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Effect of parenteral dimethyl sulfoxide (DMSO) on drug metabolizing enzyme activities in the phenobarbital pretreated rat

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Since many substrates are nonpolar compounds, small volumes of a solvent or detergent are frequently utilized to enhance their aqueous solubilization. It is recognized that the addition of such organic solvents may stimulate or inhibit biotransformation studies involving the microsomal mixed function oxygenase enzyme system [1, 2] and, therefore, misinterpretation of biologic events may occur unless the solvent effect is determined. Because we were interested in investigating drug metabolizing enzyme activities associated with experimental hepatic injury, and DMSO was to be used as the solubilizing agent for a known hepatotoxin, we assessed the effect of the *in vivo* administration of DMSO upon hepatic microsomal enzyme activities in the phenobarbital pretreated rat.

Normal Sprague-Dawley rats (240–260 g), as well as those pretreated with phenobarbital (75 mg/kg/day) intraperitoneally for 4 days, were studied. They had free access to Purina Rat Chow and water, and were caged in a constant temperature room (22°) with alternating 12 hr of light and darkness. All experiments were done in the early morning. Animals were divided into the following groups: Group 1—normal rats received a single injection of 0.55 gm (0.5 ml) undiluted DMSO (Fisher Scientific, spectral grade) intraperitoneally 72 hr prior to sacrifice; Group 2—on the day 4 of the phenobarbital pretreatment, rats received a single injection i.p. of 0.55 gm undiluted DMSO and were sacrificed 72 hr later. Control rats (no DMSO injection) were also studied in each group.

Following decapitation, blood was collected for SGOT (Sigma Kit) and serum bilirubin [3] determinations. The liver was rapidly biopsied and specimen were fixed in formalin and stained with hemotoxylin and eosin for evaluation by light microscopy. The liver was then perfused *in situ* with 75 ml of ice cold 0.15 M sodium chloride and excised. Microsomes were prepared by differential centrifugation at 105,000 *g* for 60 min in a Beckman L2-65 B centrifuge. The suspended microsomal pellet obtained from the centrifugation was used for the following assays: Cytochrome P-450 content [4], aminopyrine demethylase [5], aniline hydroxylase [6], and bilirubin glucuronyl transferase [7]. Protein concentrations were determined by the method of Lowry *et al.* [8] using bovine albumin as standard.

Statistical indices were calculated by standard methods and are expressed as the mean ∓ SEM. A *t* test of the difference between two sample means was utilized to assess the significance between parameters [9]. P values equal to or less than 0.01 were considered significant.

DMSO had no effect on the drug metabolizing enzyme activity in the normal rats (Group 1). A previous report [10], utilizing larger doses of DMSO i.p. in normal Sprague-Dawley rats, demonstrated that aniline hydroxylase activity was increased within the first 24 hr, however, this had returned to control level by 48 hr. Further, there was no change in the cytochrome P-450 levels. Therefore, the results reported here are in agreement with this report as our assays were determined 72 hr after the lower dose of DMSO administration.

The phenobarbital pretreated rats, however, responded differently to the DMSO. The cytochrome P-450 content, and the activities of aminopyrine demethylase and aniline hydroxylase were reduced by 27, 32 and 19 per cent, respectively (Table 1). All reductions were significantly different from the phenobarbital controls. To determine whether the reduction of either the aminopyrine demethylase or aniline hydroxylase activities were disproportionate to the reduction in cytochrome P-450 content, we calculated the ratio of the enzyme activity per nmole of cytochrome P-450 [11]. For aminopyrine demethylase, the ratio was essentially the same in the phenobarbital control and DMSO treated animals; 3.4 and 3.2 respectively. Similarly, the ratios for aniline hydroxylase were essentially the same (0.55) and 0.62) between control and DMSO treated animals. This would imply that the reduced enzyme activities were largely related to a diminished cytochrome P-450 content, and not due to specific alteration of the two enzyme activities. We did assess, however, the substrate spectral binding curve for DMSO in rat microsomes, and this revealed a modified Type II with a lambda maximum at 417 nm and lambda minimum at 395 nm [12]. Thus, the DMSO could competitively inhibit at both Type I (aminopyrine) and Type II (aniline) substrate binding sites

Table 1. Effect of *in vivo* DMSO on microsomal protein and enzyme activities in phenobarbital pretreated rats

Treatment	Microsomal protein	Cytochrome P-450	Aminopyrine demethylase	Aniline hydroxylase	Glucuronyl transferase
	mg/ml	n moles/mg protein	n moles HCHO formed/mg prot/min	n moles *p*-aminophenol formed/mg prot/min	ugm bilirubin formed/mg prot/min
Phenobarbital Control*	11.09 ∓ 0.59 (44)	2.33 ∓ 0.04 (22)	8.25 ∓ 0.57 (15)	1.29 ∓ 0.03 (15)	1.34 ∓ 0.05 (13)
DMSO	10.80 ∓ 0.50 (33)	1.70 ∓ 0.07 (28)	5.45 ∓ 0.43 (31)	1.05 ∓ 0.02 (27)	1.29 ∓ 0.04 (28)
P†	NS	0.001	0.001	0.001	NS

* Values given are the mean ∓ SEM with number of observations in parentheses.
† Comparison of Control with DMSO rats. NS—not significant.

on the cytochrome P-450 molecule, and this would result in reduced activities of aminopyrine demethylase and aniline hydroxylase. Such inhibition would not, however, result in a reduced cytochrome P-450 content. In contrast, the glucuronyl transferase activity was not diminished as compared with the control animals. Since this enzyme system is independent of cytochrome P-450, it would further suggest that the mixed function oxygenase enzymes were diminished secondarily to the reduction in cytochrome P-450 content.

Histologic sections revealed no evidence for hepatocellular injury as compared with normal rat liver histology. Further, there were no changes in the SGOT levels or serum bilirubin in rats given DMSO.

The i.p. administration of dimethyl sulfoxide caused a reduction in activity of the mixed function oxygenase enzyme system. DMSO has previously been reported to stimulate, inhibit, or have no effect when added *in vitro* to various enzyme systems [13, 14]. It has further been reported to stimulate aniline hydroxylase activity when given i.p. to the rat [10]. The mechanisms leading to these changes in enzyme activity are unknown, however, postulations include that DMSO may alter pH, modify protein conformation or biochemical structure [15]. Further, when DMSO is given *in vivo*, the effect of its metabolites must also be considered [16]. Because phenobarbital enhances the metabolism of DMSO [14], our results may reflect such a metabolite effect, and this is supported by the finding of the differences between the normal and phenobarbital pretreated rats. This may partly explain our reduced mixed function oxygenase activities as compared with a previous study [10]. It appeared, however, that the reduced content of cytochrome P-450 was the major factor in the diminished activity of the drug metabolizing enzyme system. It is appreciated that cytochrome P-450's catalytic activity is dependent upon its association with intracellular membranes [17]. Since DMSO has recognized effects on membranes [18], it may alter the integrity of the cytochrome P-450 by disrupting its membrane-bound position. Further, it has been shown that synthetic detergents interact with the cytochrome P-450, and this may involve either inhibition of drug binding sites on the P-450 molecule or degradation of the hemeprotein itself [19]. Thus the interaction of DMSO with the cytochrome P-450 is consistent with our results and would also explain the non interference with the glucuronyl transferase activity. We conclude that when DMSO is utilized as a solvent in drug metabolizing enzyme studies, adjustments may have to be made for its effect on enzymes in the mixed function oxygenase system if phenobarbital pretreatment is employed.

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