ELSEVIER

Antiviral Research 42 (1999) 25–33

Antiviral Research

The effects of antirhino- and enteroviral vinylacetylene benzimidazoles on cytochrome P450 function and hepatic porphyrin levels in mice

Mark J. Tebbe [a,*], Charles B. Jensen [b], Wayne A. Spitzer [a], Ronald B. Franklin [b,1], M. hristopher George [b], Diane L. Phillips [b]

[a] *Infectious Diseases Research, Eli Lilly, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA*
[b] *Department of Drug Disposition, Eli Lilly, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA*

Received 4 August 1998; accepted 6 January 1999

Abstract

In an ongoing effort to identify an orally bioavailable compound for the treatment of rhino- and enteroviral infections, a series of vinylacetylene benzimidazoles was recently examined. Previous studies demonstrated the potential for these compounds to possess both good in vitro antiviral activity as well as acceptable oral plasma concentrations in mice. Optimization of these properties led to four compounds as candidates for further evaluation. In view of the recognized potential for certain acetylenic drugs both to inhibit cytochrome P450 enzymes by mechanism-based inactivation and to possibly perturb heme metabolism, information regarding drug effects on cytochromes P450 and hepatic porphyrin levels was sought. In an initial single-dose pharmacokinetic study, the four selected compounds were given orally to mice, and both plasma concentrations and porphyrin levels were determined. Two of the compounds, **4** and **5,** caused a pronounced increase in liver porphyrin levels whereas compounds **6** and **7** exhibited almost no effect on porphyrin levels. Analysis of plasma concentrations showed that only **4** and **5** gave significant exposure and that **6** and **7** produced negligible levels of drug in the plasma even at the highest dose tested (500 mg/kg). A multiple dose study was then initiated in which compounds **4** and **5** were given for 1 week in daily oral doses to mice. Upon completion of dosing, liver was analyzed for cytochrome P450-dependent 7-ethoxyresorufin *O*-deethylase (EROD) and benzphetamine *N*-demethylase (BND) activities, total cytochrome P450 content, and porphyrin levels. Both vinylacetylenes showed dose–dependent inhibitory and induction effects on EROD and BND activities. In addition, these compounds caused a marked increase in hepatic porphyrin levels. Therefore, while all four selected compounds displayed potent antiviral activity and two of the compounds exhibited acceptable

* Corresponding author. Tel.: +1-317-2773093; fax: +1-317-2772035.
E-mail address: tebbe_mark_j@lilly.com (M.J. Tebbe)
[1] Current address: Boulder, CO, USA.

PII: S0166-3542(99)00013-3

pharmacokinetic properties, the hepatic effects of these latter two compounds suggest the potential for drug induced porphyria with multidose therapeutic use.

Keywords: Rhinoviruses; Enteroviruses; Antiviral agent; Cytochrome P450; Porphyrins

1. Introduction

The antipicornaviral benzimidazoles, which were originally discovered at Lilly Research Laboratories over 20 years ago, are a unique class of compounds with potent, broad-spectrum activity against both rhino- and enteroviruses (DeLong et al., 1978; DeLong and Reed, 1980; Wikel et al., 1980; Herrmann et al., 1981 Victor et al., 1997b). In the early 1980's, two candidates, **1** and **2**, from this series were tested in clinical trials (Fig. 1). Enviroxime (**1**) suffered from poor plasma concentrations on oral dosing ($C_{max} = 14$ ng, 100 mg dose) and marginal clinical efficacy in challenge studies as well as demonstrating some undesirable side effects in both dogs and humans (DeLong, 1984; Miller et al., 1985). Enviradene (**2**) showed improved pharmacokinetics in dogs (46% oral bioavailability) and showed no adverse toxicological events in dogs or humans. In humans, however, it gave unacceptably low plasma concentrations ($C_{max} = 69$ ng, 500 mg dose) and was, therefore, never advanced to challenge studies (Enviradene-IND, 1981a,b). Further work on this compound class was not pursued for over a decade at which time attempts were made to improve the bioavailability of the antiviral benzimidazoles by reducing metabolic clearance rates.

The initial discovery of vinylacetylene benzimidazoles (**3**) evolved from efforts to find a metabolic obstacle for the allylic oxidation that was seen at the vinyl methyl position of Enviradene (Fig. 1). The acetylene modification did indeed appear to inhibit the oxidative metabolism at the vinyl methyl position as evidenced by the improvement in oral bioavailability observed in rhesus monkeys (Enviradene-IND, 1984; Bopp and Quay, 1985). Additionally, in vitro metabolism studies carried out using hepatocytes from several different animal species, including human, demonstrated that the extent of oxidative metabolism observed in this new series was substantially less than that seen with Enviroxime or Enviradene (Victor et al., 1997a). Based on these preliminary results as well as on the resolution of several synthetic issues (Tebbe et al., 1997), efforts to synthesize new vinylacetylene benzimidazoles were renewed with an emphasis on optimizing antiviral activity while maximizing oral plasma concentrations in mice (Tebbe et al., 1997). As with their predecessors, many of these new compounds are potent antivirals, and they consistently

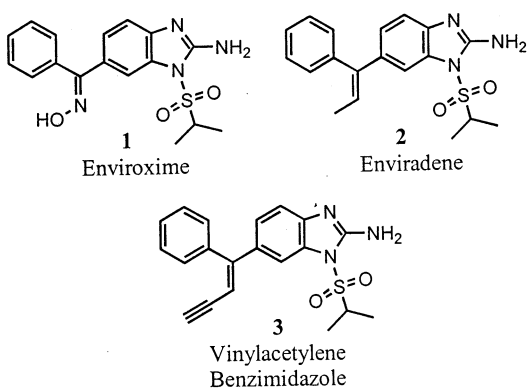

Fig. 1. Benzimidazole classes.

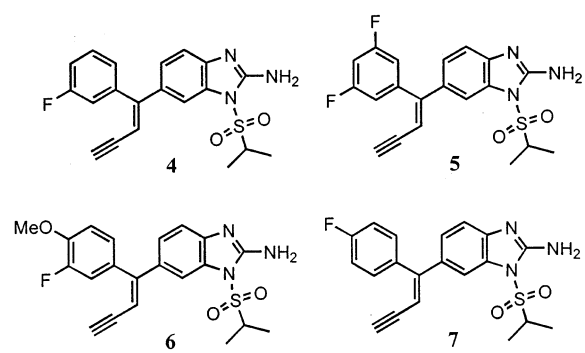

Fig. 2. Four vinylacetylene benzimidazoles in current study.

demonstrate broad-spectrum antiviral activity against a range of both rhino- and enteroviruses. Additionally, compounds dosed orally to mice during initial discovery studies demonstrated significant exposure (Tebbe et al., 1997). This research effort culminated in the selection of four compounds for further investigation (Fig. 2).

The major structural difference between this series of compounds and the earlier series studied is the incorporation of an acetylene moiety. Since the acetylene functionality is known in some cases to be metabolically labile and potentially hepatotoxic, a study was designed to assess the propensity of this series to produce these undesirable effects. The major concern with alkynes is their interactions with cytochromes P450. The cytochrome P450 monooxygenases (P450s) are a group of hemoproteins that catalyze the oxidative metabolism of endogenous substrates such as steroids and vitamins as well as xenobiotics. A variety of compounds containing an acetylene moiety have been shown to produce destruction of the heme sub-unit of several P450 isoforms leading to irreversible mechanism-based inhibition of enzymatic activity and decreases in the measurable P450 content (Oritz de Montellano and Kunze, 1980; Oritz de Montellano and Correia, 1983; Hopkins et al., 1992; Chan et al., 1993; Foroozesh et al., 1997). Further, the destruction of the heme moiety may cause depletion of the free heme pool and accumulation of porphyrin intermediates in the liver (Marks et al., 1988). Thus, repeated exposure to porphyrinogenic drugs can produce sufficient porphyrin accumulation to induce hepatic porphyria, which may ultimately lead to hepatic necrosis. Given the anticipated use of these compounds to treat a self-limiting viral infection, even a hint of hepatotoxicity would be unacceptable. The mere presence of an alkyne in a molecule is not sufficient to produce these unwanted P450 interactions, however, since molecule-enzyme interactions are dependent on the compound as a whole. In fact, there are many marketed drugs that contain alkynes and do not appear to have any detrimental effects on P450, its function, or hepatic porphyrin levels (Ross, 1997).

The current studies were conducted to assess the potential for the vinylacetylene antivirals to inactivate hepatic cytochromes P450 and cause porphyrin accumulation in mice following single or multiple oral doses of the compounds. In a single dose study, plasma concentrations of the drugs were determined to confirm adequate oral exposure in the mouse, and hepatic porphyrin levels were measured. In a more intensive multiple dose study, the animals were dosed for seven days, and porphyrin levels were determined. In addition, the possible mechanism-based destruction of hepatic cytochromes P450 was evaluated by determining the cytochrome P450-dependent 7-ethoxyresorufin *O*-deethylase and benzphetamine *N*-demethylase activities as well as the total cytochrome P450 content. Limited clinical chemistry was also performed in order to monitor for early signs of hepatotoxicity.

2. Materials and methods

2.1. Antiviral agents

Compounds **4**, **5**, **6** and **7** were synthesized at Lilly Research Laboratories as previously described (Tebbe et al., 1997).

2.2. Antiviral activity

The antiviral activity and cellular toxicity of the compounds was measured as previously described (Tebbe et al., 1997). In general, susceptible Hela cells were grown in 6-well tissue culture cluster plates. When confluent monolayers were formed, growth medium was removed and an appropriate dilution of virus was added. After adsorption for 1–2 h at room temperature, the infected cell sheet was overlaid with varying concentrations of the compounds to be tested. The compounds were dissolved in DMSO at a concentration of 20 mg/ml, and an aliquot was diluted to the desired concentration in DMSO before addition to the agar medium mixture. Plates were incubated at 34°C for rhinoviruses and 37°C for the enteroviruses until the DMSO control wells demonstrated plaques of optimal size. At this time, a solution containing 10% formalin and 2% sodium acetate was added to each well to inactivate the virus and fix the cell sheet

to the plastic surface. The fixed cell sheets were stained with 0.5% crystal violet and the plaques were counted. Results from duplicate wells at each concentration were averaged and compared with DMSO control wells.

2.3. Single dose studies

Compounds **4**, **5**, **6** and **7** were finely ground in a mortar and pestle and suspended in a vehicle of 10% acacia containing 0.2% polysorbate 80. Male CD-1 mice were administered a single dose of 125, 250, or 500 mg test article/kg body weight by gavage. Hepatic porphyrin concentrations were quantitated from two mice/dose level following collection of liver 24 h after dose administration. Plasma drug concentrations were measured from two mice/dose level. Whole blood was collected by cardiac puncture from CO_2 anesthetized mice (two per timepoint) at 2, 8, and 24 h after dosing. Plasma was obtained for analysis by centrifugation of the whole blood.

2.4. Multiple dose studies

Compounds **4** and **5** were finely ground in a mortar and pestle and suspended in a vehicle of 10% acacia containing 0.2% polysorbate 80. Male CD-1 mice were administered a single dose of 0, 5, 50, 125 or 500 mg test article/kg body weight by gavage daily for 7 days. Hepatic porphyrin concentrations were quantitated from three mice/dose level from liver collected 24 h after the final dose was administered. Livers from three mice/dose level were collected 24 h after the final dose was administered for measurement of cytochrome P450 activity. Kidney, liver and spleen weights were recorded at necropsy and limited clinical chemistry was also conducted.

2.5. Analysis of plasma drug concentrations

Test article and internal standard were extracted from plasma (0.25 ml) onto SCX solid–phase extraction cartridges (Isolute, 100 mg sorbent). Compounds were eluted using an elution solvent of 0.5% ammonium hydroxide in methanol. The solid phase extraction (SPE) eluant was evaporated to dryness under nitrogen and reconstituted in mobile phase prior to analysis by high pressure liquid chromatography (HPLC). The analytes were chromatographed on a SB-Phenyl column (4.6 mm ID $\times$ 250 mm length, 5 micron) using a mobile phase composed of 20 mM phosphoric acid/acetonitrile (1/1, v/v) delivered at a flow rate of 1 ml/min with electrochemical detection (screening voltage 0.4 V and detection voltage 0.6 V). The limit of quantitation was 5–10 ng/ml.

2.6. Microsome preparation

Liver samples from three animals per treatment group were collected and 25% (w/v) liver homogenates were prepared using ice-cold homogenization buffer, consisting of 1.15% potassium chloride (w/v) and 10 mM potassium phosphate, pH 7.4. The homogenates were centrifuged at approximately 4°C to obtain a 9000 $\times$ g supernatant (S9) fraction. A portion of the S9 fraction was further centrifuged at 225 000 $\times$ g (R_{max}), and the resulting microsomal pellet was resuspended in buffer consisting of 20% glycerol (v/v), 1 mM EDTA, 1 mM dithiothreitol, 20 (M butylated hydroxytoluene, and 100 mM potassium phosphate, pH 7.25.

2.7. Ethoxyresorufin O-deethylase assay

The rate of 7-ethoxyresorufin *O*-deethylation was measured in microsomal preparations using a fluorescent microtiter plate assay (Burke and Mayer, 1974; Glass and van Lier, 1988). The substrate, 7-ethoxyresorufin, was incubated with microsomes at 37°C in the presence of the cofactor, NADPH, and the rate of formation of the fluorescent product, resorufin, was determined fluorometrically. Enzyme activity is reported as nanomoles of resorufin produced per milligram of protein per hour. The enzyme activities obtained from this assay are used as an indication of cytochrome P450 1A activity in the liver.

2.8. Benzphetamine N-demethylase (BND) assay

The rate of BND was measured in microsomal preparations by the rate of formation of formalde-

hyde (Prough and Ziegler, 1977). The substrate, benzphetamine, was incubated with microsomes at 37°C in the presence of a NADPH regenerating system. After a 20 min incubation period, the reaction was terminated by addition of trichloroacetic acid, and the deproteinized sample was reacted with Nash reagent at 60°C to yield a colored product formed from formaldehyde. The product was measured spectrophotometrically. Enzyme activity is expressed as nanomoles of formaldehyde produced per milligram of protein per hour. The *N*-demethylation of benzphetamine is catalyzed primarily by the cytochrome P450 2B and 2C subfamilies of isozymes.

2.9. Cytochrome P450 assay

Cytochrome P450 was quantitated by a modification of the method of Omura and Sato (1964), which is based on the cytochrome P450 difference spectrum in microsomes in the presence or absence of carbon monoxide. This is a direct assay for cytochrome P450 content, and the data are expressed as nanomoles of cytochrome P450 per milligram of protein.

2.10. Hepatic porphyrin content

The total hepatic porphyrin content was determined for two animals per treatment group in the single does study and three animals per treatment group in the multiple dose study by a modification of the method of Labbe (1976). Liver samples were homogenized in extraction solvent, consisting of 20% glacial acetic acid, 80% ethyl acetate. They were then centrifuged, and the supernatant was collected. The extraction was repeated with an additional 12 vol. of solvent, and the supernatants were pooled. The total porphyrin content was measured fluorometrically with excitation and emission wavelengths of 405 and 585 nm, respectively. Protoporphyrin IX was used as a standard. The hepatic porphyrin content is expressed as μg protoporphyrin IX equivalents per g of liver.

2.11. Protein measurement

Protein was measured by the method of Lowry et al. (1951) using crystalline bovine serum albumin as a standard.

3. Results

3.1. In vitro antiviral activity

The four vinylacetylenes selected for the current investigation are the result of optimization of antiviral activity and plasma concentrations in mice using a screening model. The spectrum and potency of the compounds are illustrated in Table 1. These compounds all demonstrated good antiviral potency as well as good broad-spectrum activity in the viruses selected to test against.

3.2. Single dose study

In order to examine the effects of the acetylene functionality on liver enzymes and hepatic porphyrin levels as well as to establish exposure levels, a preliminary single dose study was conducted. Mice were administered several dose levels

Table 1
Antiviral potency and spectrum[a,b]

Compd	RV-1A	RV-2	RV-14	RV-16	PV-1	CA21	CA21M	CB3
1	0.050(8)	0.045(7)	0.10(8)	0.063(8)	0.155(7)	0.063(8)	0.085(7)	0.066(7)
4	0.027	0.069	0.060(6)	0.057	0.037	0.085	0.046	0.032
5	0.12	0.18	0.17(2)	0.26	0.12	0.19	0.23	0.14
6	0.049	0.054	0.061(3)	0.060	0.016	0.032	0.029	0.035
7	0.078	0.091	0.13(4)	0.098	0.067	0.10	0.062	0.095

[a] Values are IC_{50}'s in μg/ml, single determinations (except where noted), and were run with Enviroxime as a standard.
[b] The superscript number is the number of replicates.

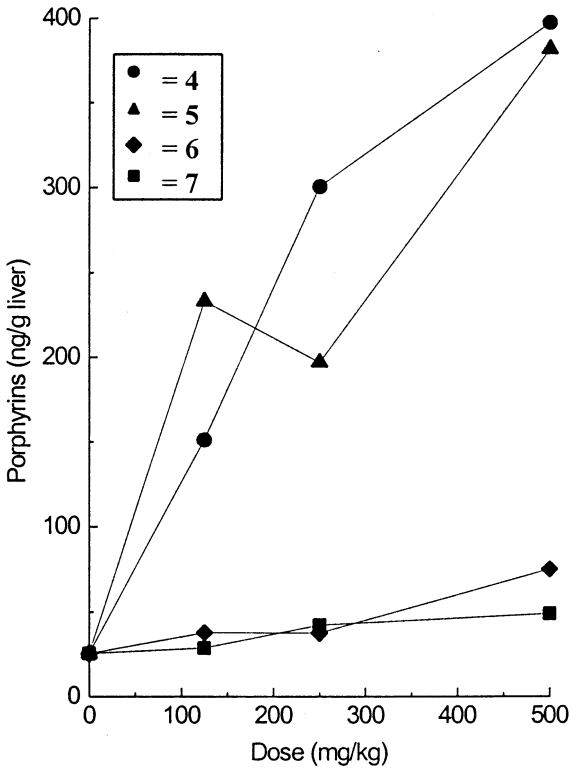

Fig. 3. Hepatic porphyrins measured 24 h after a single oral dose of compounds **4**, **5**, **6**, and **7**.

of the compounds, and the dose–response relationship for hepatic porphyrin accumulation was determined. In addition, plasma levels for the highest doses were compared for the four compounds. This was necessary since the current study was being conducted in a different vehicle from that of the original screening study. Accordingly, the compounds were reformulated from PVP-30 to 10% acacia containing 0.2% polysorbate 80. As depicted in Fig. 3, marked accumulation of porphyrins was seen in the livers of mice 24 h after dosing with compounds **4** and **5**. However, porphyrin accumulation was minimal with compounds **6** and **7**. The increases observed with compounds **4** and **5** were roughly dose–dependent, and at the highest dose hepatic porphyrin levels were elevated approximately 15-fold relative to controls. Determinations of plasma concentrations of drugs showed that only compounds **4** and **5** attained significant levels (Table 2). Of note is the fact that although compound **6** did obtain plasma concentrations at the highest dose (500 mg/kg) roughly equal to that found with compound **5** at the lowest dose (125 mg/kg), the compound did not induce porphyrins as significantly (3-fold vs. 9-fold, respectively). However, upon examination of the plasma concentration curves (data not shown) the exposure was significantly lower for compound **6** as compared to **5**.

3.3. Seven day study

Based on these preliminary single dose investigations, compounds **4** and **5** were selected for further investigation in a multiple dose study of one week duration conducted in mice. The purpose of the study was to determine if porphyrin accumulation increased with longer drug exposure. In order to establish whether the effects on heme metabolism were accompanied by destruction of cytochromes P450, several cytochrome P450-dependent enzymatic activities and the cytochrome P450 content were measured in hepatic microsomes from mice given 5, 50, 125, or 500 mg/kg oral doses of compounds **4** or **5** daily for seven days. Consistent with the effects seen after single doses, marked hepatic porphyrin accumulation was seen following multiple 125 and 500 mg/kg doses of both vinylacetylene compounds (Fig. 4). No effects of either compound **4** or **5** were seen with doses (50 mg/kg. With the 500 mg/kg dose administered for one week, hepatic porphyrin levels were approximately 2- to 3-fold higher than observed with a single 500 mg/kg dose (Fig. 3), indicating that the drug effects on

Table 2
C_{max} plasma concentrations of compounds **4**, **5**, **6** and **7** after oral administration

Dose level (mg/kg)	Peak concentration measured (ng/ml)[a]			
	4	**5**	**6**	**7**
125	10788	7893	1885	772
250	18610	21251	3161	928
500	22433	17705	7000	2469

[a] Values are a mean derived from two mice.

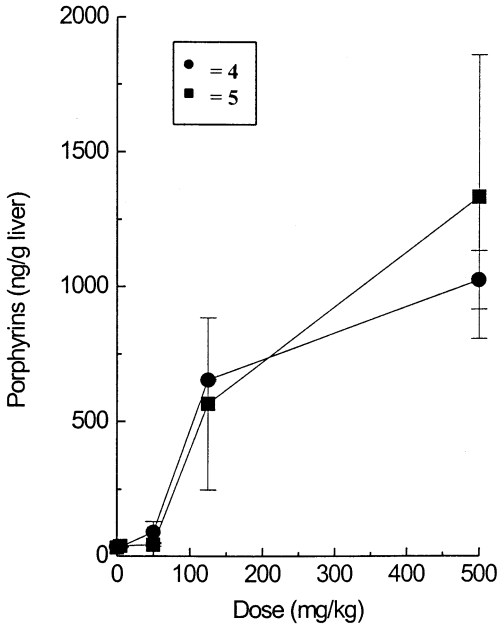

Fig. 4. Hepatic porphyrins measured on day 8 after seven daily oral Doses of 4 and 5.

hepatic heme levels increased with increasing duration of exposure to drug. These effects on heme metabolism were accompanied by losses in cytochrome P450 dependent activities (Fig. 5 and Fig. 6). In particular, loss of 7-ethoxyresorufin *O*-deethylase activity, a marker of catalytic activity for CYP1A, was seen at all doses (50 mg/kg, and maximal losses of 43% and 58% of control for compounds **4** and **5**, respectively. Interestingly, slight induction of cytochromes P450 was seen with 500 mg/kg doses. Compound **4** induced benzphetamine *N*-demethylase, a marker of catalytic activity for CYP2B by 39%, while compound **5** induced the total cytochrome P450 content by 47%. Thus, both loss of EROD activity suggestive of destruction of CYP1A, and induction of CYP2B or other cytochrome P450 isoforms were caused by compounds **4** and **5** in a dose–dependent manner.

3.4. Toxicological findings

Finally, these compounds showed effects on hepatic morphology and clinical chemistry consistent with early stage hepatotoxicity. Liver weights were increased in the multiple dose study 39 and 54% for compounds **4** and **5**, respectively, at the 500 mg/kg dose level. As well, these increases in liver weight were accompanied by hepatocellular hypertrophy and cellular vacuolization. Serum gamma-glutamyl transpeptidase (GGT) was elevated up to 3-fold for both compounds. Vinylacetylene **4** produced additional effects with approximately 2-fold increases in serum alkaline phosphatase and serum alanine aminotransferase (ALT). Taken together, these findings could be an early indication of drug induced hepatic porphyria.

4. Discussion

The antirhino- and enteroviral benzimidazoles have undergone extensive evaluations in efforts to define a series of compounds to take into the clinic to test the efficacy of an oral drug for treatment of the common cold. Two compounds were advanced into the clinic in the early 1980's to try to address this question; however, both com-

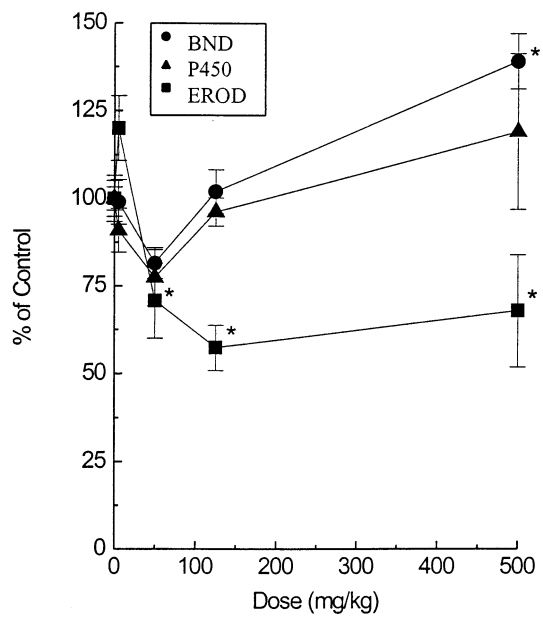

Fig. 5. Effect of 4 on P450 measured on day 8 after seven daily oral doses.

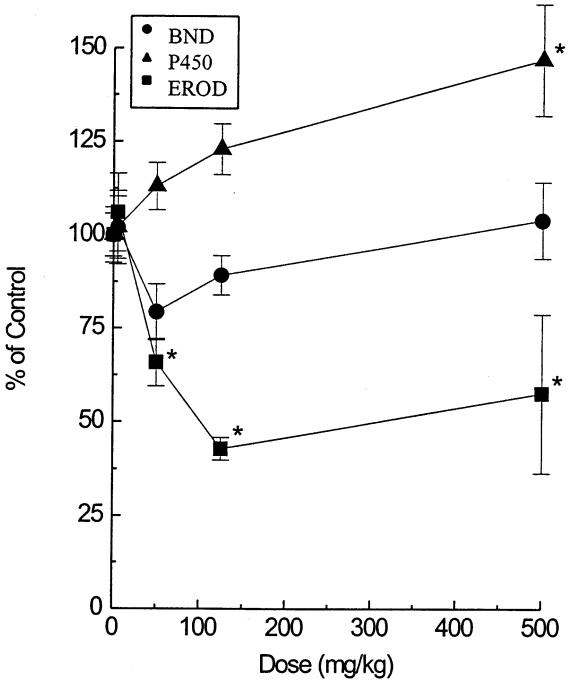

Fig. 6. Effect of 5 on P450 measured on day 8 after seven daily oral doses.

pounds showed poor oral bioavailability in humans. A series of vinylacetylene benzimidazoles was found to produce improved oral bioavailability which subsequently allowed toxicology studies to be conducted in mice. Although there were no gross clinical observations noted from these mice, single and multiple dose toxicology studies revealed a pronounced effect on liver enzymes and hepatic porphyrin levels. Additionally, the multiple dose studies showed significant increases in liver weights as well as increases in serum levels of enzymes suggestive of hepatotoxicity. These effects on the liver are almost certainly due to the acetylene moiety since the structurally similar analogs Enviroxime (**1**) and Enviradene (**2**) did not appear to show any adverse effects on the liver during their respective preclinical and clinical evaluations. Although the interaction of acetylenes with cytochromes P450 can lead to enzymatic inactivation, there are many drugs on the market which contain an acetylene functionality, and the presence of an alkyne is not commensurate with liver toxicity. Nonetheless, in this series of compounds, there appear to be toxic effects from the acetylene functionality. Based on the severe effects of these compounds on cytochromes P450 and their ensuing effects on heme metabolism as well as consideration of the mild and self-limiting nature of the disease state being targeted, further work in this series was terminated. However, the information gained in this study has served as an additional guide to subsequent research efforts.

References

Bopp, R.J., Quay J.F., 1985 Unpublished results.

Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: direct fluorometric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos. 2, 583–588.

Chan, W.K., Sui, Z., Oritz de Montellano, P.R., 1993. Determinants of protein modification versus heme alkylation: inactivation of cytochrome P450 1A1 by 1-ethynylprene and phenylacetylene. Chem. Res. Toxicol. 6, 38–45.

DeLong, D.C., 1984. Effect of enviroxime on rhinovirus infections in humans. In: Leive, L., Schlessinger, D. (Eds.), Microbiology. American Society for Microbiology, Washington, DC, pp. 431–434.

DeLong, D.C., Nelson, J.D., Wu, C.Y.E., Warren B.R., Wikel, J.H., Chamberlin, J., Montgomery, D., Paget, C.J., 1978. Virus inhibition studies with AR-336 I. Tissue Culture Activity. Abstracts of the Annual Meeting of the American Society for Microbiology, Abstracts S128, Washington, DC, p. 234.

DeLong, D.C., Reed, S.E., 1980. Inhibition of rhinovirus replication in organ culture by a potential antiviral drug. J. Infect. Dis. 141, 87–91.

Enviradene-IND, 1981a. Enviradene (IND 20204); IND Protocol No. 3, unpublished results pp. 1475–1461.

Enviradene-IND, 1981b. Eviradene (IND 20204); Virology Section, Summary, unpublished results p.58.

Enviradene-IND, 1984. Enviradene (IND 20204), Twelve-Month Report, unpublished results, pp. 1450–1456.

Foroozesh, M., Primrose, G., Guo, Z., Alworth, W.L., Guengerich, F.P., 1997. Aryl acetylenes as mechanism-based inhibitors of cytochrome P450-dependent monooxygenase enzymes. Chem. Res. Toxicol. 10, 91–102.

Glass, S.J., van Lier, R.B.L., 1988. Metabolism of 7-ethoxyresorufin by phenobarbital or Aroclor 1245 pretreated mice. Drug Metab. Dispos. 16, 333–334.

Herrmann, E.C., Jr., Herrmann, J.A., DeLong, D.C., 1981. Comparison of the antiviral effectsof substituted benzimidazoles and guanidine in vitro and in vivo. Antiviral Res. 1, 301–314.

Hopkins, N.E., Foroozesh, M.K., Alworth, W.L., 1992. Suicide inhibitors of cytochrome P450 1A1 and P450 2B1. Biochem. Pharmacol. 44, 787–796.

Labbe, R.F., 1976. Porphyrins and related compounds. In: Teitz, N.W. (Ed.), Fundamentals of Clinical Chemistry. W.B. Saunders, Philadelphia, PA, pp. 455–473.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Marks, G.S., McCluskey, S.A., Mackie, J.E., Riddick, D.S., James, C.A., 1988. Disruption of hepatic heme biosynthesis after interaction of xenobiotics with cytochrome P-450. FASEB. J. 2, 2774–2783.

Miller, F.D., Monto, A.S., DeLong, D.C., Exelby, A., Bryan, E.R., Srivastava, S., 1985. Controlled trial of enviroxime against natural rhinovirus infections in a community. Antimicrob. Agents Chemother. 27, 102–106.

Omura, T., Sato, R., 1964. The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem. 239, 2370–2378.

Oritz de Montellano, P.R., Correia, M.A., 1983. Suicidal destruction of cytochrome P-450 during oxidative drug metabolism. Annu. Rev. Pharmacol. Toxicol. 23, 481–503.

Oritz de Montellano, P.R., Kunze, K.L., 1980. Self-catalyzed inactivation of hepatic cytochrome P-450 by ethynyl substrates. J. Biol. Chem. 255, 5578–5585.

Prough, R.A., Ziegler, D.M., 1977. The relative participation of liver microsomal amine oxidase and cytochrome P-450 in *N*-demethylation reactions. Arch. Biochem. Biophys. 180, 363–373.

Ross, J., 1997. USP Dictionary of USAN and International Durg Names. United States Pharmacopeial Convention, Rockville, MD.

Tebbe, M.J., Spitzer, W.A., Victor, F., Miller, S.C., Lee, C.C., Sattelberg, T.R., McKinney, E., Tang, J.C., 1997. Antirhino/enteroviral vinylacetylene benzimidazoles: a study of their activity and oral plasma levels in mice. J. Med. Chem. 40, 3937–3946.

Victor, F., Brown, T.J., Campanale, K., Heniz, B.A., Shipley, L.A., Su, K.S., Tang, J., Vance, L.M., Spritzer, W.A., 1997a. Synthesis, antiviral activity, and biological properties of vinylacetylene analogs of enviroxime. J. Med. Chem. 40, 1511–1518.

Victor, F., Loncharich, R., Tang, J., Spitzer, W.A., 1997b. Synthesis and antiviral activity of C2 analogs of enviroxime: an exploration of the role of critical functionality. J. Med. Chem. 40, 3478–3483.

Wikel, J.H., Paget, C.J., DeLong, D.C., Nelson, J.D., Wu, C.Y.E., Paschal, J.W., Dinner, A., Templeton, R.J., Chaney, M.O., Jones, N.D., Chamberlin, J.W., 1980. Synthesis of syn and anti isomers of 6-[[(Hydroxyimino)phenyl]methyl]-1[(1-mehtylethyl)sulfonyl]-1H-benzimidazol-2-amine. Inhibitors of rhinovirus multiplication. J. Med. Chem. 23, 368–372.