0.10
C	DDS	0.12	0.18	0.21	0.26	0.14
	MADDS	1.26	0.98	0.72	0.58	0.11
D	DDS	0.10	0.20	0.26	0.29	0.18
	MADDS	1.16	0.90	0.79	0.58	0.13

cially when lower protein concentrations were used.

Discussion. These studies have shown that the dog clears MADDS from the circulation more rapidly than DDS. This is surprising in view of the higher protein-binding of MADDS than of DDS and the relatively slow deacetylation of MADDS. In man, the $T_{1/2}$ values for DDS and MADDS were essentially the same when either compound was given (2). However, quantitatively greater urinary excretion of acid-hydrolyzable DDS conjugates was observed after MADDS suggesting that MADDS was metabolized more extensively to these water soluble excretory products than was DDS (2). In the dog, a similar more rapid conversion of MADDS to such conjugates and

TABLE III. HALF-TIMES OF DISAPPEARANCE OF DDS AND MADDS IN DOGS RECEIVING THESE COMPOUNDS.

Dog	DDS[a]		MADDS[a]	
	$T_{1/2}$ (hr)	95% confidence limits (hr)	$T_{1/2}$ (hr)	95% confidence limits (hr)
A	9.2	8.1–10.7	5.7	5.1–6.4
	8.9[b]	8.1–9.7		
B	15.9	14.7–17.4	6.8	6.1–7.7
	16.5[b]	13.8–20.7		
C	10.3	9.0–12.1	6.4	5.9–6.9
D	9.2	7.9–11.0	7.0	6.5–7.6
Mean	11.7		6.5	

[a] The correlation coefficients of the regression lines were < -0.99 ($P < 0.005$).
[b] Second trial.

TABLE IV. BINDING OF DDS AND MADDS BY PLASMA PROTEINS OF DOGS.

Experiment	Drug level (μg/ml)		Protein level (mg/ml)	Binding of drug (%)
	DDS	MADDS		
In vivo[a]	0.89 ± 0.07	—	60 ± 2	71 ± 2
	—	0.96 ± 0.08	60 ± 2	84 ± 2
In vitro[b]	2.0	—	57	62
	—	2.0	57	79
	2.0	—	10	32
	—	2.0	10	55

[a] Measured in 2-hr plasma samples following iv injection of 1.0 mg DDS or 1.2 mg MADDS/kg. Values are the means (± SE) of four dogs.

[b] DDS or MADDS was added to pooled plasma from four untreated dogs to obtain the final concentrations shown. The study at the lower protein concentration was performed after dilution of the pooled plasma with 0.1 *M* phosphate buffer, pH 7.4.

their excretion could explain the shorter $T_{1/2}$ of MADDS. The demonstrated inability of the dog to acetylate DDS is consistent with the findings of others that this species cannot acetylate sulfonamides and isoniazid (14–16). Although urine was not examined for the presence of MADDS in the current studies, we later investigated both urine and plasma from dogs receiving 25 mg DDS/kg (Biggs *et al.*, unpublished studies). No MADDS (< 0.005 μg/ml) was detected in either fluid. In the mouse, deacetylation of MADDS formed from DDS was so rapid and extensive that acetylation of DDS was barely discernible (7). Rapid deacetylation of MADDS cannot explain the failure to detect acetylation in the dog.

These results show that the dog can be a useful model of man for the study of the pharmacokinetics of DDS uncomplicated by concurrent acetylation and deacetylation. The results of further studies will be summarized in subsequent reports.

Summary. Four female dogs receiving 1.0 mg dapsone (DDS)/kg iv exhibited logarithmic decline of plasma levels of DDS with a mean half-time of disappearance ($T_{1/2}$) of 11.7 hr. No evidence of acetylation of DDS to monoacetyl DDS (MADDS) was found. An equimolar dose of MADDS was deacetylated slowly to DDS by the same dogs. The mean $T_{1/2}$ of MADDS was 6.5 hr, significantly less than that of DDS. In 2-hr plasma samples after these doses of drugs, protein-binding of DDS and MADDS averaged 71 and 84%, respectively. Tests of protein-binding of the two drugs *in vitro* confirmed the observations *in vivo*.

1. Shepard, C. C., Ann. Rev. Pharmacol. **9,** 37 (1969).
2. Gelber, R., Peters, J. H., Gordon, G. R., Glazko, A. J., and Levy, L., Clin. Pharmacol. Therap. **12,** 225 (1971).
3. Peters, J. H., Gordon, G. R., Ghoul, D. C., Tolentino, J. G., Walsh, G. P., and Levy, L., Amer. J. Trop. Med. Hyg. **21,** 450 (1972).
4. Peters, J. H., Gordon, G. R., Levy, L., Storkan, M. A., Jacobson, R. R., Enna, C. D., and Kirchheimer, W. F., Amer. J. Trop. Med. Hyg. **23,** 222 (1974).
5. Biggs, J. T., and Levy, L., Proc. Soc. Exp. Biol. Med. **137,** 692 (1971).
6. Riley, R. W., and Levy, L., Proc. Soc. Exp. Biol. Med. **142,** 1168 (1973).
7. Levy, L., Biggs, J. T., Jr., Gordon, G. R., and Peters, J. H., Proc. Soc. Exp. Biol. Med. **140,** 937 (1972).
8. Ellard, G. A., Gammon, P. T., Rees, R. J. W., and Waters, M. F. R., Leprosy Rev. **42,** 101 (1971).
9. Biggs, J. T., Gordon, G. R., and Peters, J. H., Fed. Proc. **30,** 629 (1971).
10. Murray, J. F., Jr., Gordon, G. R., Ghoul, D., and Peters, J. H., Proc. Western Pharmacol. Soc. **15,** 100 (1972).
11. Gordon, G. R., Shafizadeh, A. G., and Peters, J. H., Xenobiotica **3,** 133 (1973).
12. Gordon, G. R., Peters, J. H., Gelber, R., and Levy, L., Proc. Western Pharmacol. Soc. **13,** 17 (1970).
13. Peters, J. H., Ann. Rev. Pharmacol. **8,** 427 (1968).
14. Weber, W. W., *in* "Metabolic Conjugation and Metabolic Hydrolysis" (W. H. Fishman, ed.), Vol. 3, p. 249. Academic Press, New York (1973).

15. Peters, J. H., Amer. Rev. Respirat. Diseases **81**, 485 (1960).
16. Peters, J. H., and Hayes, V. E., Arch. Int. Pharmacodyn. **159**, 328 (1966).
17. Peters, J. H., Gordon, G. R., and Colwell, W. T., Jr., J. Lab. Clin. Med. **76**, 338 (1970).
18. Waddell, W. J., J. Lab. Clin. Med. **48**, 311 (1956).
19. Murray, J. F., Jr., Gordon, G. R., and Peters, J. H., J. Lab. Clin. Med. **78**, 464 (1971).

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