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Inhibition of Microsomal Cytochromes P450 in Rat Liver by the TricycleC Antidepressant Drug Desipramine and Its Primary Oxidized Metabolites

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ABSTRACT. *N*-Monoalkyl substituted tricyclic antidepressants like desipramine (DES) undergo cytochrome P450 (P450)-mediated biotransformation in liver to produce inhibitory metabolite-intermediate (MI) complexes with the enzyme. However, additional oxidation pathways that generate isolable metabolites have also been identified, so that the relationship between MI complexation and total oxidative metabolism is unclear. The present study investigated the capacity of DES and three putative metabolites (2-hydroxy- and 10-hydroxy-DES and *N,N*-didesmethylimipramine; DIDES) to elicit MI complexation and inhibit P450-dependent activities in rat liver. MI complexation of P450 was produced by DES, but not with the three metabolites, in NADPH-supplemented microsomes. Consistent with this finding, inhibition of testosterone hydroxylation pathways was enhanced markedly by prior incubation of DES with NADPH and microsomes. Direct addition of DIDES to incubations resulted in significant inhibition of P450 activities (IC_{50}s of 35 and 29 μM against estradiol 6β- and 16α-hydroxylation mediated by P450s 3A2 and 2C11, respectively). Neither 2-hydroxy- nor 10-hydroxy-DES directly inhibited testosterone hydroxylation (IC_{50}s > 100 μM). However, after a preincubation step between these metabolites and NADPH-fortified microsomes, enhanced inhibition of reactions mediated by P450 3A2 and P450 2C11/2A1 was produced by 2-hydroxy-DES and 10-hydroxy-DES, respectively. Metabolism of DES to DIDES and 2-hydroxy-DES was estimated as 7.77 ± 0.48 nmol/mg protein/hr (10-hydroxy-DES was not detected). It is likely that secondary oxidized metabolites derived from 2-hydroxy-DES, as well as the primary metabolite DIDES, may contribute to the inhibition of P450 activity during DES biotransformation. These results indicate that the 2-hydroxy-, 10-hydroxy-, and *N*-desmethyl-metabolites of DES are not involved in MI complexation, but complexation is not the sole mechanism by which DES inhibits microsomal drug oxidation that may lead to pharmacokinetic drug interactions. BIOCHEM PHARMACOL 51;1:15–20, 1996.

KEY WORDS. tricyclic antidepressants; cytochrome P450; metabolite-intermediate complexation; pharmacokinetic interactions

Alkylamine-substituted tricyclic antidepressants, including DES† and nortriptyline, have been associated with clinically significant pharmacokinetic drug interactions related to the inhibition of P450 enzymes [1–3]. These agents are substrates for the P450 drug monooxygenase system and generate MI complexes that sequester P450s in a catalytically inactive state [4, 5]. It has been established that alkylamine biotransformation is a prerequisite for MI complexation that may be expected to result in long-lived inhibition of drug oxidation. In a recent study, it was found that MI-complexation of P450 2C11 by a reactive nortriptyline metabolite was the principal inhibitory mechanism in male rat hepatic microsomes [5]. Despite this finding, it was suggested that other metabolites of the drug, distinct from those involved in MI complexation, may contribute to the inhibition of other P450s in rat liver. Thus, it is of interest that several additional metabolites of DES and imipramine have been identified in microsomal incubations *in vitro* [6]. For example, in human liver fractions, DES is reportedly oxidized to 2-hydroxy- and 10-hydroxy-DES and *N,N*-didesmethylimipramine (DIDES). The possibility that such oxidized metabolic products may contribute to the overall extent of inhibition produced by DES provided the rationale for the present study. The principal finding to emerge from this study was that MI-complexation appears to be a metabolic pathway distinct from those that generate the 2- and 10-hydroxy metabolites and DIDES. Although this may have been anticipated, it was found that the metabolite DIDES was a reversible inhibitor of P450 reactions. Evidence was also obtained that secondary products from the oxidation of 2- and 10-hydroxy-DES may contribute to reversible inhibition processes. Thus, it is conceivable that the accumulation of such metabolites during administration of DES to patients may lead to pharmacokinetic interactions and toxicity.

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† Abbreviations: DES, desipramine; P450, cytochrome P450; MI, metabolite intermediate; DIDES, *N,N*-didesmethylimipramine.

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MATERIALS AND METHODS

Chemicals

[^{14}C]Testosterone (sp. act. 59 mCi/mmol) and [^{14}C]estradiol (sp. act. 56 mCi/mmol) were purchased from Amersham Australia (North Ryde, NSW, Australia) and New England Nuclear (Sydney, Australia), respectively. Steroid standards were from the Sigma Chemical Co. (St Louis, MO, U.S.A.), Steraloids (Wilton, NH, U.S.A.) and the MRC Steroid Reference Collection (Queen Mary's College, London, U.K.). DES and biochemicals were purchased from Sigma and the DES metabolites were generous gifts from Drs D. Scholer and L. Maitre, Ciba-Geigy AG (Basel, Switzerland). Analytical reagent-grade solvents and other chemicals were purchased from Ajax (Sydney, Australia).

Animals and Preparation of Microsomal Fractions

Male Wistar rats (~250 g) were obtained from the Department of Animal Care, Westmead Hospital and were held in wire cages under conditions of constant temperature and lighting (12-hr light-dark cycle). Animals were anesthetized and killed and washed hepatic microsomes were prepared by differential ultracentrifugation. The final microsomal pellets obtained were resuspended in 50 mM potassium phosphate buffer, pH 7.4, that contained 20% glycerol and 1 mM EDTA, snap frozen in liquid nitrogen and stored at −70° until required in experiments [7]. Microsomal protein was estimated by the Lowry procedure [8].

Assays of Microsomal Steroid Hydroxylation

Testosterone (50 μM; 0.18 μCi/0.4 mL incubation) hydroxylation reactions (2.5 min, 0.15 mg microsomal protein) were conducted at 37° in potassium phosphate buffer (0.1M, pH 7.4 containing 1 mM EDTA) [5].

Drug/metabolites (concentration range 1–200 μM) were added to the incubations in 50 μL of solvent (in aqueous dimethylformamide, which had no effect on rates of hydroxysteroid metabolite formation). The reactions were initiated by the addition of NADPH (1 mM final) and were terminated by addition of 5 mL of chloroform and removal to ice. After extraction, centrifugation, and separation, the organic phase was evaporated to dryness under N_2 and the residue was applied to TLC plates (Merck silica gel 60 F_{254} type; Darmstadt, Germany) in approximately 40 μL of chloroform. The plates were developed in dichloromethane/acetone (4:1), air dried, and then developed in chloroform/ethyl acetate/ethanol (4:1:0.7) [9]. Radioactive metabolites were located by autoradiography (Hyperfilm-MP; Amersham) over approximately 60 hr and quantified using scintillation counting (in ACS II; Amersham).

Estradiol (50 μM; 0.15 μCi/0.4 mL incubation) hydroxylation reactions were conducted in microsomes in a similar manner to that described for testosterone hydroxylation, except that the incubation was of 10-min duration. After similar application of the products of the reactions to TLC plates, development was performed in the solvent system cyclohexane/ethyl acetate/ethanol (10:9:1) [10]. The [^{14}C] estradiol used as the microsomal substrate in these experiments was purified before use by TLC in the same system.

All steroid hydroxylation measurements were conducted in duplicate. IC_{50}s were derived from semilogarithmic plots of *percentage activity remaining versus* log_{10} *inhibitor concentration*.

Analysis of the Interactions of DES and Its Metabolites with Microsomal P450 by Optical Difference Spectroscopy

For determination of the binding of DES analogues to ferric P450, microsomal protein (2 mg/mL) was divided between two cuvettes and a baseline of equal light absorbance was established in a Varian Cary 2300 spectrophotometer at 37°C. DES or its metabolites (200 μM) were added to the sample cuvette in microliter volumes of aqueous dimethylformamide and solvent was added to the reference cuvette. After each addition, the optical difference spectrum was scanned between the wavelengths of 500 and 380 nm. The difference in absorbance (ΔA) between the maximum and minimum of the binding interaction was calculated. Double-reciprocal plots of DES/metabolite concentration versus ΔA were constructed, from which the abscissal and ordinal intercepts enabled the calculation of K_s and ΔA_{max}, respectively.

The formation of MI complexes between DES analogues and P450 in rat hepatic microsomes was monitored by difference spectroscopy as described previously [5]. DES or one of its metabolites (200 μM) was added to the sample cuvette (2 mg microsomal protein/mL) and NADPH (1 mM final) was added to both cuvettes. MI complexation was indicated by the time-dependent formation of a 452 nm absorbance maximum in the optical difference spectrum; the reaction was conducted for a period of at least 10 min. After 30 min, P450 content was calculated according to the method of Omura and Sato [11]. In some experiments, potassium ferricyanide (50 μM final) was used to restore the portion of P450 that had been sequestered as an MI complex [7]. This confirmed that MI complexation and not autocatalytic destruction was the mode of mechanism-based inhibition of P450 by DES.

Assay of DES Oxidation in Rat Liver Microsomes

Microsomes (1 mg/mL; 0.2 mL) were incubated at 37°C with DES (200 μM) and NADPH (1 mM final) for 60 min, over which time the rates of metabolite formation were linear. The reactions were stopped by addition of 1 mL 1M Na_2CO_3 and removal to ice. The internal standard (imipramine, 4 μM final) and 5 mL of ethyl acetate were added to the reaction vials. After mixing and centrifugation, the organic layers were evaporated under N_2, reconstituted in 100 μL of mobile phase, and applied to an Ultrasphere-Si HPLC column (5 μm, 4.6 mm i.d. × 25 cm; Beckman, San Ramon, CA, U.S.A.) [12]. The mobile phase consisted of methanol-acetonitrile (1:5) containing 0.4% concentrated ammonia and the flow rate was 1.8 mL/min; the detection wavelength was 240 nm. Standard curves were constructed for each metabolite by parallel extraction of each of the authentic compounds from microsomes

(range 0–4 nmol/tube). Reactions were conducted in duplicate using three different microsomal samples.

Statistics

Differences between means from two or more than two treatment groups were detected using the Student's *t*-test or one-way analysis of variance in conjunction with the Student-Newman-Keuls test, respectively.

RESULTS

Effects of DES and DES Metabolites on Microsomal P450 Steroid Hydroxylases

The capacity of DES and three putative metabolites to inhibit the P450-mediated positional hydroxylation of steroid substrates in rat hepatic microsomes was investigated. In the absence of a preincubation step DES, as well as its 2- and 10-hydroxy metabolites, were relatively ineffective inhibitors of the four major pathways of testosterone hydroxylation (less than 50% inhibition of all pathways of hydroxylation was observed at DES/metabolite concentrations of 100 μM; Table 1). Consistent with previous findings, after a 20-min preincubation step to generate metabolites involved in MI complexation, the potency of the parent drug was markedly increased. Thus, after the preincubation step, DES strongly inhibited P450 2C11-mediated 2α- and 16α-hydroxylation (IC_{50}s of 3.5 and 1.7 μM, respectively) and, to a lesser degree, 2A1-mediated 7α-hydroxylation (IC_{50} of 25 μM) and 3A2-mediated 6β-hydroxylation (IC_{50} of 92 μM). Of considerable interest were the findings that similar preincubation steps enhanced the potency of 10-hydroxy-DES against activities catalyzed by 2C11 and 2A1, whereas metabolism of 2-hydroxy-DES increased the extent of inhibition of reactions mediated by 3A2 and 2C11. Indeed, after preincubation, IC_{50}s of 22 and 18 μM were determined for 10-hydroxy-DES against testosterone 2α- and 16α-hydroxylation and an IC_{50} of 38 μM against steroid 7α-hydroxylation. In contrast, after preincubation of 2-hydroxy-DES with NADPH-supplemented microsomes, IC_{50}s of 39 and 58 μM were observed against 6β- and 16α-hydroxylation, respectively (Table 1).

In the absence of preincubation, DIDES inhibited testosterone hydroxylation pathways, but IC_{50} values could not be determined because higher concentrations of the compound (>50 μM) led to the appearance of unidentified radioactive products that did not comigrate with authentic hydroxysteroid standards. Similar effects occurred with androst-4-ene-3,17-dione and progesterone, which are also steroid substrates for P450 enzymes [9, 13]. It was considered that the unidentified compounds may be products of reactions between the ketosteroid substrate and the primary amine DIDES. The problem was overcome by employing estradiol, a steroid that does not possess a keto functionality, as the substrate in place of testosterone. Thus, IC_{50}s of 35 and 29 μM were obtained for DIDES against 3A2-mediated estradiol 6β-hydroxylation and 2C11-mediated 16α-hydroxylation of estradiol to estriol, respectively. Inhibition of 2-hydroxylation was less extensive than for the other two pathways. Slight decreases in the extent of inhibition were noted after a preincubation step (Table 1).

Spectrophotometric Studies of MI Complexation of Rat Liver Microsomal P450 by DES and Its Metabolites

Incubation of DES with microsomes and NADPH for 30 min resulted in a concentration-related decrease of apparent P450 (maximal decrease of about 40%; $P < 0.01$; Fig. 1A). Despite the enhanced inhibition of P450 reactions after preincubation, neither 2-hydroxy-DES nor 10-hydroxy-DES formed MI complexes with microsomal P450. This was clear from two complementary experimental approaches. In the first approach, P450 content was measured in incubations containing the putative metabolite(s), NADPH and microsomes, and was found not to be decreased (Fig. 1A). Second, the optical difference spectra of the metabolites in microsomes did not un-

TABLE 1. *In vitro* inhibition of steroid hydroxylation in control male rat liver microsomes by DES and its metabolites

Drug/metabolite	Preincubation*	Hydroxytestosterone metabolite IC_{50} (μM)			
		2α-	6β-	7α-	16α-
Desipramine	No	>100 (6)†	>100 (42)	>100 (39)	>100 (31)
	Yes	3.5	92	25	1.7
10-OH-desipramine	No	>100 (19)	>100 (18)	>100 (0)	>100 (23)
	Yes	22	>100 (43)	38	18
2-OH-desipramine	No	>100 (25)	>100 (36)	>100 (49)	>100 (28)
	Yes	>100 (49)	39	>100 (49)	58
		Hydroxyestradiol metabolite IC_{50} (μM)			
		2	6β-	16α-	
N,*N*-didesmethylimipramine	No	>100 (31)	35	29	
	Yes	>100 (40)	>100 (45)	46	

* Preincubation step between DES or DES metabolite in NADPH-supplemented microsomes was of 20-min duration. Control activities were: testosterone 2α-, 6β-, 7α-, and 16α-hydroxylation (2.02 ± 0.21, 2.01 ± 0.07, 0.38 ± 0.03, and 2.58 ± 0.24 nmol/min/mg protein, respectively) and estradiol 2-, 6β-, and 16α-hydroxylation (0.033 ± 0.006, 0.183 ± 0.013, and 0.291 ± 0.045 nmol/min/mg protein, respectively).

† Signifies that the IC_{50} was greater than 100 μM. Values in parentheses indicate percent inhibition observed at 100 μM.

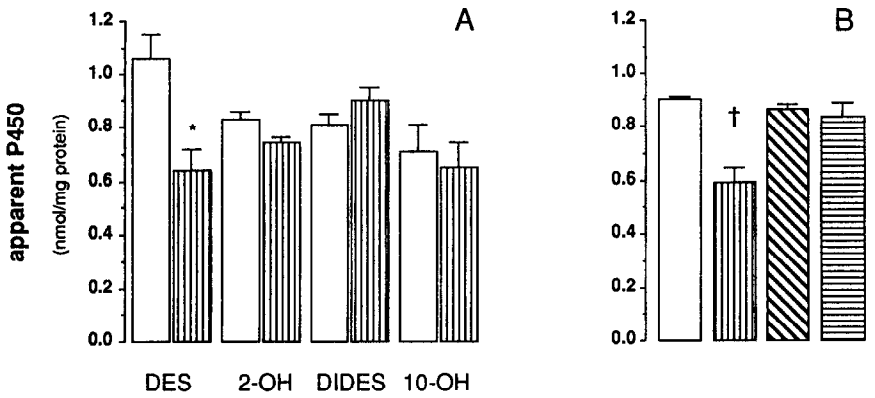

FIG. 1. (A) Changes in apparent microsomal cytochrome P450 content produced by DES and its metabolites (2-OH, 2-hydroxy-DES; DIDES, *N,N*-didesmethylimipramine and 10-OH, 10-hydroxy-DES). The four compounds (200 μM) were incubated with NADPH (1 mM) and microsomes (2 mg protein/mL) for 30 min and then P450 was determined as described in Materials and Methods. *Open bars* indicate P450 levels in control incubations (containing no drug); *vertical lines* indicate P450 levels after incubation with DES or its metabolites. (B) Restoration of P450 that had been sequestered in an MI complex with a DES metabolite by oxidation with potassium ferricyanide. *Open bar:* incubation (30 min) in absence of drug; *vertical lines:* incubation in presence of 200 μM DES; *diagonals:* incubation in absence of drug, followed by addition of potassium ferricyanide (50 μM); and *horizontal lines:* incubation in presence of 200 μM DES, followed by addition of potassium ferricyanide (50 μM). Different from control: *$P < 0.01$, †$P < 0.05$.

dergo time-dependent changes after NADPH addition. The dissociability of the MI complex formed from DES was confirmed by oxidation of the microsomes with potassium ferricyanide and the consequent restoration of total P450 to control levels (Fig. 1B).

Because the DES metabolites did not elicit MI complexation of P450, the capacity of the analogues to interact with oxidized P450 was investigated. DES and its 2- and 10-hydroxy metabolites produced type I difference spectra of quite high affinity (K_s 0.64–3.9 μM) but low capacity (ΔA_{max} 8.9–11.0 × 10^{-3} absorbance units/nmol P450; Table 2). In contrast, the primary amine DIDES interacted with the P450 in type II fashion; the spectrum was stable after reduction by sodium dithionite. This binding interaction was of somewhat lower affinity (K_s 31 μM) than those elicited by the other ligands, but a substantial proportion (~44%) of the P450 appeared to be involved in the interaction (by application of the literature extinction coefficient of 130 $mM^{-1}cm^{-1}$ [14] to the ΔA_{max} value of 57.1 × 10^{-3} absorbance units/nmol P450; Table 2).

Assessment of Metabolite Formation in Rat Liver Microsomes

The microsomal biotransformation of DES was assessed in rat hepatic microsomes in relation to the potential significance of the inhibition studies. Thus, an HPLC separation of DES and its putative metabolites was achieved [12]. Retention times in this system were: imipramine (internal standard 8.0 min), DIDES (12.2 min), 10-hydroxy-DES (14.7 min), DES (26.0 min), and 2-hydroxy-DES (32.4 min). At a DES concentration of 200 μM, the metabolites detected were 2-hydroxy-DES (6.45 ± 0.38 nmol/mg/60 min) and DIDES (1.32 ± 0.07 nmol/mg/60 min); 10-hydroxy-DES was not detected after 60 minutes of metabolism (Table 3). Thus, total formation of isolable metabolites was 7.77 ± 0.48 nmol/mg protein/hr (N = 3 separate microsomal fractions).

TABLE 2. Parameters of the spectral binding interactions of DES and its metabolites with ferric P450 in male rat hepatic microsomes

Drug/metabolite	Binding spectrum	K_s (μM)	ΔA_{max} (absorbance units/nmol P450) × 10^3
DES	type I	1.4 ± 0.1	8.9 ± 2.0
10-hydroxy-DES	type I	3.9 ± 0.2	11.0 ± 1.0
2-hydroxy-DES	type I	0.64 ± 0.01	9.8 ± 1.3
DIDES	type II	31 ± 2	57.1 ± 0.5

Values were derived from experiments conducted in three separate microsomal fractions.

DISCUSSION

A number of studies with alkylamine substituted drugs have demonstrated their capacity to inhibit P450 function as a result of MI complexation. Thus, complexes are formed between P450 and metabolites of macrolide antibiotics, such as erythromycin and troleandomycin [15], and the antiparkinsonian agent orphenadrine [16]. It has been suggested that MI complexation may be the mechanism responsible for the documented drug interactions between erythromycin and warfarin [17], carbamazepine [18], and alfentanil [19]. Previous work has established that MI complexes are also generated during

TABLE 3. Microsomal metabolism of DES in male rat liver *in-vitro*

Metabolite	Amount formed (nmol/mg protein/hour)
2-hydroxy-DES	6.45 ± 0.38
10-hydroxy-DES	ND*
DIDES	1.32 ± 0.07

* ND, not detected. Data are derived from experiments conducted in three separate microsomal fractions. The substrate (DES) concentration was 200 μM and the protein concentration was 1 mg/mL.

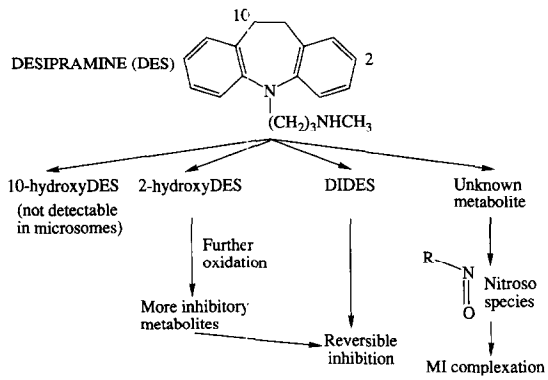

FIG. 2. Scheme showing the relationship between the enzymic conversion of DES in rat hepatic microsomes to a P450-MI complex and other metabolites.

the oxidative metabolism of tricyclic antidepressants containing *N*-monomethyl substituents [4]. Thus, DES and nortriptyline were more potent inhibitors of P450 reactions after preincubation with NADPH and microsomes. This is consistent with the notion that the *N*-alkylamine substituent must undergo biotransformation to a reactive metabolite, probably the nitroso species, and that this forms a quasi-covalent ligand with the heme iron of P450 [20].

Tricyclic antidepressants are known to produce several metabolites. For example, apart from the metabolite involved in MI complexation, DES is also converted in P450 reactions in human hepatic microsomes to the 2- and 10-hydroxy metabolites and the *N*-demethylated product, DIDES [6, 21]. It has not been considered previously that such metabolites may contribute significantly to P450 inhibition by tricyclic antidepressants, but an earlier study reported that removal of free metabolites produced during the microsomal oxidation of nortriptyline decreased the observed extent of inhibition of P450 3A2 [5]. Thus, it appeared that metabolites other than those involved in MI complexation may exert significant inhibition of P450 reactions.

The principal finding to emerge from the present study is that DIDES, but not 2- or 10-hydroxy-DES, may contribute to P450 inhibition mediated by DES. Whereas DES was noninhibitory unless preincubated with NADPH-supplemented microsomes, the direct addition of DIDES to microsomal incubations led to effective inhibition of 2C11-mediated 16α-hydroxylation and 3A2-mediated 6β-hydroxylation of estradiol. Because DIDES effected inhibition in the absence of a preincubation step, the inhibitory mechanism does not appear to involve MI complexation. It also appears from this study that the 2- and 10-hydroxy-metabolites of DES were relatively weak inhibitors of P450 activities, but that the preincubation step in NADPH-supplemented microsomes enhanced their effectiveness somewhat. Thus, after the preincubation procedure, 2-hydroxy-DES was able to inhibit the P450 3A2 reaction (testosterone 6β-hydroxylation) with an IC_{50} of 39 μM. Biotransformation of the 10-hydroxy-metabolite appeared to enhance its inhibitory capacity against steroid hydroxylations mediated by P450s 2C11 and 2A1 (2α-/16α- and 7α-hydroxylations, respectively). Because DIDES and the 2-hydroxy-metabolite of DES, but not 10-hydroxy-DES, were detected in rat microsomal incubations in the present study, some of the inhibitory properties of these DES metabolites and their further oxidation products may have potential significance.

The data derived from the experiments using UV/visible spectroscopy confirmed that the incubation of DES with NADPH-supplemented microsomes led to a significant decrease in spectrally apparent P450 (~40% P450 loss). An absorbance maximum at 452 nm was observed in the optical difference spectrum produced in NADPH-fortified microsomes by DES. In contrast, the three major metabolites of DES had little effect on P450 levels and typical absorbance maxima associated with alkylamine MI complexes, such as that seen with DES, were not observed after incubation of the DES metabolites with NADPH and microsomes.

It has been established that the inhibition of P450 enzyme activity can occur as a result of binding to oxidized P450 (that is, the native or "resting" state) [22, 23]. This process modifies the first step of the P450 cycle involving substrate binding to the enzyme and in which there is a shift in the spin equilibrium leading to reduction of the substrate-coordinated cytochrome by an electron from NADPH. Substrate or inhibitor binding to P450 can be detected from perturbations in the optical difference spectrum of microsomes [24, 25]. In this study, the capacity of DES and its metabolites to interact with microsomal P450 was analyzed spectrophotometrically. Such experiments revealed that DIDES was a type II ligand (which reflects a shift in the high-to-low spin equilibrium of ferric P450), whereas DES and its 2- and 10-hydroxy metabolites generated type I spectra (generally associated with a spin state shift from the low-to-high spin configuration). Although DIDES interacted with P450 with slightly lower affinity than the other two metabolites, the interaction involved a substantial proportion of the total P450 (as reflected by the relatively large ΔA_{max}/nmol P450 value). Because type II ligands are generally nitrogenous bases that can occupy the sixth axial ligand position in ferric P450 efficiently and impede reduction of the cytochrome, they are often found to be quite potent inhibitors [26]. These properties of DIDES are probably responsible for the capacity of this metabolite to inhibit P450 reactions directly.

The present findings eliminate the two principal metabolites formed from DES in microsomal incubations, and their further biotransformation products, from involvement in MI complexation of P450. This is consistent with previous suggestions that the MI complex occurs as a result of oxidation at the alkylamine nitrogen [20]. This suggestion has arisen from work with model hydroxylamines (R-NHOH) that are oxidized in NADPH-supplemented microsomes and generate MI complexes rapidly [20, 26]. Rates of complex formation are more rapid than those observed with the parent alkylamines, which suggests that hydroxylamines may be more proximate to the complex-forming species. A recent report confirmed that this is also the case with the hydroxylamine derivative of DES [27].

In summary, the present findings suggest that an as yet unidentified metabolite(s) of DES, perhaps the nitroso deriv-

ative, is responsible for the major inhibitory effect that the drug has on P450 after its own metabolism. The scheme outlined in Fig. 2 indicates that MI complexation is a separate metabolic route from those leading to formation of the isolable metabolites. It has emerged from this work that DES metabolites, such as DIDES and the products from the further enzymic oxidation of 2-hydroxy-DES, may produce significant inhibition of P450 activity. Thus, it is conceivable that, in some circumstances, DIDES may contribute to pharmacokinetic problems produced by the drug.

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