Disposition of the Antileprosy Drug, Dapsone, in the Mouse[1] (36584)

LOUIS LEVY, JOHN T. BIGGS, JR.,[2] G. ROSS GORDON, AND JOHN H. PETERS

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

Because the causative microorganism of human leprosy, *Mycobacterium leprae,* has not been successfully cultivated *in vitro*, almost all of our knowledge of the properties of this organism has resulted from studies carried out in the mouse foot pad infection first described by Shepard (1). Early work demonstrated that dapsone (4,4′-diaminodiphenyl sulfone, DDS) inhibited multiplication of *M. leprae* in the mouse (2); the minimal inhibitory concentration (MIC) in this system has recently been shown to be < 10 ng/ml of plasma and nonhepatic tissues (3, 4). The mouse foot pad infection is useful for the screening of antimicrobial drugs and for some pharmacological studies; in immunologically intact mice it is limited, however, and the mice do not develop leprosy. After the number of organisms increases from $10^{3.7}$ to approximately 10^6/foot pad, the *M. leprae* are rapidly killed by the mouse without the assistance of an antimicrobial drug (5). Studies on chemotherapy of leprosy cannot, therefore, be carried out in mice and must be performed in man. The need to study some properties of *M. leprae* in mice and to pursue chemotherapeutic studies in man requires that we know the disposition of DDS in the two species.

Recent work in our laboratories has elucidated many of the characteristics of the disposition of DDS in man (6–9). Monoacetyldapsone (4-amino-4′-acetamidodiphenyl sulfone, MADDS) has been shown to be the major circulating metabolite. Human subjects acetylate DDS polymorphically and readily deacetylate MADDS. DDS is relatively long-lived in man; the half-time of disappearance ($T_{½}$) from plasma ranged from 14 to 53 hr. No relationship between $T_{½}$ and acetylation capacity was discerned. DDS was found to be moderately bound to plasma proteins; MADDS was more strongly bound. No difference in the degree of protein-binding was found in the two acetylator phenotypes.

In contrast to the situation in man, our knowledge of the disposition of DDS in the mouse is quite limited. Ellard *et al.* (4) have recently reviewed this subject. Available information indicates only that the $T_{½}$ of DDS in mice is relatively short (about 3 hr). No evidence for the acetylation of DDS to MADDS was found, nor was the disposition of MADDS investigated.

We have conducted a number of studies on the disposition of DDS and MADDS in two strains of mice. The disappearance rates of DDS and MADDS and the acetylation of DDS and the deacetylation of MADDS were investigated. Binding of DDS and MADDS to plasma proteins of the mouse was also determined both *in vivo* and *in vitro*. An early finding that mouse plasma readily deacetylates MADDS led us to examine, in a preliminary way, the kinetics of this deace-

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[2] Present address: Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63117.

tylation *in vitro*. In addition, we studied the deacetylation of diacetyldapsone (4,4′-diacetamidodiphenyl sulfone, DADDS). This latter sulfone is now in use as a repository form of DDS therapy for man (10, 11).

Methods. The DDS, MADDS (6) and DADDS (12) used in these studies were described previously. Two strains of mice were employed: BALB/c mice were bred in our laboratories; and B6C3F1 mice were obtained from Dr. C. C. Congdon, Oak Ridge National Laboratory, Oak Ridge, TN. DDS or MADDS was administered ip in small volumes of 10% aqueous ethanol (150 μg/ml). Groups of adult mice were exsanguinated by heart puncture or by the axillary pouch method after administration at the intervals shown in the tables. The blood was collected in heparinized syringes and for some experiments transferred immediately to tubes containing NaF in amount yielding a final concentration of 0.2 *M* to inhibit deacetylation (13). After separation, plasma samples from groups of mice were pooled and stored at −20°. All plasma samples were analyzed within 2 weeks of collection. DDS, MADDS (14) and protein (15, 16) concentrations were determined by published methods.

The degree of binding of DDS and MADDS to plasma proteins was measured by an ultrafiltration technique (9). Binding *in vivo* was examined in heparinized plasma from mice receiving DDS ip. Binding *in vitro* was measured after the addition of DDS or MADDS (2.0 μg/ml) to heparinized plasma samples of untreated mice and after incubation for 30 min at 25°. In the studies *in vitro*, binding was measured both in undiluted plasma and in plasma diluted to a protein concentration of 10 mg/ml with 0.1 *M* phosphate buffer, pH 7.4. Because plasma from mice was found to deacetylate MADDS, the studies with this compound *in vitro* were carried out in the presence of 0.2 *M* NaF.

Deacetylation of MADDS and DADDS was studied in plasma from untreated mice. Reaction mixtures contained plasma diluted to 10 mg protein/ml, and 2.00 μg MADDS or 2.56 μg DADDS/ml. They were incubated at 37° for the times indicated in the tables. Samples of the reaction mixtures containing MADDS initially were analyzed for DDS and MADDS, and those containing DADDS initially, for DDS, MADDS and DADDS with slight modifications of the published procedure (14) to increase the sensitivity for DADDS. Studies using DDS as substrate had demonstrated no acetylation to MADDS or DADDS during 4 hr and the added DDS was recovered quantitatively.

The $T_{½}$ values of DDS were calculated from the regression lines representing the logarithmic decay of the concentration of DDS with time. Differences between $T_{½}$ values found in the various experiments were determined by comparing the slopes of the regression lines.

Results. Table I summarizes our observations in mice receiving 1.0 mg DDS/kg. No evidence for acetylation of DDS was observed in male BALB/c mice unless fluoride was present in the collection tubes to inhibit deacetylation of MADDS. Even under those circumstances, acetylation occurred only to a very small extent as shown by the second experiment in this table. Female BALB/c mice also exhibited a very limited capacity to acetylate DDS. Similar results were obtained in the B6C3F1 mice, *viz.*, very little acetylation occurred without the use of fluoride and only slightly more when fluoride was employed. These results indicate that these strains of mice acetylated DDS only to a slight extent. DADDS could not be detected in any plasma sample of any experiment. The calculated $T_{½}$ value for each experiment is shown in the last column. No significant differences were discerned in these values between the sexes or strains.

The results of similar studies in these mice using an equimolar dose of MADDS are shown in Table II. Again, experiments were performed with and without the addition of fluoride to the blood after collection. In this case, the percentage deacetylation was calculated. The results show, in all cases, extremely rapid and nearly quantitative deactylation of the administered MADDS to DDS, with fluoride having little effect on the deacetylation measured at the various times.

TABLE I. Plasma Levels of DDS and MADDS in Mice Receiving 1.0 mg DDS/kg Intraperitoneally.

Experimental conditions	Sampling time (hr)	Plasma level (μg/ml)		% Acetylation[a]	$T_{1/2}$ of DDS[b] (hr)
		DDS	MADDS		
BALB/c, ♂	1.0	0.28	<0.01	0	2.6
5/time period	2.0	0.22	<0.01	0	
	4.0	0.17	<0.01	0	
	6.0	0.06	<0.01	0	
	8.0	0.05	<0.01	0	
BALB/c, ♂	1.0	0.55	0.02	4	2.4
8/time period[c]	2.0	0.34	0.02	6	
	3.5	0.24	0.03	8	
	5.0	0.14	0.01	7	
	6.5	0.11	0.01	8	
BALB/c, ♀	1.0	0.72	0.06	6	2.9
8/time period[c]	2.0	0.58	0.08	11	
	4.0	0.35	0.05	10	
	6.0	0.24	0.02	8	
	8.0	0.13	0.02	13	
B6C3F1, ♂	1.0	0.75	0.02	2	2.6
8/time period	2.0	0.64	0.04	4	
	3.5	0.35	0.01	3	
	5.0	0.25	0.02	7	
	6.5	0.18	0.01	5	
B6C3F1, ♂	1.0	0.65	0.08	8	3.4
8/time period[c]	2.0	0.52	0.06	9	
	3.5	0.37	0.05	10	
	5.0	0.28	0.04	10	
	6.5	0.21	0.02	10	

[a] % acetylation = MADDS (as DDS equivalents) × 10^2/DDS + MADI

[b] Half-lives were calculated from regression lines. Correlation coefficie these lines were significant ($p < .01$).

[c] Collection tubes contained NaF in amounts to yield 0.2 *M* in the blood.

DADDS could not be detected in any plasma sample. Because of the rapidity of deacetylation, $T_{1/2}$ values for MADDS could not be calculated. Therefore, the last column shows the $T_{1/2}$ values of DDS in these mice. These values did not differ significantly between the sexes or strains, nor did they differ from the results found after DDS administration.

That deacetylation of MADDS occurs in plasma *in vitro* is shown by the upper half of Table III. This process was relatively slow, achieving about 90% of theoretical after incubation for 3 to 4 hr at 37°. These data also show that deacetylation by plasma was almost completely inhibited by 0.2 *M* fluoride but only partially by 0.01 *M* fluoride. Thus, deacetylation of MADDS occurred largely *in vivo*, and was not a procedural artifact. Additional studies have shown that the deacetylase activity of whole blood is entirely accounted for by that of plasma. Furthermore, washed or hemolyzed red blood cells did not deacetylate MADDS.

The lower half of Table III shows that mouse plasma was also capable of deacetylating DADDS. The finding of both MADDS and DDS as products suggests that the deacetylation of DADDS was stepwise. However, the deacetylation of DADDS was less extensive than that of MADDS, attaining only 34% in 4 hr. Again, fluoride was found to inhibit almost completely the deacetyla-

TABLE II. Plasma Levels of DDS and MADDS in Mice Receiving 1.2 mg MADDS/kg Intraperitoneally.

Experimental conditions	Sampling time (hr)	Plasma level (μg/ml)		% Deacetylation[a]	$T_{1/2}$ of DDS[b] (hr)
		DDS	MADDS		
BALB/c, ♂	1.0	0.30	0.02	95	2.4
5/time period	2.0	0.30	0.01	97	
	4.0	0.12	<0.01	100	
	6.0	0.10	<0.01	100	
	8.0	0.04	0.01	82	
BALB/c, ♂	1.0	0.80	0.08	93	2.1
8/time period[c]	2.0	0.50	0.04	94	
	3.5	0.29	0.03	94	
	5.0	0.18	0.01	95	
	6.5	0.13	0.01	93	
BALB/c, ♀	1.0	0.87	0.15	87	3.0
8/time period[c]	2.0	0.45	0.05	93	
	4.0	0.36	0.06	88	
	6.0	0.22	0.02	90	
	8.0	0.14	0.01	92	
B6C3F1, ♂	1.0	0.75	0.08	93	4.3
8/time period	2.0	0.72	0.06	94	
	3.5	0.55	0.03	96	
	5.0	0.43	0.02	96	
	6.5	0.32	0.02	94	
B6C3F1, ♂	1.0	0.78	0.18	84	2.8
8/time period[c]	2.0	0.46	0.04	94	
	3.5	0.37	0.03	95	
	5.0	0.24	0.02	92	
	6.5	0.19	0.02	90	

[a] % deacetylation = DDS $\times$ 10^2/DDS + MADDS (as DDS equivalents).

[b] Half-lives were calculated from regression lines. Correlation coefficients of these lines were significant ($p < .05$).

[c] Collection tubes contained NaF in amounts to yield 0.2 *M* in the blood.

tion of DADDS. Heat inactivation studies showed that the deacetylase activity of mouse plasma was unaffected by incubation at 50° for 30 min, whereas activity was completely abolished when the incubation was carried out at 60°.

The results of the binding studies are shown in Table IV. Binding of DDS to plasma proteins was extensive *in vivo*; but that of MADDS could not be measured because of its virtual absence from plasma after administration of DDS. The studies *in vitro* showed, however, that MADDS was more extensively bound than was DDS. This difference is shown more strikingly by the studies in diluted plasma, in which DDS binding was only 7%, whereas that of MADDS was 61%.

Discussion. Ellard *et al.* (4) found that female P-strain albino mice exhibited $T_{1/2}$ values for DDS of 2.7 and 3.7 hr after administration ip of 10 and 50 mg DDS/kg, respectively. These values are similar to those we observed in our strains of mice receiving a substantially smaller dose. In addition, they could not detect MADDS in the plasma in any of their studies. However, their analytical method could not have detected MADDS in the presence of DDS if the ratio of DDS to MADDS was larger than 6:1. Our results, in comparable experiments, show that the ratio of DDS to MADDS always exceeded 10:1 (Table I).

The ability of mouse plasma to deacetylate

TABLE III. Deacetylation of MADDS and DADDS by Plasma of Female BALB/c Mice *in vitro*.

Compound added (μg)	Incubation time (hr)	Compound found (μg)			% Deacetylation[a]	% Recovery of total sulfones[b]
		DADDS	MADDS	DDS		
MADDS (2.00)	0.5	—	1.53	0.42	23.5	101
	1.0	—	1.27	0.82	36.5	112
	2.0	—	0.60	1.17	70.0	98.2
	2.0[c]	—	1.89	0.07	5.5	98.5
	3.0	—	0.29	1.40	85.5	96.5
	3.0[d]	—	1.29	0.34	20.7	81.2
	4.0	—	0.16	1.52	92.0	97.1
DADDS (2.56)	0.5	2.23	0.24	0.12	12.9	104
	1.0	2.14	0.29	0.16	16.4	105
	2.0	1.96	0.32	0.22	23.4	102
	2.0[c]	2.44	0.14	0.15	4.7	109
	3.0	1.87	0.34	0.34	26.9	106
	4.0	1.69	0.33	0.45	34.0	104

[a] Added compound deacetylated × 10^2/added compound.
[b] Total sulfone found × 10^2/sulfone added (all in DDS equivalents).
[c] Incubation mixture contained 0.2 *M* NaF.
[d] Incubation mixture contained 0.01 *M* NaF.

MADDS and DADDS is unique. A survey of other species (unpublished studies) demonstrated that this activity for MADDS was completely absent from the plasma or red blood cells of rats, rabbits, dogs, and man. Also, it was not found in plasma of rhesus and squirrel monkeys. The finding of this plasma deacetylase in the mouse may be contrasted with the ubiquity of enzymes for deacetylation of acylarylamides in various tissues of numerous species (17). The only previous report of a plasma deacylase is that of Gleason and Vogh (18). They found that plasma from ICR mice deformylated 4, 4′-diformamidodiphenyl sulfone readily. In addition, this activity was also observed in plasma from rats and a rabbit. They did not detect deacetylation of DADDS by mouse plasma, as we have. Nevertheless, extensive deacetylation of DADDS was observed *in vivo* in the mouse by these authors. It would appear, at this time, that the deacetylase activity we have measured is different from the deformylase activity reported by Gleason and Vogh. Finally, our inability to detect DADDS when either DDS or MADDS was the substrate in the *in vitro* experiments coupled with the near quantitative recovery of the total sulfone compounds as DDS and MADDS when MADDS was the substrate, shows that acetylation of either DDS or

TABLE IV. Binding of DDS and MADDS to Plasma Proteins of BALB/c Mice.

Expt.	Plasma protein (g %)	DDS bound (%)	MADDS bound (%)
In vivo[a]	4.7	76 ± 1[b]	—[c]
In vitro[d]	4.7	54 ± 2	83 ± 1[e]
	1.0[f]	7 ± 3	61 ± 2[e]

[a] These measurements were based on a study of the pooled plasma of 40 mice to which 1 mg DDS/kg had been administered ip 30 to 90 min earlier. Plasma DDS concentration = 1.27 μg/ml.
[b] Mean ± SE. All measurements were performed in quadruplicate.
[c] MADDS levels were too low to permit measurements of binding.
[d] These measurements are based on a study of the pooled plasma of 40 mice. DDS or MADDS was added to yield a final concentration of 2.0 μg/ml.
[e] NaF was added in a final concentration of 0.2 *M* to inhibit deacetylation.
[f] Pooled plasma was diluted with 0.1 *M* phosphate buffer, pH 7.4.

MADDS to DADDS does not occur in mouse plasma. In man receiving DADDS, all three compounds, DDS, MADDS and DADDS, are present in the circulation, but no DADDS could be detected in the plasma of human subjects receiving DDS, suggesting that, in man also, no acetylation of DDS or MADDS to DADDS occurs (12).

Numerous differences between mice and man in regard to the disposition of DDS and MADDS are now apparent. Mice do not appear to acetylate DDS to a significant extent; man acetylates the compound polymorphically, yielding rapid and slow acetylator phenotypes (6). Also, mice deacetylate MADDS rapidly and extensively, whereas man effects this reaction to a lesser extent. In man, an apparent steady state of acetylation–deacetylation is reached almost immediately after administration of DDS but this state is attained only 4 to 6 hr after the administration of MADDS. The question of the possible antimycobacterial activity of MADDS remains open. In earlier tests in which MADDS was fed to mice infected with *M. leprae* we found that it had the same activity, on a molar basis, as that of DDS (unpublished experiments). The current work shows that administering MADDS was equivalent to giving DDS because the mice deacetylated so extensively. Thus, no conclusion can be reached regarding the possible inherent antileprosy activity of MADDS.

The $T_{1/2}$ of DDS in the mice was found to be about one-tenth of that in man (8). This may reflect the known differences in metabolic rates (19) and in clearance rates for other drugs (20) of the two species. The finding that DDS is bound to plasma proteins to about the same extent in the two species (9) suggests that differences in $T_{1/2}$ are not attributable to this phenomenon.

Because the disposition of DDS in the mouse is so different from that in man, chemotherapeutic studies performed in the mouse would have only limited application to the treatment of leprosy patients. We anticipate similar limitations to the use of the immunosuppressed mouse which develops a progressive infection with *M. leprae* (21). The immunosuppressed rat may prove to be a better model of man for chemotherapeutic studies of DDS. This animal permits progressive infection with *M. leprae* (22) and the disposition of DDS and MADDS in this species is more similar to that of man (23).

Summary. The $T_{1/2}$ of DDS ranged from 2 to 4 hr in male and female BALB/c and in male B6C3F1 mice. It was independent of strain or sex. The $T_{1/2}$ of MADDS could not be measured because of minimal acetylation of administered DDS and nearly complete deacetylation of administered MADDS. Thus, mice are poor acetylators of DDS and very efficient deacetylators of MADDS. Studies *in vitro* showed that mouse plasma deacetylated MADDS and DADDS but at a rate insufficiently rapid to account for the observed deacetylation of MADDS *in vivo*. Examination of the binding of DDS and MADDS to plasma proteins both *in vivo* and *in vitro* showed considerable binding of both compounds, as is the case in man.

The disposition of DDS in the mouse is much different from that in man. Although one may apply some information from studies in the mouse to the clinical use of DDS in the treatment of human leprosy, many therapeutic variables important to human treatment cannot be meaningfully studied in the mouse.

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1. Shepard, C. C., J. Exp. Med. **112**, 445 (1960).
2. Shepard, C. C., and Chang, Y. T., Proc. Soc. Exp. Biol. Med. **109**, 636 (1962).
3. Ozawa, T., Shepard, C. C., and Karat, A. B. A., Amer. J. Trop. Med. Hyg. **20**, 274 (1971).
4. Ellard, G. A., Gammon, P. T., Rees, R. J. W., and Waters, M. F. R., Lepr. Rev. **42**, 101 (1971).
5. Levy, L., Proc. Soc. Exp. Biol. Med. **135**, 745 (1970).
6. Gelber, R., Peters, J. H., Gordon, G. R., Glazko, A. J., and Levy, L., Clin. Pharmacol. Ther. **12**, 225 (1971).
7. Peters, J. H., and Levy, L., Ann. N.Y. Acad. Sci. **179**, 660 (1971).
8. 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).
9. Biggs, J. T., and Levy, L., Proc. Soc. Exp. Biol. Med. **137**, 692 (1971).

10. Shepard, C. C., Tolentino, J. G., and McRae, D. H., Amer. J. Trop. Med. Hyg. **17**, 192 (1968).

11. Shepard, C. C., Levy, L., and Fasal, P., Amer. J. Trop. Med. Hyg. **21**, 446 (1972).

12. Murray, J. F., Jr., Gordon, G. R., and Peters, J. H., J. Lab. Clin. Med. **78**, 464 (1971).

13. Gutmann, H. R., Seal, U. S., and Irving, C. C., Cancer Res. **20**, 1072 (1960).

14. Peters, J. H., Gordon, G. R., and Colwell, W. T., Jr., J. Lab. Clin. Med. **76**, 338 (1970).

15. Gornall, A. G., Bradawill, C. J., and David, M. M., J. Biol. Chem. **177**, 751 (1949).

16. Waddell, W. J., J. Lab. Clin. Med. **48**, 311 (1956).

17. Weber, W. W., *in* "Handbook of Experimental Pharmacology, Concepts in Biochemical Pharmacology" (B. B. Brodie and J. R. Gillette, eds.), Vol. 28/2, p. 564. Springer-Verlag, New York (1971).

18. Gleason, L. N., and Vogh, B. P., Biochem. Pharmacol. **20**, 2409 (1971).

19. Kleiber, M., "The Fire of Life," p. 177. Wiley, New York (1961).

20. Brodie, B. B., Clin. Pharmacol. Ther. **3**, 374 (1962).

21. Rees, R. J. W., Nature (London) **211**, 657 (1966).

22. Fieldsteel, A. H., and McIntosh, A. H., Proc. Soc. Exp. Biol. Med. **138**, 408 (1971).

23. Gordon, G. R., Murray, J. F., Jr., Ghoul, D. C., Peters, J. H., Fieldsteel, A. H., and Levy, L., Int. J. Lepr., in press.

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