Binding of Dapsone and Monoacetyldapsone by Human Plasma Proteins[1] (35647)

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Although dapsone (4,4′-diaminodiphenylsulfone, DDS) is currently the most important drug in the treatment of leprosy (1), studies of its disposition in man have been undertaken only recently (2–5). This work has demonstrated that acetylation of DDS to monoacetyldapsone (4-amino-4′-acetamidodiphenylsulfone, MADDS), an important metabolic process in the disposition of this drug, represents another genetic polymorphism. Rapid acetylators of DDS are distinguished from slow acetylators only by the ratio of the plasma concentration of MADDS to that of DDS (MADDS/DDS); the mean ratio was 5 times larger in 9 rapid acetylators of isoniazid and sulfamethazine than in 10 slow acetylators. The two acetylator phenotypes could not be readily distinguished by the quantity of MADDS excreted in the urine nor by the half-time of disappearance of DDS from plasma.

In these earlier studies, the MADDS/DDS characteristic of the individual was achieved within 15 min after the oral administration of DDS, and remained constant thereafter for at least 60 hr. The MADDS/DDS after administration of MADDS was the same as that after DDS, but was achieved only 4 to 6 hr after MADDS was administered. Very large plasma concentrations of MADDS were encountered during the period between MADDS administration and establishment of the characteristic ratio; peak plasma MADDS concentrations achieved after MADDS administration were 3 to 6 times greater than the peak plasma DDS concentrations after DDS administration to 2 subjects of each phenotype. The half-time of MADDS was the same as that for DDS, regardless of which compound was administered; the mean half-time of DDS and MADDS after administration of each compound to 2 subjects of each phenotype was 21 hr. Very little MADDS was excreted in the urine; 3 subjects of each phenotype, studied on 4 separate occasions after administration of DDS, excreted 0.17 to 0.70% of the dose as MADDS during the first 24 hr after the dose, and 4.2 to 11.1% of the dose as DDS; during this same period, 24.9 to 38.1% of the administered DDS could be accounted for in the urine as DDS, MADDS, and compounds yielding DDS or MADDS on hydrolysis. Thus, although MADDS represented 12 to 55% of the sum of DDS plus MADDS in plasma [no other DDS metabolites have been identified in human plasma (Peters, J. H., personal communication), urinary excretion of MADDS was negligible.

A study of the binding of DDS to bovine serum albumin (6) and a preliminary study of the binding of DDS and MADDS to human serum albumin (7) have demonstrated that both compounds are strongly bound, with MADDS the more strongly bound. A more detailed study has been undertaken to learn if rapid and slow acetylator subjects exhibit different binding characteristics for the two compounds, and to what extent the disposition of DDS might be determined by the binding of DDS and MADDS to plasma proteins. The degree of protein binding of DDS and MADDS at therapeutic concentrations has been measured, both *in vivo* and *in vitro*, in plasma from both rapid and slow acetylators of DDS. And the binding of one

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compound in the presence of the other has been investigated.

Methods. Plasma protein binding of DDS (K and K Laboratories, Inc., Hollywood, Calif.) and of MADDS (Parke, Davis and Co., Ann Arbor, Mich.) was measured by ultrafiltration (8). Four-ml samples of plasma were placed in 27/32-in. cellulose dialysis tubing (A.H. Thomas Co., Philadelphia, Pa.) which had been soaked 30 min in distilled water and then blotted dry. The dialysis bags were then suspended in 50-ml centrifuge tubes and centrifuged 20 min at 500*g* to remove water from the dialysis tubing. The centrifugate was discarded; the bags were resuspended in clean tubes and centrifuged for 2 hr at 500*g*. The volume of centrifugate equaled about 10% of the initial volume; protein leakage through the dialysis membrane represented less than 0.5% of the original protein concentration (9). Centrifugation caused an increase in temperature of about 2°. The pH was maintained at 7.4 by gassing the samples with 95% O_2–5% CO_2 prior to centrifugation.

The degree of drug binding was calculated by assuming that the drug concentration in the filtrate equaled the concentration of unbound drug in the original sample. No correction was necessary for the small increase in protein concentration within the dialysis bag after centrifugation. The possibility that significant amounts of the compounds were bound to the dialysis membrane was studied by substituting 0.1 *M* phosphate buffer, pH 7.4, for plasma. The concentrations of the compounds in the centrifugate were found to be no smaller than those in the tubing, indicating the absence of drug binding to the membrane. DDS and MADDS concentrations were determined fluorometrically (10). All measurements of binding were made in duplicate; the mean difference between duplicates for DDS binding was 3.2% (SD 3.9%) and for MADDS binding 0.8% (SD 1.1%). No evidence of acetylation of DDS to yield MADDS, nor of deacetylation of MADDS to DDS was found to have occurred in any of the experiments.

Fresh heparinized plasma was obtained from human volunteers who had previously been phenotyped as rapid or slow acetylators with sulfamethazine and DDS (5). All of the subjects were found to have plasma protein concentrations within normal limits. Binding *in vivo* was determined in plasma samples obtained 4 hr after oral administration of 50 mg of DDS. Binding *in vitro* was measured after addition to the plasma samples of one or the other of the compounds dissolved in a volume of ethanol smaller than 1% of the volume of the plasma sample followed by incubation for 30 min at 25°. The incubation step was omitted for the studies of binding *in vivo*. Some of the studies of binding *in vitro* were performed on plasma samples which were diluted with a 0.1 *M* potassium phosphate buffer, pH 7.4.

TABLE I. Binding of DDS and MADDS to Human Plasma Proteins 4 hr After Ingestion of 50 mg of DDS.

	Plasma (μg/ml)		Bound (%)	
Subject	[DDS]	[MADDS]	DDS	MADDS
	Slow acetylators			
DGR	1.26	0.15	79	100
JBI	1.02	0.38	76	100
SFE	1.44	0.22	79	100
Mean			78[a]	100[a]
	Rapid acetylators			
LLE	1.18	0.65	75	100
NMO	1.48	1.66	72	100
LMU	1.03	0.63	81	100
Mean			76	100

[a] This value is not significantly different from the corresponding value for rapid acetylators at the 95% level of confidence.

Results. Binding of DDS and MADDS *in vivo* was measured in 6 subjects after ingestion of DDS. Three of the subjects were slow acetylators and 3 were rapid acetylators, as demonstrated by the plasma MADDS/DDS (Table I). These subjects had previously been phenotyped after 100 mg of DDS orally, with almost identical values for the MADDS/DDS resulting (5). The results of these studies demonstrate that the degree of binding *in vivo* of DDS and MADDS was identical for slow and rapid acetylators of DDS.

The degree of binding of both drugs, added

TABLE II. Binding of DDS and MADDS in Plasma of Rapid and Slow Acetylators *in Vitro*.

Subject	Bound (%) DDS	MADDS
Slow acetylators		
PJA	76	100
DGR	70	98
JBI	73	99
SFE	75	99
SON	78	97
DDR	80	99
Mean	75[a]	99[a]
Rapid acetylators		
LLE	78	99
LIL	76	98
NMO	73	99
LMU	79	99
Mean	77	99

[a] This value is not significantly different from the corresponding value for rapid acetylators at the 95% level of confidence.

in a final concentration of 2 μg/ml to plasma samples obtained from 10 human subjects, is shown in Table II. Six of the subjects are those in whom binding *in vivo* was studied (Table I). The 4 additional subjects whose plasma binding of DDS and MADDS was studied *in vitro* also had been phenotyped (5). Previously measured plasma MADDS/DDS after an oral dose of 100 mg of DDS were: PJA, 0.17; SON, 0.14; DDR, 0.17; and LIL, 0.76. These studies *in vitro* yielded results very similar to those done *in vivo*; there was no difference in the degree of binding to plasma proteins of DDS or of MADDS between slow and rapid acetylators.

In a study of the influence of drug concentration on the degree of binding, binding of DDS and of MADDS, added separately in final concentrations of 2 or 20 μg/ml, was measured in the plasma of 2 subjects (Table III). MADDS was completely bound at both concentrations studied. DDS, on the other hand, was more completely bound at the lower concentration; although the difference in degree of binding between the two concentrations is significant, it is obviously not great. These data suggest that, at the plasma concentrations achieved in therapy, not only is MADDS bound more completely than is DDS, but also that the capacity of human plasma proteins to bind MADDS is greater than their capacity to bind DDS.

The possibility of competition between DDS and MADDS for binding sites on plasma proteins was initially investigated in undiluted human plasma, but the limited solubility of both compounds in plasma prevented adequate variation of drug concentration. Plasma samples were diluted 1:1 and 1:10 with 0.1 *M* phosphate buffer, pH 7.4, to permit varying the ratio of drug concentration to the number of binding sites over a wider range. As shown in Table IV, the degree of DDS binding was decreased in the presence of MADDS; whereas MADDS binding was not significantly changed by the presence of DDS. The decrease in the degree of MADDS binding in the plasma diluted 1:10 may have resulted from saturation of binding sites.

Discussion. The results of this study indicate that both DDS and MADDS are bound to plasma proteins, and that MADDS is the more strongly bound compound. Binding of MADDS may well account for its limited excretion in urine (2–5). Binding of MADDS may also exclude this compound from sites of deacetylation, thus accounting for the large plasma concentrations of this drug encountered shortly after its oral administration. On the other hand, differences in the degree of binding of DDS and MADDS to plasma proteins do not account for individual variations in the MADDS/DDS. DDS bind-

TABLE III. Plasma Protein Binding of DDS and MADDS *in Vitro*.

Subject	DDS or MADDS added (μg/ml)	Bound (%) DDS	MADDS
LLE	2	77 ± 1[a,b]	98 ± 0
	20	69 ± 0	99 ± 0
JBI	2	73 ± 1[b]	100 ± 0
	20	66 ± 0	99 ± 0

[a] Each value represents the mean ± standard error of 4 determinations.

[b] This value is significantly greater than the corresponding value for the higher concentration of DDS, at the 95% level of confidence.

TABLE IV. Effect of MADDS on the Binding of DDS *in Vitro*.

Added (μg/ml)		Bound (%)	
DDS	MADDS	DDS[a]	MADDS
	Plasma diluted 1:1		
10	0	63	—
0	10	—	99
10	10	56	98
10	20	46	98
	Plasma diluted 1:10		
10	0	25	—
0	10	—	91
10	10	15	91
10	20	7	75

[a] The slope of the regression of % DDS bound on the concentration of added MADDS was significantly different from 0 at the 95% level of confidence.

ing is reduced in the presence of MADDS when plasma is diluted and large concentrations of the two compounds are employed, but the similarity in DDS binding between rapid and slow acetylators speaks against important displacement of DDS by MADDS at the concentrations of these compounds encountered in therapy. The great affinity of these compounds for plasma proteins may account for their long half-life in man, but preliminary studies of the degree of plasma protein binding of DDS and MADDS in species known to have widely differing DDS half-lives suggests the importance of other factors. The contribution of biliary recirculation remains to be evaluated.

Summary. Binding of dapsone and monoacetyldapsone to human plasma proteins has been investigated by means of an ultrafiltration technique. When binding was studied *in vivo* or *in vitro* at therapeutic concentrations of the drugs, dapsone was 70 to 80% bound, and monoacetyldapsone 98 to 100%. Rapid and slow acetylators of dapsone bind both compounds to the same degree. Protein binding of monoacetyldapsone may well account for the limited excretion of this compound in the urine. The contribution of protein binding to the long plasma half-lives of these compounds must be evaluated in the light of future studies.

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