TERATOLOGY 59:81–87 (1999)

Teratological Interaction Between the Bis-Triazole Antifungal Agent Fluconazole and the Anticonvulsant Drug Phenytoin

GIAN MARIO TIBONI,[1]* ELISABETTA IAMMARRONE,[1] FRANCA GIAMPIETRO,[1] DEBORA LAMONACA,[1] UMBERTO BELLATI,[1] AND CARMINE DI ILIO[2]

[1]*Dipartimento di Medicina e Scienze dell'Invecchiamento, Sezione di Ostetricia e Ginecologia, Facoltà di Medicina e Chirurgia, Università "G. d' Annunzio," Ospedale SS. Annunziata, 66100 Chieti, Italy*

[2]*Dipartimento di Scienze Biomediche, Sezione di Biochimica, Facoltà di Medicina e Chirurgia, Università "G. d' Annunzio," 66100 Chieti, Italy*

ABSTRACT Previous studies implicated the cytochrome P450 (CYP) system as critical in the teratogenic bioactivation of phenytoin (PHT). Fluconazole (FCZ) is an antifungal bis-triazole with potent inhibitory effect on the principal CYP-dependent metabolic pathway of PHT. In this study an in vivo experimental model was used to evaluate the potential ability of FCZ (2, 10, or 50 mg/kg intraperitoneally) to modulate PHT (65 mg/kg intraperitoneally) teratogenesis on day 12 (plug day = day 1) Swiss mice. PHT alone elicited embryocidal and malformative effects, with cleft palate as the major malformation. Pretreatment with the non-embryotoxic dosage of 10 mg FCZ/kg potentiated PHT-induced teratogenesis, as indicated by a twofold (from 6.2% to 13.3%) increment of cleft palate incidence ($P < 0.05$). Combined treatment with 50 mg FCZ/kg plus PHT resulted in a statistically significant ($P < 0.05$) increment of the resorption incidence recorded after PHT-alone exposure, but possibly as a consequence of the increased embryolethality, in the loss of the potentiative effect on PHT teratogenesis. Although the mechanistic nature of teratological interaction between FCZ and PHT remains to be established, these results may not support CYP system-mediated metabolic conversion as the mechanistic component of PHT teratogenesis. *Teratology 59:81–87, 1999.*

The anticonvulsant drug phenytoin (PHT; diphenylhydantoin sodium) has been significantly implicated as causative factor in human teratogenesis (Hanson and Smith, '75; Strickler et al., '85; Buehler et al., '90; Scolnik et al., '94; Nulman et al., '97), and is a proven animal teratogen (Massey, '66; Harbison and Becker, '74; McClain and Langhoff, '80).

Mechanistically, several theories have been postulated to explain developmental consequences of PHT exposure (reviewed in Hansen, '91; Wells and Winn, '95; Juchau, '97), but for none of them has conclusive evidence been provided. Great interest has been prompted over the years by the hypothesis that PHT elicits teratogenesis via bioactivation to highly reactive intermediates, such as an epoxide (arene oxide), capable of electrophilic attack on embryonic macromolecules with resultant altered cellular function (Martz et al., '77; Pantarotto et al., '82; Strickler et al., '85; Buehler et al., '90; Roy and Snodgrass, '90). This hypothesis achieved prominence following the finding that inhibition of epoxide hydrolase increases the covalent binding of PHT metabolites to embryonic macromolecules, and the incidence of orofacial cleft (Martz et al., '77). The pharmacokinetic profile of PHT is characterized by an extensive cytochrome P450 (CYP) system-mediated metabolism (90%), with hydroxylation presumed to occur via an arene oxide intermediate (Hansen, '91), so that the CYP enzymes catalyzing this reaction are believed to bioactivate PHT in the process. Therefore, studies which intended to evaluate a potential role for reactive intermediates in PHT-induced teratogenesis included teratological interaction studies between PHT and modulators of CYP system activity. But these studies yielded conflicting results (Harbison and Becker, '70; Finnell et al.,'92, '93, '94).

Fluconazole (FCZ) is a bis-triazole antifungal agent with a potent inhibitory effect on the principal CYP-dependent pathway responsible for PHT metabolism (Levy, '95). In this light, we reasoned that FCZ could represent a useful probe to further investigate the potential role of the CYP system in the teratogenic bioactivation of PHT.

MATERIALS AND METHODS

Drugs

PHT (purity approximately 99%) was purchased from Sigma Chemical Co. (St. Louis, MO). Dosing solutions

Grant sponsor: Progetto Prevenzione dei Fattori di Rischio per la Salute Materno-Infantile, Istituto Superiore di Sanità.

*Correspondence to: Gian Mario Tiboni, M.D., Dipartimento di Medicina e Scienze dell'Invecchiamento, Sezione di Ostetricia e Ginecologia, Facoltà di Medicina e Chirurgia, Università "G. d'Annunzio," Ospedale SS. Annunziata, Via Valignani, 66100 Chieti, Italy.
E-mail: tiboni@unich.it

Received 30 March 1998; Accepted 23 September 1998

TABLE 1. Effect of FCZ-PHT interaction on maternal parameters in the Swiss mouse

	Vehicle + vehicle	FCZ* (10 mg/kg) + vehicle	FCZ (50 mg/kg) + vehicle	Vehicle + PHT**	FCZ (2 mg/kg) + PHT	FCZ (10 mg/kg) + PHT	FCZ (50 mg/kg) + PHT
No. of pregnant females	14	14	17	28	20	24	19
Percent maternal lethality (no.)							5.26 (1)
Maternal body weight (g ± SEM) at term	65.80 ± 3.03	59.39 ± 2.07	69.32 ± 2.44	63.64 ± 1.58	60.17 ± 1.46	60.50 ± 1.67	59.84 ± 1.36
Maternal corrected body weight (g ± SEM)***	45.46 ± 1.75	40.64 ± 1.60	48.24 ± 1.38	46.47 ± 0.95	42.52 ± 0.74	45.22 ± 1.06	45.13 ± 0.61
Maternal weight gain (g ± SEM) (days 12–19)	19.45 ± 1.53	18.46 ± 1.39	21.99 ± 1.41	17.04 ± 1.13	16.49 ± 1.20	14.26 ± 1.03	14.28 ± 1.30

*Administered intraperitoneally on gestational day 12, 3 hr before PHT administration.
**65 mg/kg administered intraperitoneally.
***Maternal body weight at term − gravid uterine weight.

were prepared shortly before administration by dissolving PHT in 0.9% (w/v) NaCl containing 0.002 M NaOH at a final pH of 10.7–10.9. The FCZ used in the study was the compound commercially available for intravenous infusion (Diflucan®, Pfizer), formulated as an iso-osmotic solution, and containing 2 mg of FCZ and 9 mg of NaCl per ml.

Animals and breeding procedure

The animals used in the study were sexually mature Swiss outbred mice, obtained from the animal quarters of the Catholic University of the Sacred Heart (Rome, Italy). Animals were housed in solid-bottom polycarbonate cages with stainless steel tops, containing heat-treated hardwood chips. Standard rodent laboratory food and tap water were provided ad libitum throughout the study. Animals room was maintained at 22°C ± 1°C with a relative humidity of 55% ± 5%. A 12-hr light/dark cycle was used. For breeding, one male was housed with 4–5 nulliparous females throughout the dark cycle. At the end of this period, the presence of a vaginal plug was taken as an index of copulation. The morning the copulation plug was identified was considered day 1 of gestation. Plug-positive females were group housed (3/cage maximum) until the gestational age scheduled for treatments.

Teratological study

Experimental procedures were carried out on gestational day 12. As has been previously shown, this gestational stage brackets the window of maximal susceptibility to PHT-induced cleft palate in the mouse (Lum and Wells, '86). At 0900 hr, females were injected intraperitoneally with 0 (vehicle), 2, 10, or 50 mg/kg of FCZ. Three hours later, mice received an intraperitoneal dose of 0 (vehicle; only animals treated with 0, 10, or 50 mg/kg of FCZ) or 65 mg/kg of PHT. The study protocol, therefore, consisted of seven experimental groups. These FCZ doses inhibit the CYP enzyme system in the mouse (La Delfa et al., '89; Morita et al., '92). PHT was administered at 65 mg/kg, since this dosage was previously found to be teratogenic in the mouse (Wells et al., '89). On the morning of gestational day 19, females were weighed and then sacrificed. After hysterectomy, the uterus was weighed, and uterine contents were evaluated for number of implantations, resorptions, and dead and live fetuses. Viable fetuses were weighed, sexed, and carefully examined for gross malformations, including cleft palate. Approximately half of the fetuses from each litter were double-stained with alizarin red and alcian blue, according to the method proposed by Inouye ('76) and Kimmel and Trammel ('81) as modified by Kuczuk and Scott ('84), and examined for skeletal abnormalities. The remaining fetuses were fixed in Bouin's solution and evaluated for soft-tissue abnormalities, using the free-hand razor blade section technique devised by Wilson ('65). All morphological evaluations were conducted with the aid of a stereo microscope (Stemi SV11, Zeiss, Oberkochen, Germany).

Statistical analysis

Continuous data were compared by one-way analysis of variance (ANOVA) followed, if significant, by a Student-Newman-Keuls multiple range test. Binomial data were compared using the chi-square test with Yates correction. All numerical data reported are expressed as the mean ± SEM. The null hypothesis was rejected at the 5% significance level ($P < 0.05$).

RESULTS

FCZ alone

Administration of FCZ at 10 or 50 mg/kg neither induced appreciable effects on maternal well-being, nor affected the maternal body weight parameters shown in Table 1. Maternal treatment with FCZ at 10 mg/kg did not reveal embryocidal effects (Fig. 1). Conversely, FCZ

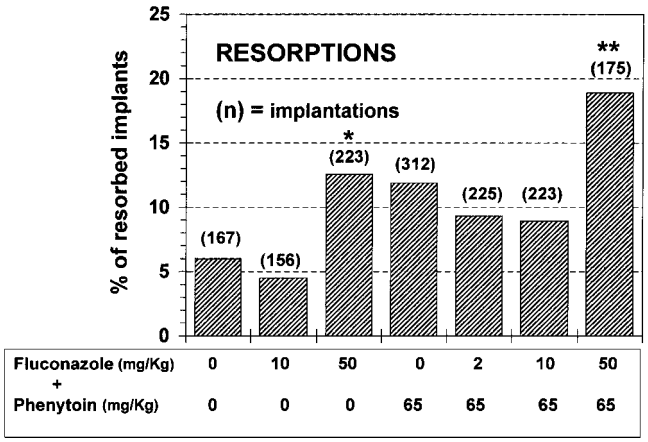

Fig. 1. Effect of fluconazole pretreatment on phenytoin-induced resorptions in the Swiss mouse. Fluconazole was given intraperitoneally at 0900 hr on gestational day 12 mice. Three hours later, mice received an intraperitoneal dose of phenytoin (65 mg/kg). Uterine contents were examined on gestational day 19. *Significantly different vs. control (vehicle) group ($P < 0.05$). **Significantly different vs. phenytoin-alone group ($P < 0.05$).

at 50 mg/kg significantly reduced embryo viability, as evidenced by the increase of resorption incidence from 5.98% (vehicle group) to 12.55% ($P < 0.05$) (Fig. 1). The mean fetal weights of FCZ-exposed litters (either 10 and 50 mg/kg) were comparable to the control value (Table 2). Morphological evaluation revealed that fetuses exposed at 50 mg/kg of FCZ had an increased incidence ($P < 0.05$) of rib abnormalities (full supernumerary ribs and short supernumerary ribs) in comparison to control fetuses (Fig. 2). The highest dose of FCZ also caused a low but measurable incidence of dilated renal pelvis (Fig. 3). No other morphological anomalies were noted in FCZ-exposed fetuses.

PHT alone

Intraperitoneal administration of PHT (65 mg/kg) rapidly induced signs of maternal nervous system impairment, including sedation, ataxia, and in some instances, opisthotonus. These signs persisted for several hours. Despite distress, maternal weight gain was not affected by PHT in comparison to the control group (Table 1). PHT treatment nearly doubled the embryolethality of the control group (Fig. 1), but this increment reached only borderline statistical significance ($P = 0.058$). Mean fetal body weight was not lessened by PHT (Table 2). As shown in Figure 4, maternal PHT administration significantly ($P < 0.05$) increased the low cleft-palate incidence observed in vehicle-exposed fetuses, from 1.28% to 6.20%. Additional morphological anomalies were significantly ($P < 0.05$) increased by PHT with respect to controls, including rib abnormalities (Fig. 2) and dilated renal pelvis (Fig. 3). Without statistical relevance were the remaining defects observed, including exencephaly (1.45%), ectrodactyly (0.36%), and tail defect (0.36%) (data not shown).

FCZ + PHT

Pregnant animals pretreated with FCZ (at any dose level) did not show appreciable modifications of clinical sequelae (maternal nervous system impairment) induced by PHT administration. For unknown reasons, one dam of the group given FCZ 50 mg/kg plus PHT died during the night of the treatment day (Table 1). Maternal and fetal weight values of groups pretreated with FCZ (2, 10, or 50 mg/kg) were comparable to values recorded after treatment with PHT alone (Tables 1 and 2, respectively). In terms of embryolethality, the combined administration of 50 mg FCZ/kg plus PHT resulted in a 37% increase ($P < 0.05$) of embryonic loss in comparison with the PHT-alone group (Fig. 1). A similar resorption rate increase (33.4%) was noted between groups treated with 50 mg/kg of FCZ alone and 50 mg/kg FCZ plus PHT, but in this case statistical analysis did not reveal significance (Fig. 1). In terms of malformations, a significant interaction between agents was found in response to a lower dose of FCZ. In fact, as shown in Figure 3, FCZ at 10 mg/kg induced a twofold (from 6.2% to 13.3%) increment of cleft palate incidence ($P < 0.05$). In contrast with expectations, pretreatment with FCZ at the maximal dose did not significantly enhance PHT-induced cleft palates (Fig. 4). FCZ pretreatment also caused an approximately twofold increment of PHT-induced dilated renal pelvis (when given at 10 or 50 mg/kg; Fig. 3), and a decreased frequency of fetuses with rib anomalies (when given at 2, 10, or 50 mg/kg; Fig. 2). But these changes were not significant statistically. Sporadic malformations found (data not shown) included cases of exencephaly (0.99%; in the group pretreated with 5 mg FCZ/kg), and gastoschisis and open eyelids (0.49%; in the group pretreated with 50 mg FCZ/kg).

DISCUSSION

In the present study it was found that FCZ, at the nonembryotoxic level of 10 mg/kg, significantly potentiated PHT-induced teratogenesis, as evidenced by a twofold increase in cleft palate incidence.

In treatment of fungal infections, FCZ is prescribed at a maximum recommended daily dose of 1,600 mg (Debruyne, '97). Assuming a human patient of 60 kg, this dosage corresponds to 26 mg/kg/day. Thus, the FCZ dosage that we found effective in enhancing the teratogenic effect of PHT (10 mg/kg) is within the human therapeutic range. This idea is also supported by major differences in the pharmacokinetic profiles existing between humans and mice. Peak plasma concentrations of FCZ, normalized to a 1 mg/kg dose level following oral administration, were found to be twofold higher in humans (1.4 μg/ml) than in mice (0.7 μg/ml) (Humprey et al., '85). Differences in half-lives were more than fourfold (22 hr in humans vs. 4.8 hr in mice) (Humprey et al., '85). On the other hand, it should be considered that a single dose given all at once is likely to result in plasma concentrations higher than those achieved with several doses spread over a day. With

TABLE 2. Effect of FCZ-PHT interaction on reproductive outcome in the Swiss mouse

	Vehicle + vehicle	FCZ* (10 mg/kg) + vehicle	FCZ (50 mg/kg) + vehicle	Vehicle + PHT**	FCZ (2 mg/kg) + PHT	FCZ (10 mg/kg) + PHT	FCZ (50 mg/kg) + PHT
No. of litters	14	14	17	28	20	24	18
Mean ± SEM of implantations/litter	11.93 ± 1.15	11.14 ± 1.01	13.12 ± 1.04	11.14 ± 0.76	11.25 ± 0.88	9.29 ± 0.75	9.72 ± 0.87
Mean ± SEM of viable fetuses/litter***	11.14 ± 1.01	10.57 ± 1.09	11.41 ± 0.84	9.79 ± 0.70	10.10 ± 0.82	8.46 ± 0.70	7.83 ± 0.83
No. of dead fetuses (%)	1 (0.63)	1 (0.67)	1 (0.51)	1 (0.36)	2 (0.98)		1 (0.70)
Mean fetal weight (g ± SEM)/litter	1.38 ± 0.04	1.36 ± 0.03	1.39 ± 0.02	1.30 ± 0.02	1.30 ± 0.01	1.31 ± 0.02	1.37 ± 0.04

*Administered intraperitoneally on gestational day 12, 3 hr before PHT administration.
**65 mg/kg administered intraperitoneally.
***Fetuses evaluated on gestational day 19.

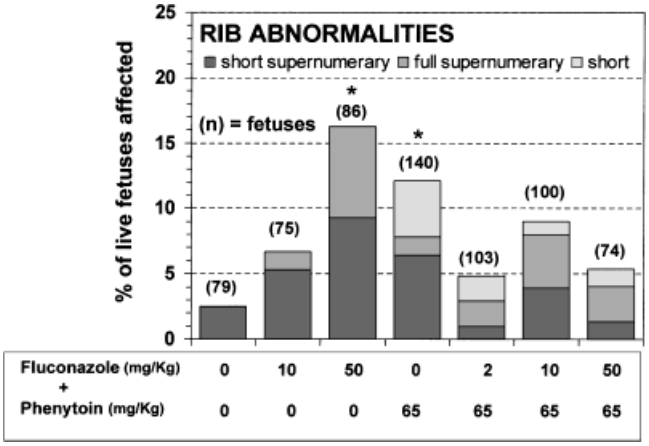

Fig. 2. Effect of fluconazole pretreatment on phenytoin-induced rib abnormalities in the Swiss mouse. The term "supernumerary rib" indicates the presence of an additional rib with respect to the usual number. Ribs were considered as short supernumerary, instead of full supernumerary, if they were less than half the length of the preceding rib. Fluconazole was given intraperitoneally at 0900 hr on gestational day 12 mice. Three hours later, mice received an intraperitoneal dose of phenytoin (65 mg/kg). Uterine contents were examined on gestational day 19. *Significantly different vs. control (vehicle) group ($P < 0.05$).

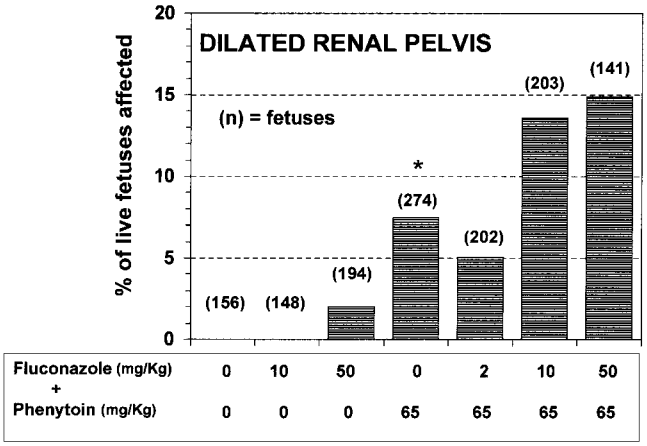

Fig. 3. Effect of fluconazole pretreatment on phenytoin-induced dilated renal pelvis in the Swiss mouse. Fluconazole was given intraperitoneally at 0900 hr on gestational day 12 mice. Three hours later, mice received an intraperitoneal dose of phenytoin (65 mg/kg). Uterine contents were examined on gestational day 19. *Significantly different vs. control (vehicle) group ($P < 0.05$).

respect to PHT, assuming again a patient of 60 kg, the dosage administered to Swiss mice (65 mg/kg) was approximately four times the maximal human dosage (1,000 mg/day) (McNamara, '95), although it has been reported that an oral administration of 75 mg/kg of PHT in the mouse results in plasma drug concentrations in the human therapeutic range (Sulik et al., '79).

Interestingly, FCZ increased cleft palate incidence without altering the incidence of remaining PHT-induced developmental disorders. This may suggest that FCZ promotes selective modifications of molecular imbalances that compound PHT embryotoxicity. Alternatively, it is possible that a single dose of PHT at a single gestational time may have missed the critical times of susceptibility for the other developing primordia.

In contrast with a dose-response model, increment of FCZ exposure to 50 mg/kg failed to maintain cleft palate incidence at a significantly higher level in comparison to the PHT-alone-treated group. This *inverted* teratogenic dose-response relationship may be a consequence of embryonic loss increment that followed 50 mg FCZ/kg-PHT coadministration, with a resulting reduced survival of affected conceptuses. In this regard it is worth remembering that embryolethality precluding the recognition of other developmental disorders is a well-characterized phenomenon in teratogenesis (Wilson, '77). The finding of an increased embryo mortality after the combined treatment 50 mg/kg of FCZ plus PHT appears explainable in terms of a nearly additive interaction between agents, considering that both exhibited a 12% incidence of embryolethality when separately administered, and a 19% incidence of embryolethality when given in combination. The evidence that FCZ is per se capable of eliciting embryotoxicity is not surprising considering that the embryopathic effects of this triazole have been shown by in vivo (Tachibana et al., '87; data on file at the Roerig Division, U.S. Pharmaceuticals Group, Pfizer, New York, NY) and in vitro

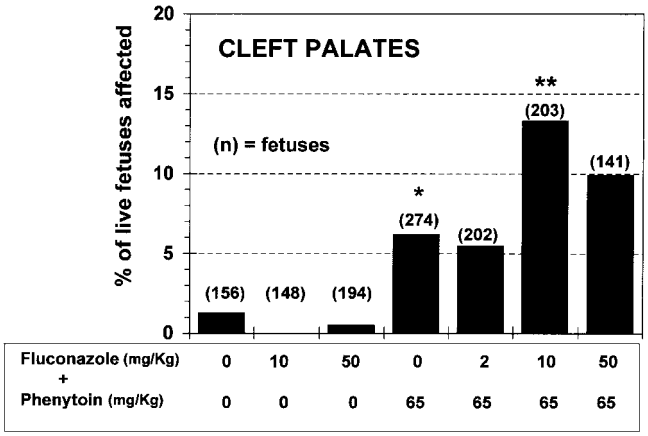

Fig. 4. Effect of fluconazole pretreatment on phenytoin-induced cleft palate in the Swiss mouse. Fluconazole was given intraperitoneally at 0900 hr on gestational day 12 mice. Three hours later, mice received an intraperitoneal dose of phenytoin (65 mg/kg). Uterine contents were examined on gestational day 19. *Significantly different vs. control (vehicle) group ($P < 0.05$). **Significantly different vs. phenytoin alone group ($P < 0.05$).

(Tiboni, '93) studies. Possible developmental effects of FCZ appear to deserve attention in view of a recently postulated fetal fluconazole embryopathy (Lee et al. '92; Pursley et al., '95; Aleck and Bartley, '97).

The primary molecular mechanism of action of FCZ is inhibition of ergosterol biosynthesis, leading to inhibition of fungal cell growth and division (Vanden Bossche, '85; Saag and Dismukes, '88; Como and Dismukes, '94). FCZ mediates this effect via competition for oxygen at the catalytic heme iron atom of CYP enzymes, thereby decreasing C-14 demethylation of lanosterol, the precursor intermediate of ergosterol (Vanden Bossche, '85; Saag and Dismukes, '88; Como and Dismukes, '94). In contrast to imidazoles, FCZ has shown a high specificity for fungal CYP enzymes while binding only weakly to the mammalian CYP enzymes (Como and Dismukes, '94). On the other hand, FCZ has been recently recognized as a potent inhibitor of the mammalian CYP system. Morita et al. ('92), using the ratio of 6β-hydroxycortisol to cortisol in urine as an indicator of hepatic oxidative drug-metabolizing capacity in humans, found that therapeutic doses of FCZ (200 mg/day) decreased up to 50% of the original level. In the same study, FCZ (1–10 mg/kg given intraperitoneally) significantly prolonged pentobarbital sleeping time in mice in a dose-dependent manner. La Delfa et al. ('89), employing the [^{14}C] antipyrine breath test, provided evidence supporting FCZ (1 or 10 mg/kg administered by gavage) as a potent, partially selective, and reversible inhibitor of the CYP-dependent enzyme system in mice. Of particular relevance to the present study, FCZ was found to be a potent inhibitor of CYP 2C9 (Levy, '95; Kunze et al., '96; Black et al., '96), the CYP isoform that, catalyzing parahydroxylation of PHT to *p*-hydroxyphenytoin, accounts for 70–90% of total PHT clearance (Levy, '95). Consistent with this notion, symptomatic PHT toxicity has resulted from concomitant administration of FCZ (Howitt and Oziemsky, '89; Mitchell and Holland, '89; Cadle et al., '94). Moreover, coadministration of FCZ and PHT in healthy volunteers induced a marked increase in the mean PHT area under the concentration-time curve (Lazar and Wilner, '90; Blumm et al., '91; Touchette et al., '92) without affecting FCZ levels (Lazar and Wilner, '90).

From this perusal of the literature, it is tempting to infer a causal link between the inhibitory properties of FCZ on the CYP enzyme system and the FCZ-induced potentiation of PHT teratogenesis found in the present study. If so, then our findings would argue against a role for CYP system-catalyzed bioactivation in the mechanism of PHT teratogenesis. A possible explanation of the enhancement of PHT teratogenesis by FCZ is that inhibition of maternal PHT hydroxylation resulted in an enhanced maternal plasma concentration of PHT and a transfer to the developing conceptus. Since FCZ has been shown to cross rodent placenta with fetal tissue concentrations approximately equal to maternal plasma concentration (Diflucan, Drug Information Fulltext, American Society of Health-System, Pharmacist Inc., Bethesda, MD), it is also possible that inhibition of the low activities of embryonic CYP enzymes contributed to the potentiation of PHT teratogenesis. Our study appears congruent with data provided by Harbison and Becker ('70). These investigators found that a pharmacological stimulus apparently effective in enhancing PHT metabolism (pretreatment with phenobarbital) was associated with a decrement of PHT embryotoxicity and that, on the other hand, a pharmacological exposure (pretreatment with SKF 525A) consistent with inhibition of PHT metabolism resulted in enhancement of PHT embryotoxicity. Conversely, Finnell et al. ('93, '94) found that preadministration of stiripentol, an anticonvulsant with broad inhibiting effects on CYP enzymes, significantly reduced the overall incidence of PHT-induced fetal defects in three strains of mice. These two studies differed in route of drug administration, duration of administration, and strains of mice used, partly accounting for resultant discrepancies. It also appears possible that the protective effect elicited by stiripentol on PHT dysmorphogenesis was of a toxicodynamic (more than toxicokinetic) nature, as indicated by the fact that stiripentol coadministration did not increase PHT maternal plasma concentrations.

The pharmacokinetic profile of PHT is characterized by an extensive binding to plasma protein (~90%), with both efficacy and toxicity dependent on the plasma concentration of the unbound drug (McNamara, '95). It is well-known that factors altering plasma protein binding can critically modulate teratogenesis, with only the unbound drug expected to equilibrate with the embryo (Nau, '87). It is therefore possible that FCZ elicited its effect by displacing PHT from protein binding. However, this mechanism appears unlikely, as suggested by the notion that only 12% of FCZ is bound to plasma protein in the mouse (Humphrey et al., '85).

It has been postulated that azole compounds may also affect the fungal cell by inhibition of cytochrome *c* oxidative and peroxidative enzymes, with a resultant increase in intracellular peroxide generation (Vanden Bossche, '85; Saag and Dismukes, '88). This pathway, if not specific for fungal cells, may possibly be involved in the potentiation elicited by FCZ on PHT teratogenicity. In fact, peroxides (hydrogen peroxide and/or lipid hydroperoxides) have been recently postulated to play a role in teratological initiation of PHT (Ozolins et al., '96). Of note, this study represents part of the growing evidence implicating free radicals, generated via lipoxygenases or prostaglandin synthetase activities, as the ultimate embryotoxic intermediates of PHT (reviewed by Wells and Winn, '96).

LITERATURE CITED

Aleck KA, Bartley DL. 1997. Multiple malformation syndrome following fluconazole use in pregnancy: Report of an additional patient. Am J Med Genet 72:253–256.

Black DJ, Kunze KL, Wienkers LC, Gidal BE, Seaton TL, McDonnell ND, Evans JS, Bauwens JE, Trager WF. 1996. Warfarin fluconazole II. A metabolically based drug interaction: In vivo studies. Drug Metab Dispos 24:422–421.

Blumm RA, Wilton JH, Hilligoss DM, Gardner MJ, Henry EB, Harrison NJ, Schentag JJ. 1991. Effect of fluconazole on the disposition of phenytoin. Clin Pharmacol Ther 49:420–425.

Buehler BA, Delimont D, Vanwaes M, Finnell RH. 1990. Prenatal prediction of risk of the fetal hydantoin syndrome. N Engl J Med 322:1567–1572.

Cadle RM, Zenon GJ, Rodriguez-Barradas MC, Hamill RJ. 1994. Fluconazole-induced symptomatic phenytoin toxicity. Ann Pharmacother 28:191–195.

Como JA, Dismukes WE. 1994. Oral azol drugs as systemic antifungal therapy. N Engl J Med 330:263–272.

Debruyne D. 1997. Clinical pharmacokinetics of fluconazole in superficial and systemic mycoses. Clin Pharmacokinet 33:52–77.

Finnell RH, Buehler BA, Kerr BM, Ager PL, Levy RH. 1992. Clinical and experimental studies linking oxidative metabolism to phenytoin-induced teratogenesis. Neurology [Suppl] 42:25–31.

Finnell RH, van Waes M, Musselman A, Kerr BM, Levy RH. 1993. Differences in the pattern of phenytoin-induced malformations following stiripentol coadministration in three inbred mouse strains. Reprod Toxicol 7:439–448.

Finnell RH, Kerr BM, van Waes M, Steward RL, Levy RH. 1994. Protection from phenytoin-induced congenital malformations by coadministration of the antiepileptic drug stiripentol in a mouse model. Epilepsia 35:141–148.

Hansen DK. 1991. The embryotoxicity of phenytoin: An update on possible mechanisms. Proc Soc Exp Biol Med 197:361–368.

Hanson JW, Smith DW. 1975. The fetal hydantoin syndrome. J Pediatr 87:285–290.

Harbison RD, Becker BA. 1970. Effect of phenobarbital or SKF 525-A pretreatment on diphenylhydantoin teratogenicity in mice. J Pharmacol Exp Ther 175:283–288.

Harbison RD, Becker BA. 1974. Diphenylhydantoin teratogenicity in rats. Toxicol Appl Pharmacol 22:193–200.

Howitt KM, Oziemsky MA. 1989. Phenytoin toxicity induced by fluconazole. Med J Aust 151:603–604.

Humphrey MJ, Jevons S, Tarbit MH. 1985. Pharmacokinetics evaluation of UK 49,858, a metabolically stable triazole antifungal drug, in animals and humans. Antimicrob Agents Chemother 28:648–653.

Inouye M. 1976. Differential staining of cartilage and bone in fetal mouse skeleton by alcian blue and alizarin red S. Cong Anomal 16:171–173.

Juchau MR. 1997. Chemical teratogenesis in human: Biochemical and molecular mechanism. Prog Drug Res 49:25–92.

Kimmel CA, Trammel C. 1981. A rapid procedure for routine staining of cartilage and bone in fetal and adult animals. Stain Technol 56:271–273.

Kuczuk MJ, Scott WJ. 1984. Potentiation of acetazolamide-induced ectrodactyly in SWV and C57BL/mice by cadmium sulfate. Teratology 29:427–435.

Kunze KL, Wienkers LC, Thummel KE, Trager WF. 1996. Warfarin-fluconazole I. Inhibition of the human cytochrome P450-dependent metabolism of warfarin by fluconazole: In vitro studies. Drug Metab Dispos 24:414–421.

La Delfa I, Zhu QM, Mo Z, Blaschke TF. 1989. Fluconazole is a potent inhibitor of antipyrine metabolism in vivo in mice. Drug Metab Dispos 17:49–53.

Lazar JD, Wilner KD. 1990. Drug interactions with fluconazole. Rev Infect Dis [Suppl] 12:327–333.

Lee BE, Feinberg M, Abram JJ, Murthy ARK. 1992. Congenital malformations in an infant born to a woman treated with fluconazole. Pediatr Infect Dis J 11:1062–1064.

Levy RH. 1995. Cytochrome P450 isozymes and antiepileptic drug interactions. Epilepsia [Suppl] 36:8–13.

Lum JT, Wells PG. 1986. Pharmacological studies on the potentiation of phenytoin teratogenicity by acetaminophen. Teratology 33:53–72.

Martz F, Failinger C, Blake D. 1977. Phenytoin teratogenesis: Correlation between embryopathic effect and covalent binding of putative arene oxide metabolite in gestational tissue. J Pharmacol Exp Ther 203:231–239.

Massey MD. 1966. Teratogenic effects of diphenylhydantoin sodium. J Oral Ther Pharmacol 2:380–385.

McClain RM, Langhoff C. 1980. Teratogenicity of diphenylhydantoin in New Zealand white rabbits. Teratology 21:371–379.

McNamara JO. 1995. Drugs effective in the therapy of the epilepsies. In: Gilman AG, Goodman LS, editors. The pharmacological basis of therapeutics, 9th ed. New York: Macmillan. p 461–486.

Mitchell AS, Holland JT. 1989. Fluconazole and phenytoin: A predictable interaction. Br Med J [Clin Res] 298:1315.

Morita K, Konisby H, Shimakawa H. 1992. Fluconazole, a potent inhibitor of cytochrome P-450 dependent drug metabolism in mice and humans in vitro: Comparative study with ketoconazole. Chem Pharm Bull (Tokyo) 40:1247–1251.

Nau H. 1987. Species differences in pharmacokinetics, drug metabolism, and teratogenesis. In: Nau H, Scott WJ, editors. Pharmacokinetics in teratogenesis. Volume 1. Boca Raton: CRC Press. p 81–106.

Nulman I, Scolnik D, Chitayat D, Farkas LD, Koren G. 1997. Findings in children exposed in utero to phenytoin and carbamazepine monotherapy: Independent effects of epilepsy and medications. Am J Med Genet 68:18–24.

Ozolins TRS, Sikasay DL, Wells PG. 1996. Modulation of embryonic glutathione peroxidase activity and phenytoin teratogenicity by dietary deprivation of selenium in CD-1 mice. J Pharmacol Exp Ther 277:945–953.

Pantarotto C, Arboix M, Sezzano P, Abbruzzi R. 1982. Studies on 5, 5-diphenylhydantoin irreversible binding to rat liver microsomal proteins. Biochem Pharmacol 31:1501–1507.

Pursley TJ, Blomquist IK, Abram J, Andersen HF, Bartley JA. 1996. Fluconazole-induced congenital anomalies in three infants. Clin Infect Dis 22:336–340.

Roy D, Snodgrass WR. 1990. Covalent binding of phenytoin to protein and modulation of diphenylhydantoin metabolism by thiols in A/J mouse liver microsomes. J Pharmacol Exp Ther 252:895–900.

Saag MS, Dismukes WE. 1988. Azole antifungal agents: Emphasis on new triazoles. Antimicrob Agents Chemother 32:1–8.

Scolnik D, Nulman I, Rovet J, Gladstone D, Czuchta D, Gardner HA, Gladstone R, Ashby P, Weksberg R, Einarson T, Koren G. 1994. Neurodevelopment of children exposed in utero to phenytoin and carbamazepine monotherapy. JAMA 271:767–770.

Strickler SM, Miller MA, Andermann E, Dansky LV, Seni MH, Spielberg SP. 1985. Genetic predisposition to phenytoin-induced birth defects. Lancet 2:746–749.

Sulik KK, Johnston MC, Ambrose LJ, Dorgan D. 1979. Phenytoin (Dilantin)-induced cleft lip and palate in A/J mice: A scanning and transmission electron microscopic study. Anat Rec 195:243–256.

Tachibana M, Noguchi Y, Monro AM. 1987. Toxicology of fluconazole in experimental animals. In: Fromtling RA, editor. Recent trends in the discovery, development and evaluation of antifungal agents. Barcelona, Spain: Prous Science Publishers. p 93–102.

Tiboni GM. 1993. Second branchial arch anomalies induced by fluconazole, a bis-triazole antifungal agent, in cultured mouse embryos. Res Commun Chem Pathol Pharmacol 79:381–384.

Touchette MA, Chandrasekar PH, Milad MA, Edwards DJ. 1992. Contrasting effects of fluconazole and ketoconazole on phenytoin and testosterone disposition in man. Br J Clin Pharmacol 34:75–78.

Vanden Bossche H. 1985. Biochemical targets for antifungal azole dervatives: Hypothesis on the mode of action. Curr Top Med Mycol 1:313–351.

Wells PG, Zubovits JT, Wong ST, Molinari LM, Ali S. 1989. Modulation of phenytoin teratogenicity and embryonic covalent binding by ASA, caffeic acid and α-phenyl-N-t-butylnitrone: Implications for bioactivation of prostaglandin synthetase. Toxicol Appl Pharmacol 97:192–202.

Wells PG, Winn LM. 1995. Free radical-mediated mechanisms of anticonvulsant teratogenicity. Eur J Neurol [Suppl] 2:5–29.

Wells PG, Winn LM. 1996. Biochemical toxicology of chemical teratogenesis. Crit Rev Biochem Mol Biol 31:1–40.

Wilson JC. 1965. Methods for administering agents and detecting malformations in experimental animals. In: Wilson JG, Warkany J, editors. Teratology: Principles and techniques. Chicago: University of Chicago Press. p 262–277.

Wilson JC. 1977. Current status of teratology—general principles and mechanisms derived from animal studies. In: Wilson JG, Fraser FC, editors. Handbook of teratology: General principles and etiology. New York: Plenum Press. p 47–74.