The Anticarcinogen 3,3'-Diindolylmethane is an Inhibitor of Cytochrome P-450

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ABSTRACT: Dietary indole-3-carbinol inhibits carcinogenesis in rodents and trout. Several mechanisms of inhibition may exist. We reported previously that 3,3'-diindolylmethane, an in vivo derivative of indole-3-carbinol, is a potent noncompetitive inhibitor of trout cytochrome P450 (CYP) 1A-dependent ethoxyresorufin O-deethylase with Ki values in the low micromolar range. We now report a similar potent inhibition by 3,3'-diindolylmethane of rat and human CYPlA1, human CYPlA2, and rat CYP2B1 using various CYP-specific or preferential activity assays. 3,3'-Diindolylmethane also inhibited in vitro CYP-mediated metabolism of the ubiquitous food contaminant and potent hepatocarcinogen, aflatoxin B1. There was no inhibition of cytochrome c reductase. In addition, we found 3,3'-diindolylmethane to be a substrate for rat hepatic microsomal monoxygenase(s) and tentatively identified a monohydroxylated metabolite. These observations indicate that 3,3'-diindolylmethane can inhibit the catalytic activities of a range of CYP isoforms from lower and higher vertebrates in vitro. This broadly based inhibition of CYP-mediated activation of procarcinogens may be an indole-3-carbinol anticarcinogenic mechanism applicable to all species, including humans. © 1995 John Wiley & Sons, Inc.

KEY WORDS: Indole-3-Carbinol, 3,3'-Diindolylmethane, Aflatoxin B1, Cytochrome P-450, CYPlA, CYP2B1.

INTRODUCTION

Indole glucosinolates are found in high concentrations in the Cruciferae family of vegetables (1), whose members include cabbage, Brussels sprouts, broccoli, and cauliflower. Glucobrassicin is the most abundant of all glucosinolates and, after enzymatic hydrolysis by the enzyme myrosinase, yields indole-3-carbinol (I3C), glucose, and isothiocyanate anion. When fed to experimental animals, I3C has been shown to possess potent tumor-modulating properties, in most cases inhibiting tumor incidence (2–4), but with some protocols, I3C enhances tumor incidence (5,6) or preneoplastic lesions (7). The primary breakdown product of I3C in aqueous solution is a dimer of I3C, 3,3'-diindolylmethane (I33') (Figure 1) (8), and I33' can be prepared in high yield by simply refluxing I3C in neutral solution (9). Under acidic conditions, as found in the stomach, I3C quickly and irreversibly condenses with itself to yield, in addition to I33', several other oligomeric derivatives as major products (10,11). Thus, I33' has been found as a major product present in vivo after oral administration of I3C, whereas I3C was not detected (12,13) or was found in much lesser amounts (14). When aflatoxin B1 (AFB1) was coadministered with I33' or I3C into rainbow trout embryos, a profound reduction in hepatic AFB1-DNA binding and tumor incidence was observed (15), but only in animals given I33'. Wattenberg and Loub (16) observed that oral administration of I3C or I33' to rats inhibited mammary tumor formation induced by 7,12-dimethylbenz[a]anthracene in female Sprague–Dawley rats and neoplasia of the forestomach induced by benzo[a]pyrene in female ICR/Ha mice. These data show that I33' itself can be anticarcinogenic and that I33' may be a major factor in the anticarcinogenicity observed with orally administered I3C.

Several anticarcinogenic mechanisms may exist for I33'. Previous studies have shown that oral (17,18) or i.p. (19) administration of I33' can induce hepatic CYPs

*Abbreviations: I3C, indole-3-carbinol; I33', 3,3'-diindolylmethane; AFB1, aflatoxin B1; CYP, cytochrome P-450; QR, quinone reductase; UDPGT, uridine diphosphate glucuronosyl transferase; GST, glutathione S-transferase; PB, phenobarbital; BNF, β-naphthoflavone; AFB1, aflatoxin M1; AFO, aflatoxin G1; AFB1, aflatoxin M1; AFO, aflatoxin G1; PROD, pentoxysorbinsulfate; EROD, ethoxyresorufin O-deethylase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; BHA, butylated hydroxyanisole; EI, electron impact; PAH, polycyclic aromatic hydrocarbons; DBA, dibenz(a,h)anthracene.
FIGURE 1. Structure of 3,3'-diindolylmethane (I33'), a primary acid condensation product of I3C.

1A1 and 1A2 or associated activities in rats. CYP1A2 protein or activity can also be induced in trout embryos microinjected with I33' (20), or in primary cultures of rat and monkey hepatocytes exposed to I33' in the culture media (21,22). It has been suggested that this induction is responsible for altered carcinogen metabolism leading to reduced tumor incidence (23). In addition, administration of I33' to rats was found to induce CYP1A2 and microsomal estradiol 2-hydroxylation when given i.p. (19) and 4-androstenedione metabolism, when given i.p. or p.o. (24). Enhancement of estradiol 2-hydroxylation or 4-androstenedione metabolism are suggested as mechanisms responsible for the antiestrogenic effects of I3C given orally (19,24).

Inhibition of phase II enzymes, such as quinone reductase (QR), uridine diphosphate glucuronosyl transferase (UDPGT), and glutathione S-transferase (GST), is an established mechanism of protection against carcinogenesis (25,26). Treatment of rat or monkey hepatocytes in primary cell culture with I33' resulted in induction of QR and UDPGT (12,22), but not GST (21,22). However, administration of I3C orally (and therefore also I33' and other I3C oligomers) to trout or mice failed to induce monooxygenases (27-29), GST, or UDPGT activity (28,30), yet protection against carcinogen-DNA binding or tumorigenesis was observed. It was recently observed that I33' could inhibit mutagenesis induced by AFB, 8,9-epoxide in the Salmonella mutagenesis assay, suggesting a role for direct electrophile trapping as a means of protection (31).

CYP comprises a superfamily of enzymes that have various endo- and xenobiotics as substrates (32). In general, CYPs oxidize xenobiotics to more polar, nontoxic products; however, activation to carcinogenic metabolites is sometimes a sequela (33). When tested in vitro, I33' exhibited inhibition of CYP activity (20,24,34,35). To further characterize the effects of I33' as an in vitro inhibitor, we examined inhibition of rat and human CYP activities. In addition, we examined the potency of I33' to modulate the in vitro oxidative metabolism of the ubiquitous food contaminant and potent hepatocarcinogen AFB₃ (36). Limited studies on in vitro microsomal metabolism of [3H]-I33' were also performed. Our results show that, in addition to trout, I33' is a potent nonspecific in vitro inhibitor of rat and human-CYP activities. In all three species, inhibition constants are in the low to submicromolar range. In vivo levels of I33' after administration of an anticarcinogenic dose of I3C to rat or trout are near inhibition constant values suggesting that, under these conditions, I33' should be an inhibitor of CYP in vivo as well. Thus, I33' inhibition of CYP-mediated activation may be an additional mechanism of anticarcinogenesis that could apply to all animal species, including humans.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats were obtained as weanlings from Simonsen's (Gilroy, CA) and housed at the Laboratory Animal Resource Center at Oregon State University. Rats were maintained on a AIN-76A semipurified diet (U.S. Biochem. Corp., Cleveland, OH) and received diet and drinking water ad libitum. To induce CYP1A, rats received BNF (40 mg/kg) suspended in approximately 0.5 mL of corn oil, by i.p. injection for 4 consecutive days, were starved on day 4, and sacrificed on day 5. To induce CYP2B1, rats received drinking water containing 0.1% PB for seven consecutive days, were starved on day 7, and sacrificed on day 8 by CO₂ asphyxiation.

Chemicals

Ethoxyresorufin, pentoxyresorufin, and resorufin were obtained from Molecular Probes Inc. (Eugene, OR). Aflatoxins B₇, Q₇, M₇, and G₇, cytochrome C, NADPH, and BNF were obtained from Sigma Chemical Co. Ltd (St. Louis, MO). Aflatoxin B, 8,9-epoxide-glutathione conjugate was a gift of Dr. David Eaton of the University of Washington. Aflatoxin B, 8,9-epoxide was a gift of Dr. Thomas Harris of Vanderbilt University. I33' was synthesized according to the method of Leete and Marion (9) in a neutral solution and shown to be pure by HPLC. Acetanilide, 3-OH acetanilide, 4-OH acetanilide, and I3C were purchased from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were purchased from Sigma. The method of Dash-
wood et al. (37) was used to tritium-label I3C at the 5 position. The [3H]-I3C was diluted with cold carrier to an approximate specific activity of 270 mCi/mmol. An acid reaction mixture of products originating from [3H]-I3C was generated according to the method of Bjeldanes et al. (38). We isolated [3H]-I33' from this mixture by HPLC using a Beckman ODS 5u 4.6 × 250 mm analytical column (Palo Alto, CA). Starting solvent concentrations were 20% acetonitrile and 80% Milli-Q water (Millipore Corp., Bedford, MA). These conditions were held for 30 seconds before increasing to 85% acetonitrile over the next 29.5 minutes (linear gradient). After elution for 5 minutes with 85% acetonitrile, the most hydrophobic products were eluted by increasing to 100% acetonitrile over the following 5 minutes, holding for an additional 5 minutes, and then returning to starting conditions over the next 10 minutes. The mobile phase flow rate was 1 mL/min. Metabolites were monitored by UV absorbance at 280 nm using a Shimadzu SPD-6AV spectrophotometer detector (Kyoto, Japan). The retention time for I33' under these conditions was routinely 24.4 minutes.

Preparation of Microsomal and Cytosolic Enzymes

Microsomes were prepared from rat liver by differential centrifugation according to the method of Guengerich (39). Cytosol from BHA-induced mice, used to quantify production of AFB1, 8,9-epoxide, was a gift from Dr. David Eaton. Protein concentrations of cytosol and microsomes were determined according to the method of Lowry et al. (40). Control, CYP1A1, CYP1A2, and microsome products, prepared from a human lymphoblastoid cell line, were purchased from Gentest (Woburn, MA). CYP1A1 microsome products were prepared from cells treated with dibenz(a,h)anthracene (DBA) that greatly induces the low levels of natively expressed CYP1A1. Any CYP1A2 present in the CYP1A1 microsome product that might be induced (as occurs in hepatic tissue following exposure to CYP1A1 inducers) is estimated at less than 10% the content of CYP1A1 (Dr. C. Crespi, Gentest Corp., personal communication). CYP1A2 microsome product was prepared from cells transfected with human CYP1A2 cDNA. Microsomes from the native, uninduced cell line that expresses only low levels of CYP activity (CYP1A1) were used as control for CYP1A2-mediated acetaniline 4-hydroxylase assay.

Enzyme Assays

The EROD assay was conducted essentially as described by Burke et al. (41), but modified to include MgCl2 (5 mM) and bovine serum albumin (1.6 mg/mL) in the reaction buffer as suggested by Pohl and Fouts (42) to enhance activity. EROD determinations were performed using liver microsomes from BNF-treated rats, or microsomes from a DBA-induced human lymphoblastoid cell line. The assay was conducted at 37°C. The PROD assay was conducted according to the method of Burke et al. (41), at 37°C using microsomes from PB-treated rats. For both EROD and PROD, the slight quenching of fluorescence by I33' was compensated for by including the inhibitor in the standard curve solutions at the concentrations used in the assay. Cytochrome c reductase activity, which we used as an indirect measure of microsomal NADPH-cytochrome P450 reductase, was determined according to the method of Yasukochi and Masters (43).

The metabolism of AFB1 was determined essentially as described by Monroe and Eaton (44) using liver microsomes from BNF-treated rats. This assay allows simultaneous quantification of hydroxylated metabolites and AFB1, 8,9-epoxide by trapping the latter as a stable glutathione conjugate. In the presence of mouse cytosol, trapping efficiency of the epoxide as the conjugate has been reported to be greater than 99% (44). I33' was added to the reaction mixture at final concentrations of 10, 50, or 100 μM in DMSO (final DMSO concentration was 2% v/v) and preincubated at 37°C for 5 minutes before initiating the reaction with AFB1. The final reaction mixture included 1 mg/mL microsomal protein, 3 mg/mL BHA-induced mouse cytosolic protein, 16 or 124 μM AFB1, 5 mM GSH, 1 U/mL glucose-6-phosphate dehydrogenase, 5 mM glucose-6-phosphate, and 1 mM NADP+ in a buffer containing 190 mM sucrose, 60 mM potassium phosphate, 80 mM Tris, 15 mM NaCl, 5 mM KCl, and 4 mM MgCl2, pH 7.6 in a reaction volume of 250 μL. After 10 minutes [within the linear range for metabolite production (45)], the reaction was terminated by the addition of 50 μL 2 M acetic acid and 10 μL methanol containing the internal standard. The mixture was then frozen for at least 2 hours. The frozen mixture was then subjected to centrifugation at 14,000 g for 4 minutes at ambient temperature to thaw the mixture and to pellet the precipitated protein. Metabolites were resolved by HPLC on a 4.6 × 250 mm, C18 Econosphere cartridge column (Alltech Associates, Deerfield, IL) and detected by UV absorption at 362 nm. Quantification of metabolites was achieved with a Shimadzu Chromatopac integrator (Kyoto, Japan) using an AFB1 standard curve and AFG, as an internal standard to correct for recovery. The mobile phase consisted of a combination of 0.1% ammonium phosphate, pH 3.5 (solvent A) and 95:5 methanol:THF (solvent B). From 0 to 2 minutes, the concentration of solvent B was increased from 10 to 24%, then further increased to 38% solvent B at 13 minutes 60% at 16 minutes, and 90% at 17 minutes. At 20
minutes, the mobile phase was returned to starting conditions over a period of 5 minutes. All changes in the composition of the mobile phase were accomplished using a linear gradient. The flow rate was kept constant at 1.0 mL/min. The concentration of the AFB, stock solution (prepared in DMSO) was determined by UV absorbance spectrophotometry using an extinction coefficient of 21.8 mM⁻¹ cm⁻¹. The final concentration of AFB, in the assay was calculated by adding a known amount of an AFB, stock solution. When mouse cytosol was included in the absence of microsomes, no AFB, 8,9-epoxide-GSH conjugate was detected.

CYP1A2-mediated 4-hydroxylation of acetanilide was conducted essentially as described by Liu et al. (46). The final reaction mixture contained 0.1-3.0 mM acetanilide, 0-100 μM I33' delivered in DMSO (final concentration DMSO 1% v/v), 1 U/mL glucose-6-phosphate dehydrogenase, 10 mM glucose-6-phosphate, 0.5 mM NADPH, 10 mM potassium phosphate (pH 7.4), 1 mM MgCl₂, and 0.2 mM EDTA. Following incubation at 37°C for 2 minutes, 0.1 mg microsomal protein was added to initiate the reaction. The final volume of the incubation was 100 μL. Incubations in the absence of microsomes, in the presence of heat-inactivated microsomes, or lacking an NADPH-regenerating system were run concurrently as controls. After 30 minutes, the reaction was terminated by the addition of 100 μL cold acetonitrile, and the mixture was cooled on ice for 30 minutes prior to centrifugation at 10,000 g for 10 minutes at 4°C. The mixture was frozen until analysis of the supernatant by HPLC under the conditions described previously for the isolation of ['H]-I33'. Metabolites were monitored at a wavelength of 280 nm and by radioisotope detection using an online Beckman M171 radioisotope detector (liquid cell) using 3a70B liquid scintillation cocktail (Research Products International, Mount Prospect, IL) at a flow rate of 2 mL/min.

**Mass Spectrometry**

Low-resolution electron-impact (EI) mass spectral analysis was performed on a Finnigan model 4023 quadrupole mass spectrometer upgraded with a model 4500 source and a Varian model 3400 gas chromatograph at the Environmental Health Sciences Center at Oregon State University. The mass spectrometer is controlled by a Galaxy 2000 data system (LGC Co., San Jose, CA). Chromatography was performed using an Alltech (Deerfield, IL) SE-54 column (10 m × 0.25 mm i.d. with a 0.25 μm coating) operated at injector pressures of 3 psi. EI mass spectra were obtained at an electron energy of 70 eV at a source temperature of 140°C. To facilitate GC/MS analysis, active hydrogens on the indole or alcohol of the putative hydroxylated metabolite of I33' were silylated by dissolving the anhydrous sample in 20 μL of anhydrous pyridine followed by 20 μL of N-methyltrimethylsilyl trifluoroacetamide solution (Pierce Biochemical Co., Rockford, IL). The septum-topped vial was heated at 60°C for 0.5 hour prior to injection onto the GC (usually 4 μL).

**Kinetic Analysis**

Kᵢ and Kᵥ values were determined by nonlinear regression using computer analysis (47). Data were fit to a competitive or noncompetitive inhibition model, based on visual examination of 1/S vs. 1/v plots. Inhibition was deemed either noncompetitive or com-
FIGURE 2. Lineweaver–Burke plot of inhibition of rat liver microsomal EROD by 3,3'-diindolylmethane (133'). Incubations were performed as described in the Materials and Methods section and included 5 μg/mL protein. Points represent the means of three determinations ±SE. $V_{\text{max}} = 20.3 \pm 6.0 \text{ nmol/min/mg protein}$; $K_\text{s} = 205 \pm 24 \text{ nM}$; $K_\text{m} = 2.2 \pm 0.2 \text{ μM 133'}$.

FIGURE 3. Lineweaver–Burke plot of inhibition of human CYP1A1-catalyzed EROD by 3,3'-diindolylmethane (133'). Incubations were performed as described in the Materials and Methods section and included 50 μg/mL protein. Points represent means of three determinations ±SE or means of two determinations ± range. $V_{\text{max}} = 114 \pm 7.0 \text{ pmol/min/mg protein}$; $K_\text{s} = 301 \pm 50 \text{ nM}$; $K_\text{m} = 7.4 \pm 2.0 \text{ μM 133'}$; $K_\text{m} = 13 \pm 2.7 \text{ μM 133'}$.

Figure 4 depicts 133' noncompetitive inhibition of rat liver microsomal PROD, an activity associated with

**Results**

**In Vitro Inhibition of EROD and PROD**

In a recent study, we found 133' to be a potent noncompetitive inhibitor of trout liver microsomal EROD, with a $K_\text{s}$ value of $2.7 \pm 0.4 \text{ μM 133'}$ and a $K_\text{m}$ value of $13.7 \pm 2.2 \text{ μM 133'}$ (35). With rat microsomes (Figure 2), inhibition appeared competitive and a $K_\text{m}$ value of $2.2 \pm 0.2 \text{ μM 133'}$ was determined. Ethoxyresorufin-O-deethylase is catalyzed primarily by CYP1A in trout (49) and CYP1A1 in rat (50) when animals have been induced by BNF or similar inducer. I3C (100 μM) had a much weaker, though measurable, inhibitory effect on resorufin production (data not shown); however, inhibition due to 133' (formed from decomposition of I3C in an aqueous medium) could not be ruled out, and no attempt was made to determine $K_\text{s}$ values for this compound.

Figure 3 shows I33' inhibition of human CYP1A1-mediated EROD. The activity of these microsomes was relatively low compared with BNF-induced animals [$V_{\text{max}} = 114 \text{ pmol/min/mg protein vs. } 600 \text{ pmol/min/mg protein (trout) and } 20,300 \text{ pmol/min/mg (rat)}$]. Though these data are somewhat imprecise, the fitted lines are more compatible with noncompetitive inhibition and yield a $K_\text{s}$ value of $7.4 \pm 2.0 \text{ μM 133'}$ and a $K_\text{m}$ value of $13 \pm 2.7 \text{ μM 133'}$.

In some cases, the correct model choice was not unequivocal since computer-drawn regression curves reflect experimental error.

**Statistics**

Data for inhibition of AFB$_1$ metabolite production and inhibition of cytochrome $c$ reductase were analyzed by one-way analysis of variance, and differences between specific means were compared using least sign-
CYP2B1. Kinetic constants were determined to be $K_{in} = 0.62 \pm 0.08 \mu M$ I33' and $K_{iu} = 1.2 \pm 0.60 \mu M$ I33'.

Inhibition of Acetanilide 4-Hydroxylase

Figure 5 shows the inhibition of expressed human CYP1A2-mediated acetanilide 4-hydroxylase at concentrations of 10 and 50 $\mu M$ I33'. While there is clear evidence for inhibition, the data intersects are inadequate to suggest inhibitory mechanism unequivocally. Competitive inhibition was judged to best fit the data, and a $K_i$ value of 7.6 $\pm$ 4.1 $\mu M$ I33' was determined. No acetanilide 4-hydroxylase activity was observed in incubations containing control microsomes that express only a low level of CYP1A1 activity (see the Materials and Methods section).

Inhibition of In Vitro AFB1 Metabolism

Figures 6A and 6B depict the results of an experiment assessing inhibition of in vitro metabolism of AFB1 by I33' using BNF-induced rat liver microsomes. Inhibition was examined at two concentrations (16 and 124 $\mu M$) of AFB1, and three concentrations (10, 50, and 100 $\mu M$) of I33'. At the low concentration of AFB1, the velocity of CYP1A1-catalyzed conversion of AFB1 to AFM1 (51) was inhibited 14, 32, and 46% at the three concentrations of inhibitor tested (Figure 6A). Inhibition of AFB1, 8,9-epoxide-glutathione conjugate (i.e., AFB1, 8,9-epoxide) formation occurred in a nearly identical concentration-dependent manner (9, 31, and 47% inhibition) (Figure 6A).

At the high concentration of AFB1 used (124 $\mu M$), I33' inhibition of AFM1 production was not as strong as that seen at the lower concentration. Inhibition was 11, 14, and 29% at 10, 50, and 100 $\mu M$ I33', respectively (Figure 6B). In contrast to the findings at the lower concentration of AFB1, the degree of inhibition of AFB1, 8,9-epoxide-glutathione conjugate production at the higher concentration was nearly twice that of AFM1 (Figure 6B). Calculated noncompetitive inhibition constants for AFB1, 8,9-epoxidation were $K_\text{in} = 138 \pm 43 \mu M$ I33', $K_\text{iu} = 52 \pm 11 \mu M$. Competitive inhibition was apparent with AFM1 formation, and the calculated $K_i$ value was $128 \pm 24 \mu M$ I33'. At this concentration of AFB1, we were able to accurately quantify production of AFQ1. Its production, mediated by CYP3A, was inhibited 17, 42, and 54% at the three concentrations of I33' used (Figure 6B). Other peaks, representing AFP and two unidentified metabolites, also showed inhibition, but peak areas could not be reliably quantified.
Inhibition of AFB, 8,9-epoxide-glutathione conjugate formation by I33' was not observed when AFB, 8,9-epoxide was added in the presence of increasing concentrations of mouse cytosol (data not shown).

I33' (100 μM) had no apparent effect on the ability of PB-induced rat liver microsomes to reduce cytochrome c (data not shown), indicative of lack of inhibition of NADPH-cytochrome P-450 reductase.

### Metabolism of [3H]-I33'

When microsomes from BNF-treated rats were incubated with 100 μM [3H]-I33' in the presence of NADPH for 30 minutes, a major metabolite with an HPLC retention time of 18.7 minutes was produced at the rate of 49 ± 13 pmol/min/mg protein. Electron-impact mass spectral analysis of this peak revealed a compound possessing an m/z ratio of 478, and a fragmentation pattern consistent with a trimethylsilylated derivative of monohydroxylated I33' (Figure 7). Mass spectral data for the N-trimethylsilyl derivative of the putative hydroxylated I33': m/z 479 (86), 478 (100, M⁺), 405 (15), 333 (1.4), 290 (4.2), 202 (8.3). The fragmentation pattern did not permit the assignment of the position of the putative hydroxyl moiety.

### DISCUSSION

**Inhibition of CYP Isoform-Specific Reactions**

According to Wattenberg (25), chemopreventive agents, compounds that prevent the occurrence of cancer, may be classified on the basis of mechanism of action. One category is inhibitors of enzymes, such as CYP, which activate procarcinogens. Results presented here and elsewhere (35) show that I33', a nonenzymatic product of I3C, is a potent inhibitor of reactions catalyzed preferentially by CYP1A1/CYP1A2 (trout, rat, or human), CYP2B1, and CYP3A (rat), enzymes known to activate carcinogens. In liver microsomes from BNF-induced rats, EROD activity is attributed primarily to CYP1A1, with a minor contribution from CYP1A2 (50). CYP1A is also responsible for EROD activity in trout (49,52). Human CYP1A1 is essentially the only CYP enzyme present in the microsomes from the lymphoblastoid cell line, and therefore the inhibition of EROD by I33' is due to inhibition of this isoform. We also found I33' to strongly inhibit the activity of human CYP1A2. Expression of CYP1A2 is largely confined to hepatic tissue in humans (53), whereas CYP1A1 appears to be expressed only in extrahepatic tissues (53–55). Inhibition of CYP1A proteins by I33' in these tissues would be expected to protect against carcinogens that are CYP1A-activated, including PAHs, heterocyclic amines (33,54,56), and the mycotoxin, AFB, (57).

In hepatic microsomes from PB-induced rat, CYP2B1 is most probably responsible for the high level of PROD activity observed (41). This isoform can activate a number of carcinogens (33), including 4-(methylisothiocyanate)-1-(3-pyridyl)-1-butanone (MNK) (58). Though present only in low levels in liver, it is constitutively expressed in mammalian lung (59,60). Potent inhibition of CYP2B might therefore contribute to the
I3C inhibition of NNK-induced lung tumorigenesis in mice (3).

We also examined the ability of I33’ to inhibit activation of the hepatocarcinogen AFB, a common contaminant of human food supplies. As shown previously, the contributions of the various CYP enzymes to AFB, microsomal metabolism vary with initial substrate concentration (45,57,61), particularly in animals pretreated with I3C or BNF (61). However, in the rat it appears that primarily CYP2C11 (62,63) and CYP3A (63) contribute to activation reactions. CYP1A and 3A are associated with detoxication reactions that form AFM, (64) and AFQ, (65), respectively.

Failure of I33’ to inhibit NADPH-cytochrome P-450 reductase, at up to 100 μM I33’, is consistent with inhibition of catalytic activity via interaction with the hemoprotein component of CYP. Indeed, we observed that I33’ is a substrate of a microsomal and NADPH-dependent enzyme, forming a putative monohydroxylated form of I33’, consistent with a typical CYP-catalyzed product.

I33’ Inhibition of CYP as a Mechanism of Protection against AFB1 Carcinogenesis

Dietary I3C is a potent inhibitor of AFB, carcinogenesis in the trout (2) and rat (3). A decrease in hepatic in vivo AFB,-DNA binding is associated with induction of specific phase I and phase II AFB,-metabolizing enzymes by I3C in the rat (61,66), but not in trout (28), suggesting that other mechanisms apply for the latter species. Takahashi et al. (31) have recently shown inhibition of trout microsome-catalyzed AFB,-DNA binding by I33’ in vitro, and we extend those results to the rat by an alternate method, showing inhibition of formation of the major genotoxic AFB, metabolite, AFB, 8,9-epoxide. It is possible that the decrease in epoxide formation is attributable to nucleophilic trapping of the epoxide by I33’. However, this occurrence seems unlikely under these conditions, given the AFB, 8,9-epoxide trapping efficiency of mouse cytosol in the presence of glutathione (44). Rather, I33’ may inhibit in vivo CYP activation of AFB, in the rat, as was concluded for the trout model (15). This mechanism of protection could occur in addition to induction of GST (66). Because I33’ inhibits human CYP1A2, oral administration of I33’ may offer a means of inhibiting CYP1A2 activation of AFB, (57). However, interpretation of our data is not unequivocal because I33’ also inhibits phase I detoxication pathways (i.e., AFM, and AFQ, formation). In addition, under conditions of sus-

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