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ORIGINAL ARTICLE

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N,N-diethyl-2-[4-(phenylmethyl)phenoxy] ethanamine (DPPE), a chemopotentiating and cytoprotective agent in clinical trials: interaction with histamine at cytochrome P450 3A4 and other isozymes that metabolize antineoplastic drugs

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Abstract *Purpose*: *N*,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine · HCl (DPPE), an intracellular histamine (HA) antagonist with chemopotentiating and cytoprotective properties, is currently in phase 2 and 3 clinical trials in breast and prostate cancer. DPPE modulates growth at in vitro concentrations that antagonize HA binding to cytochromes P450 in rat liver microsomes. HA inhibits P450 metabolism of some drugs. Recent in vitro studies in human colon cancer cells have linked DPPE enhancement of paclitaxel, doxorubicin and vinblastine cytotoxicity to inhibition of the P-glycoprotein (P-gp) pump. Many substrates of P-gp are also substrates of CYP3A4, a P450 isozyme that metabolizes a variety of antineoplastic agents and is highly expressed in some malignant tissues. Therefore, we assessed whether (a) DPPE and HA interact at CYP3A4 and other P450 human isozymes, and (b) DPPE inhibits the catalytic activity of CYP3A4. *Methods*: Using spectral analysis, we measured DPPE and HA binding to insect microsomes that express human P450 isozymes 1A1, 2B6, 2D6 or 3A4. Employing thin-layer chromatography, we assessed the metabolism of DPPE by each isozyme and DPPE inhibition of testosterone metabolism by CYP3A4 and by rat liver microsomes. *Results*: (1) DPPE evoked "type I" (substrate site binding) absorbance-difference spectra with CYP2D6 ($K_s = 4.1 \pm 0.4$ μM), CYP3A4 ($K_s = 31 \pm 15$ μM) and CYP1A1 ($K_s = 40 \pm 9$ μM), but not with CYP2B6. (2) In correspondence with the binding studies, DPPE was metabolized by CYP2D6, CYP3A4 and CYP1A1; no metabolism occurred with CYP2B6. (3) HA evoked "type II" (heme iron binding) absorbance-difference spectra with all four isozymes, with K_s values in the range 80–600 μM. DPPE inhibited HA (600 μM) binding to CYP2D6 ($IC_{50} = 4$ μM, 95% CI = 1.8–8.9 μM) and CYP1A1 ($IC_{50} = 135$ μM: 95% CI = 100–177 μM), but stimulated HA (500 and 1000 μM) binding to CYP3A4 ($EC_{50} = 155$ μM, 95% CI = 104–231 μM). DPPE did not affect HA binding to CYP2B6. (4) DPPE inhibited the metabolism of testosterone by CYP3A4. The concentration/effect curve was biphasic: DPPE inhibited metabolism by 30% at the first site ($IC_{50} = 3$ μM, 95% CI = 0.5–25.5 μM), and an additional 70% inhibition occurred at the second site ($IC_{50} = 350$ μM, 95% CI = 215–570 μM). A similar result was observed with rat liver microsomes. *Conclusion*: DPPE is a substrate for CYP3A4, CYP2D6 and CYP1A1, but not CYP2B6. DPPE inhibits testosterone metabolism by interacting at two sites on CYP3A4, the first correlating with its K_s value to bind the substrate site and the second, with its EC_{50} value to enhance HA binding to the heme iron. We postulate that (1) the inhibitory effect of DPPE on CYP3A4 activity is mediated directly at the substrate site and indirectly by its enhancement of the binding of HA to the heme moiety; (2) in tumor cells that express high constitutive levels of CYP3A4, potentiation of chemotherapy cytotoxicity by DPPE results, in part, from inhibition of CYP3A4-mediated metabolism and P-gp-mediated efflux of antineoplastic drugs; (3) in normal cells that express low constitutive levels of the isozyme, cytoprotection by DPPE results, in part, from induction of CYP3A4 and P-gp, resulting in an increase both in metabolism and efflux of antineoplastic drugs.

Key words Chemopotentiation · Cytoprotection · P450 · CYP3A4 · Intracellular histamine · DPPE · Testosterone metabolism

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Introduction

N,*N*-diethyl-2-[4-(phenylmethyl)phenoxy]ethanamine · HCl (DPPE) is an arylalkylamine derivative [3] with structural similarity to various H_1-antihistamines, neuroleptics and triphenylethylene antiestrogens [5]. DPPE is a potent ligand for so-called antiestrogen (tamoxifen) binding sites (AEBS) [3], antagonizes the binding of histamine (HA) to AEBS-related sites in microsomes and nuclei [7], and modulates cell proliferation in vitro [4] and in vivo [6]. A major proportion of the microsomal sites with affinity for DPPE and HA represents cytochrome P450 monooxygenases [11]. Nebert [26] has proposed that, through their metabolism of endogenous lipid mediators such as steroid hormones and prostanoids, P450 enzymes regulate cell homeostasis and growth. A similar role for HA was postulated by Kahlson and Rosengren [17] 30 years ago. In support of their hypothesis, we have observed that HA binds directly to the heme iron of P450 [20], and have found a strong correlation among drug potencies to inhibit HA binding to P450 in rat liver microsomes, P450-mediated demethylation of aminopyrine and mitogenesis, and to modulate experimental tumor growth in rodents [19]. We have proposed that HA is a modulator of the catalytic activity of P450 enzymes and that perturbation of the HA/P450 complex by DPPE and other AEBS ligands may disrupt steady-state levels of monooxygenase-generated mediators of proliferation [11, 19].

In rodents, DPPE protects the bone marrow from toxic doses of doxorubicin and 5-fluorouracil [8] and the gut from noxious physical and chemical stimuli [13]. In contrast, DPPE potentiates cytotoxicity in cancer cells exposed to various chemotherapeutic agents, including doxorubicin, resulting in an increased cure rate of experimental tumors [8]. In phase 2 trials in humans with various refractory malignancies [9], including prostate and breast cancer [2, 10], increased response rates, usually with low systemic toxicity, have been observed when DPPE is combined with single antineoplastic drugs such as cyclophosphamide [10] or doxorubicin [2].

Recent studies have yielded potentially valuable new insights into possible mechanism(s) of action of DPPE. In the multidrug-resistant human colon cancer cell line, HCT 116/mdr1$^+$, DPPE potentiates the cytotoxicity of doxorubicin, paclitaxel and vinblastine, inhibits the P-glycoprotein (P-gp) pump (MDR1), decreases mitochondrial membrane potential and depletes cellular ATP [22]. Most substrates and/or inhibitors of P-gp are also substrates and/or inhibitors of CYP3A4 [29], a P450 isozyme that accounts for between 30% and 70% of the total P450 content in liver microsomes, and metabolizes many hormones and drugs [15], including various antihistamines, tamoxifen and antineoplastic agents, such as taxanes, vinca alkaloids, epipodophyllotoxins, cyclophosphamide, ifosfamide, and some anthracycline analogs [29]. Moreover, levels of P-gp and CYP3A4 are coordinately up- or downregulated by the same chemical agents in LS180/AD50 human colon cancer cells [27]. Based on these reports, we carried out studies to assess (a) the interaction of DPPE and HA with CYP3A4 and other P450 isozymes that metabolize a wide range of drugs, including antineoplastic agents and (b) the inhibition of the catalytic activity of CYP3A4 by DPPE.

Materials and methods

Reagents

DPPE was synthesized, as described previously [3]. ^{3}H-DPPE (35.6 Ci/mmol) was custom-synthesized by Dupont-New England Nuclear (Boston, Mass.). ^{3}H-Testosterone (55 Ci/mmol) was purchased from Dupont-NEN. Testosterone propionate and histamine dihydrochloride were purchased from Sigma Chemical Co. (Oakville, Ont.).

Purified P450 isozymes

The following insect (Baculovirus-infected drosophila) microsomes that express cDNA for a single human P450 isozyme were obtained from Gentest Corporation (Woburn, Mass.): CYP1A1 (metabolizes many aryl hydrocarbons and procarcinogens), CYP2B6 + cytochrome b5 (metabolizes cyclophosphamide), CYP2D6 (metabolizes many arylalkylamines, including tamoxifen), and CYP3A4 + cytochrome b5 (metabolizes many arylalkylamines and antineoplastic agents).

Preparation of rat liver microsomes

Livers were removed from freshly killed male Sprague-Dawley rats (University of Manitoba Central Animal Facility), minced, suspended (10% wt/vol) in 0.25 *M* sucrose, homogenized with six to eight passes in a motor-driven Teflon-pestle glass vessel and centrifuged (13,000 *g*, 15 min, 4 °C). The supernatant was decanted and centrifuged (144,000 *g*, 95 min, 4 °C). The resulting pellet was resuspended in 5 m*M* TRIS buffer (pH 8.5) at a final concentration of 4 mg protein/ml, centrifuged (144,000 *g*, 35 min, 4 °C), resuspended in TRIS buffer (10–20 mg protein/ml) and stored at −80 °C until used.

Spectral analysis

The absorbance-difference spectrum of P450-bound DPPE or HA was measured with a Pharmacia Ultraspec 4000 spectrophotometer. A Swift II computer software program controlled and operated the unit and collected and plotted the spectral data. To obtain spectra, P450 isozyme (final P450 concentration about 150 p*M*/ml; protein concentration about 0.6 mg/ml) was suspended in 5 m*M* TRIS buffer (pH 8.5; final volume 0.1 ml) containing DPPE (30–100 μ*M*) or HA (30–1000 μ*M*) or both. Actual K_s values were determined by employing the LIGAND program [25] to analyze absorbance-difference spectra. IC_{50} values for inhibition by DPPE of HA binding to the isozymes were determined using PRISM (Version 2; GraphPad, San Diego, Calif.), a curve-fitting program employing weighted nonlinear regression analysis.

Measurement of DPPE metabolism

To measure metabolism of DPPE by the various P450 isozymes, 0.2 ml of a mixture containing a final concentration of 40 μ*M* DPPE, 20 n*M* ^{3}H-DPPE, 50 m*M* glucose-6-phosphate, 3 m*M* NADP, 2 U glucose-6-phosphate dehydrogenase and insect microsomes, adjusted to a P450 concentration of 0.1 μ*M*, in 50 m*M*

K_3PO_4 and 3.3 m*M* $MgCl_2$ buffers (pH 7.4) was added to replicate 1.5 ml Eppendorf tubes. Following incubation (4 h, 37 °C) the reaction was stopped by the addition of 1 ml EtOH and the tubes centrifuged (15,000 *g*, 10 min, 4 °C). A 1-ml aliquot of each supernatant was dried under a nitrogen stream, and the residues were dissolved in 0.1 ml EtOH and applied to 20 × 20 cm 250 μm hard-surfaced silica gel thin layer chromatography (TLC) plates. The plates were developed in a toluene/triethylamine (9:1) mixture, air-dried for 10 min and scanned with a Bioscan Systems 200 imaging scanner. The amount of each product was determined by measuring the radioactivity under the peak and expressed as percentage of the total radioactivity.

Measurement of testosterone metabolism

To assess the effect of DPPE on the CYP3A4-mediated conversion of testosterone to 6β-OH-testosterone, a 0.2-ml mixture containing DPPE (0–1000 μ*M*, final concentration), 100 μ*M* cold testosterone, 26 n*M* ^{3}H-testosterone (DuPont NEN), 50 m*M* glucose-6-phosphate, 3 m*M* NADP, 2 U glucose-6-phosphate dehydrogenase and 5 pmol purified isozymes or 20 μg rat liver microsome protein in 50 m*M* K_3PO_4 and 3.3 m*M* $MgCl_2$ (pH 7.4) was added to replicate 1.5 ml Eppendorf tubes. Following incubation (10 min, 37 °C) the reaction was stopped by the addition of 1 ml EtOH and the tubes centrifuged (15,000 *g*, 10 min, 4 °C). A 1-ml aliquot of each supernatant was dried under a nitrogen stream, the residues dissolved in 0.1 ml EtOH and applied to 20 × 20 cm 250 μm hard-surfaced silica gel TLC plates. The plates were developed in a dichloromethane/acetone (4:1) mixture, air-dried for 10 min, developed a second time in the same solvent, air-dried and scanned with a Bioscan Systems 200 imaging scanner. The amount of 6β-OH-testosterone was determined by calculating the radioactivity under the peak, and expressed relative to the total radioactivity. The IC_{50} values for inhibition by DPPE of testosterone metabolism were determined by PRISM (Version 2). The threshold *P*-value was set to 0.05 to determine significance for a two-site fit.

Results

The addition of DPPE to insect microsomes expressing CYP2D6, CYP3A4 or CYP1A1 produced a "type I" spectrum, with a peak at approximately 385 nm and a trough at approximately 420 nm (Fig. 1), typical of ligands that bind to the substrate site in the heme cavity. K_s values for DPPE binding to each isozyme (Table 1) were as follows: 4.1 ± 0.4 μ*M* (CYP2D6), 31 ± 15 μ*M* (CYP3A4), 40 ± 9 μ*M* (CYP1A1). DPPE did not generate a spectrum with CYP2B6 (data not shown).

In contrast to DPPE, addition of HA to rat liver microsomes, or to individual isozymes, produced a "type II" absorbance-difference spectrum (Fig. 2) with a trough at approximately 390–410 nm and a peak at approximately 425–435 nm, typical of compounds, including imidazoles, that bind to the heme moiety of the enzyme [19]. With equivalent concentrations (P450/mg protein) of CYP3A4, CYP1A1, CYP2B6 or CYP2D6, a saturating concentration of HA (1000 μ*M*) produced absorbance-difference spectra with peak-to-trough distances (DA) ranging between 0.0039 and 0.0052 Absorbance Units (Fig. 2). Actual K_s values for HA binding to each isozyme (Table 1) were as follows: 80 ± 16 μ*M* (CYP1A1), 114 ± 29 μ*M* (CYP3A4), 356 ± 76 μ*M* (CYP2D6), and 600 ± 200 μ*M* (CYP2B6).

DPPE antagonized HA binding to CYP2D6 (IC_{50} = 4 μ*M*, 95% CI = 1.8–8.9 μ*M*) and CYP1A1 (IC_{50} = 137 μ*M*, 95% CI = 100–187 μ*M*; Fig. 3). In contrast, DPPE enhanced HA binding to CYP3A4 (EC_{50} = 155 μ*M*, 95% CI = 104–231 μ*M*). This stimulatory effect was maximal at a DPPE concentration of 600 μ*M* and then diminished at higher concentrations (Fig. 3). DPPE did not affect HA binding to CYP2B6 (data not shown).

Correlating with the spectral data for DPPE affinities for the isozymes (Table 1), incubation of DPPE with CYP2D6, CYP3A4 and CYP1A1 led to the formation of at least five products (Fig. 4); CYP2B6 was inactive except for the production of a small amount of product 4 (Fig. 4). Based on the radioactivity in the product peaks, CYP2D6 was most avid to metabolize DPPE, followed by CYP3A4 and CYP1A1, which were approximately equal to one another in potency. Component 1, also present at the origin when control microsomes were used (Fig. 4), had been previously determined to contain 4-hydroxydiphenylmethane, a starting product of DPPE synthesis. The observed increase in this component in the presence of the isozymes may represent oxidation products that, like the impurity,

Fig. 1 Absorbance-difference spectra produced by the addition of DPPE (20–50 μ*M*) to insect microsomes expressing various human isozymes (100–150 pmol P450/ml). DPPE evoked a "type I" spectrum with (a) CYP2D6, (b) CYP3A4 and (c) CYP1A1, but not with CYP2B6. The peak at approximately 385 nm and trough at approximately 420 nm is typical for ligands that bind to the substrate site on the enzyme

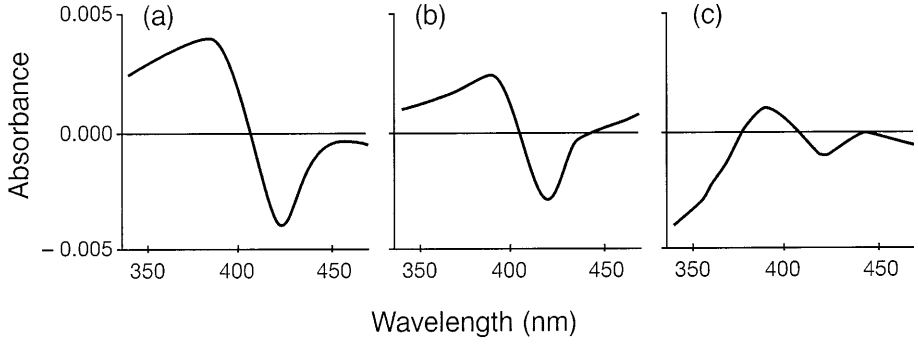

Table 1 Interactive binding of DPPE and HA to expressed human P450 isozymes (IC_{50} concentration of DPPE inhibiting HA binding by 50%, EC_{50} effective concentration of DPPE increasing HA binding by 50%, *ND* no Δ absorbance detected)

Isozyme	K_s (DPPE) (μ*M*)		K_s (HA) (μ*M*)		IC_{50}/EC^*_{50} (μ*M*)	
	Mean	SD	Mean	SD	Mean	95% CI
CYP2D6	4.1	0.4	356	76	4	1.8–8.9
CYP3A4	31	15	114	29	55	104–231
CYP1A1	40	9	80	16	137*	100–187
CYP2B6	ND		600	200	ND	

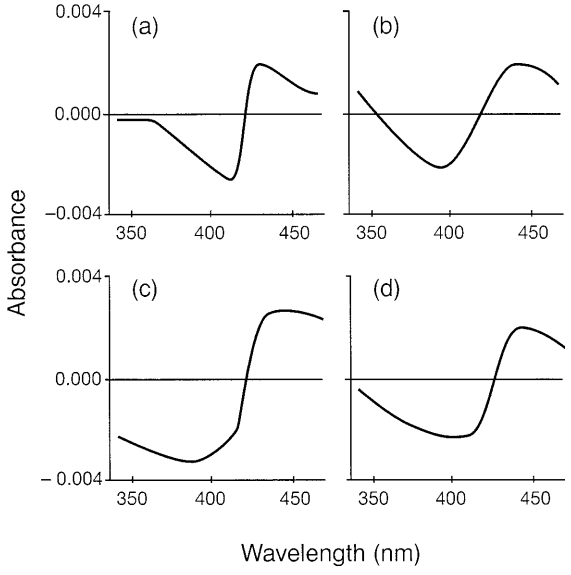

Fig. 2 Absorbance-difference spectra produced by the addition of HA (1000 μ*M*) to insect microsomes (128 pmol P450/ml) expressing (a) CYP3A4, (b) CYP2D6, (c) CYP1A1 or (d) CYP2B6. HA produced a "type II" spectrum with each isozyme, with a trough at approximately 400 nm and a peak at approximately 430 nm, typical for imidazole compounds that bind to the heme iron

remained at the origin. The two largest radioactive peaks (4 and 5) were believed to represent the major metabolites, 4-hydroxy-DPPE and *N*-desmethyl-DPPE.

The conversion of testosterone to 6β-OH-testosterone by CYP3A4 was inhibited by DPPE (Fig. 5). The concentration/effect curve was biphasic ($IC_{50} = 3$ μ*M*, 95% CI = 0.5–25.5 μ*M*, and $IC_{50} = 350$ μ*M*, 95% CI = 215–570 μ*M*), suggesting that DPPE acted, at least, at two sites. In rat liver microsomes, where testosterone was metabolized into five general products (Fig. 6), DPPE inhibited the formation of metabolites 1–4 with equal potency. As with purified CYP3A4, the concentration/effect curve for DPPE was biphasic ($IC_{50} = 23$ μ*M*, 95% CI = 5.3–103 μ*M*, and $IC_{50} = 385$ μ*M*, 95% CI = 127–1160 μ*M*). DPPE inhibited the formation of metabolite 5 much more weakly ($IC_{50} = 900$ μ*M*, 95% CI = 640–1300 μ*M*).

Discussion

The parameters of HA binding to the four human P450 isozymes (K_s range: 80–600 μ*M*) are consistent with our previous observations in rat liver microsomes that HA binds to a composite of P450 enzymes ($K_s = 90 \pm 17$ μ*M*) [11]. Based on the data presented here, the potency of HA to bind rat microsomal P450 [11] may largely reflect its K_s values for binding to CYP2D6, CYP3A2 (the rat ortholog of rat CYP3A4) and CYP1A1 (Table 1), the three most abundant rat liver P450 isozymes. Our data suggest, also, that microsomal "AEBS" represents the substrate site, located mainly on CYP2D6, CYP3A4, and CYP1A1 that recognizes DPPE, tamoxifen and other arylalkylamine drugs that interact with HA at rat microsomal P450 [19].

The excellent correlation between the potency of DPPE to bind the substrate site on CYP2D6 and potency to inhibit HA binding suggest that DPPE

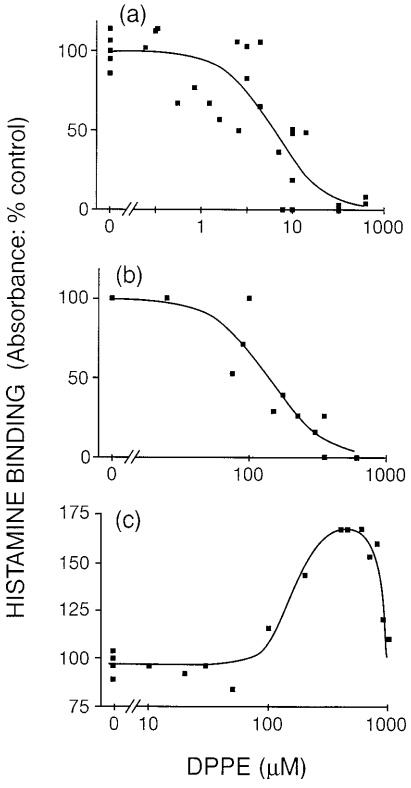

Fig. 3 Effect of DPPE on the absorbance-difference spectrum produced by the addition of 500–1000 μ*M* HA to insect microsomes (about 125 pmol P450/ml) expressing (a) CYP2D6, (b) CYP1A1 or (c) CYP3A4. DPPE inhibited HA binding to CYP2D6 ($n = 4$) and CYP1A1 ($n = 3$), and enhanced HA binding to CYP3A4 ($n = 2$). DPPE did not affect HA binding to CYP2B6 ($n = 3$; data not shown)

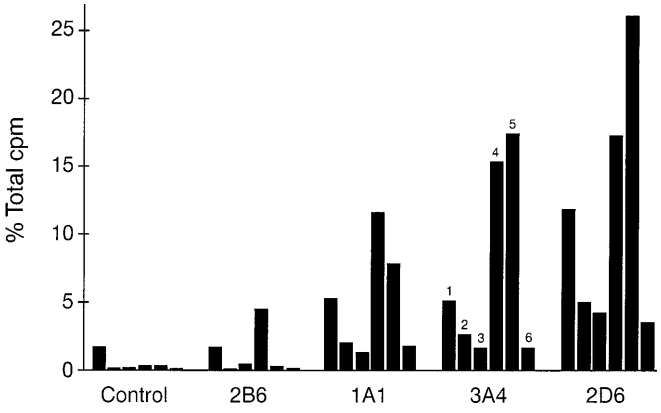

Fig. 4 Metabolism of DPPE (40 μg) after 4 h incubation (37 °C) by control (no P450 present) and CYP2B6-, CYP1A1-, CYP3A4- or CYP2D6-expressing insect microsomes (100 pmol P450/ml) (*bars*, representing product peaks, are labelled on CYP3A4; the DPPE peak is not shown). Total radioactivity in each isozyme experiment averaged 3000 ± 400 c.p.m. Radioactivity in the parent DPPE lanes were as follows: CYP2D6 520 c.p.m. (20% of total radioactivity); CYP3A4 1370 c.p.m. (50% of total radioactivity); CYP1A1 1990 c.p.m. (90% of total radioactivity); CYP 2B6 3100 c.p.m. (91% of total radioactivity). Control microsomes do not show any metabolite-containing peaks; the *bar* at the origin corresponding to peak 1 represents a previously identified impurity (4-hydroxydiphenylmethane, a starting product of DPPE synthesis). Microsomes expressing CYP1A1, CYP3A4, or CYP2D6 show five radioactive peaks (*bars* 2–6) representing DPPE derivatives. The increase in product 1, as compared to control microsomes, may have resulted from the production of oxidation products that, like 4-hydroxydiphenylmethane, remained at the origin. The number and location of products were identical for CYP3A4 and CYP2D6; however, CYP2D6 produced higher radioactive peaks than CYP3A4. Products 4 and 5 are thought to represent the major metabolites, 4-hydroxy-DPPE and *N*-desmethyl-DPPE. Product 4 was higher relative to product 5 in microsomes expressing CYP1A1, while product 5 was higher relative to product 4 in microsomes expressing CYP3A4 or CYP2D6. Microsomes expressing CYP2B6 show a minor increase in product 4, the only observed change as compared to control microsomes

allosterically inhibited HA binding to the heme iron. A weaker correlation was observed at CYP1A1. Moreover, despite binding with approximately equal potency ($K_s = 31$ and 40 μ*M*, respectively) to the substrate sites on CYP3A4 and CYP1A1, DPPE inhibited HA binding to CYP1A1 ($IC_{50} = 137$ μ*M*) but enhanced HA binding to CYP3A4 ($EC_{50} = 155$ μ*M*). These findings suggest differences in amino acid sequence among isozymes that could alter the proximity of the substrate site to the HA site, resulting in the observed differences in interactive binding potencies and determining whether DPPE antagonizes or enhances HA binding.

The concentration/effect curve for DPPE to inhibit testosterone metabolism by CYP3A4 (Fig. 5) suggests two sites of action. At the first site ($IC_{50} = 3$ μ*M*, 95% CI = 0.5–25.5 μ*M*), 30% inhibition occurs within the range of K_s values (31 ± 15 μ*M*) for DPPE to bind the substrate site. The serum concentration in patients receiving DPPE by infusion has been measured at 3–5 μ*M* [9], suggesting that substrate site inhibition of CYP3A4 also occurs at achievable concentrations of DPPE

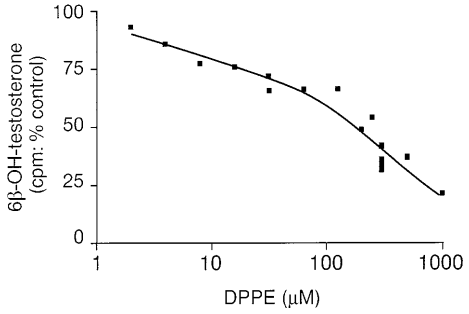

Fig. 5 Inhibition by DPPE of the conversion of testosterone to 6β-hydroxy-testosterone by insect microsomes expressing CYP3A4. Control radioactivity = 1700 c.p.m. Analysis of the concentration/effect curve showed that DPPE inhibited the enzyme at 2 sites ($n = 2$; $P < 0.05$)

in vivo. At the second site ($IC_{50} = 350$ μ*M*, 95% CI = 215–570 μ*M*), an additional 70% inhibition occurs within the range of IC_{50} values (104–231 μ*M*) for DPPE to enhance HA binding to the heme iron moiety of the isozyme. HA inhibits P450-mediated 9-hydroxylation and *O*-demethylation of metoprolol ($IC_{50} = 160$ and 240 μ*M*, respectively) [24]. Thus, DPPE may inhibit CYP3A4-mediated metabolism of testosterone, in part, by competing for the hormone at the substrate site and, in part, by increasing HA binding to the heme iron.

CYP3A4 expression reportedly is increased in many human tumors [29]. Thus, the demonstration that DPPE is a substrate for, increases HA binding to, and inhibits the catalytic activity of, CYP3A4 may explain, in large part, its observed potentiation of antineoplastic drugs that are metabolized, also, by CYP3A4. That DPPE is also an inhibitor/substrate of the P-gp pump [22] is in accordance with the reported overlap of drug affinities for both CYP3A4 and P-gp [29]. In addition, the reported depletion of cellular ATP by DPPE [22] suggests another mechanism underlying inhibition of the P-gp pump, which is ATP-driven.

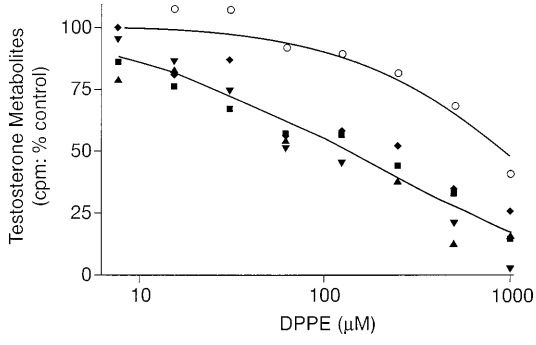

Fig. 6 Inhibition by DPPE of the metabolism of testosterone by rat liver microsomes (containing a full complement of P450 isozymes) (control radioactivity 4100 c.p.m.). DPPE inhibited the formation of peaks 1–4 with equal potency. Analysis of the concentration/effect curve showed that this inhibition occurred at two sites ($P < 0.05$). DPPE inhibited the formation of peak 5 much more weakly

We propose that in tumor cells, which may express high constitutive levels of certain P450 isozymes [29], enhancement by DPPE of chemotherapy-induced cytotoxicity results from its inhibition of both CYP3A4-mediated drug metabolism and P-gp pump-mediated drug efflux. In normal cells, which, with the exception of liver and adrenal tissue, have low constitutive levels of P450, the cytoprotective effects of DPPE result from the induction of both CYP3A4 and P-gp, thereby increasing drug metabolism and/or P-gp-mediated drug efflux. Drug induction of P450 isozymes, specifically CYP3A4, occurs in gut endothelial cells [29] and blood leukocytes [21], tissues that appear to be protected by DPPE from the effects of cytotoxic agents [8, 13]. Induction of P450s in gastrointestinal epithelium may also account for the finding that the level of prostacyclin (PGI_2) in gastrointestinal juice is increased almost sevenfold in DPPE-treated rats [14].

DPPE neither binds to, nor is significantly metabolized by, CYP2B6, a P450 isozyme also implicated in the metabolism of cyclophosphamide [12]. Thus, intratumoral potentiation of this chemotherapeutic agent by DPPE [10] may be mediated exclusively at CYP3A4. Since cyclophosphamide is a prodrug that is activated by CYP2B6 and, to a lesser extent, by CYP3A4 in the liver [12], induction by DPPE of hepatic CYP3A4 may help to augment the conversion of the drug to its active form, thereby further enhancing its systemic antitumor effect.

The ability of DPPE to decrease mitochondrial function [22] also could result from inhibition of mitochondrial P450 enzymes, including CYP3A4 [1]. Both an inhibitory effect on mitochondria and depletion of cellular ATP [22] may account for the reported potentiation by DPPE of cis-platinum [16], and other drugs [2, 9] not metabolized by P450. An alternative and as-yet-untested hypothesis is that DPPE might modulate the activity of glutathione-S-transferase, a non-P450 enzyme implicated in cell resistance to drugs such as cis-platinum [18].

DPPE has higher affinity for CYP2D6 than for CYP3A4 (Table 1). Polymorphism of CYP2D6 is found in 8% of humans [28]. High tissue levels of this isozyme would be expected to metabolize DPPE and limit access to CYP3A4, rendering the drug less effective to potentiate antineoplastic agents. In such cases, the concomitant administration of a selective CYP2D6 substrate, such as quinidine [23], might enhance the availability of DPPE to bind CYP3A4.

Previously, we have shown that, in addition to DPPE and other arylalkylamine drugs that stimulate or inhibit cell growth [19], the polyamines (spermine and spermidine) demonstrate potent interactive binding with HA at P450 in rat liver microsomes [11]. On the basis of our studies and those of others [25], we have postulated that HA may modulate the catalytic activity of the monooxygenases [11] and that the HA/P450 complex may be perturbed by a variety of growth-modulatory endogenous biogenic amines and exogenous chemicals, including drugs [11, 19]. Overall, our findings point to an important role for cytochromes P450, and agents that modulate their activity, in determining the response to, and toxicities of, cancer chemotherapy.

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