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ACID REACTION PRODUCTS OF INDOLE-3-CARBINOL AND THEIR EFFECTS ON CYTOCHROME P450 AND PHASE II ENZYMES IN RAT AND MONKEY HEPATOCYTES

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Abstract—The effects of three acid condensation products of indole-3-carbinol (I3C), i.e. 3,3'-diindolylmethane (DIM), 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b'*:7,8-*b''*]tri-indole (CTI) and 2,3-bis[3-indolylmethyl]indole (BII), on cytochrome P450 and phase II enzymes were studied in primary cultures of rat and cynomolgus monkey liver cells. In rat hepatocytes all three indole derivatives dose-relatedly induced the ethoxyresorufin O-dealkylation (EROD) activity (to 24-fold) and 7α-hydroxylation of testosterone (to 4-fold), whereas all three decreased the 16α- and 2α-testosterone hydroxylation (DIM to 60%, CTI and BII to a mere 5% of the control cells). Treatment of monkey hepatocytes with DIM and BII enhanced the EROD activity to 6- and 9-fold, respectively. Furthermore, BII decreased the 6β-hydroxylation of testosterone (to 60% of the untreated cultures) in monkey cells. Phase II enzymes were also affected. In rat hepatocytes DIM, CTI and BII enhanced DT-diaphorase (DTD) (= NAD(P)H-quinone reductase) activity, and DIM and BII the glucuronidation of 1-naphthol. In monkey cells BII only enhanced DTD, and no changes were observed in the glucuronidation of 1-naphthol after treatment with either DIM or BII. The indole derivatives did not affect glutathione *S*-transferase activity and sulfation of 1-naphthol in either rat or monkey hepatocytes. These results identify two novel acid condensation products of I3C, CTI and BII, as potent compounds in affecting biotransformation in rat as well as in monkey hepatocytes.

The human diet contains a number of compounds that affect the carcinogenic process. Apart from carcinogens our food contains compounds which prevent tumorigenesis, among them minor non-nutrient constituents of vegetables and fruits, such as terpenes, aromatic isothiocyanates, phenols, flavones and indoles [1–3]. Indoles are hydrolysis products of indolylmethyl-glucosinolates, a group of compounds in cruciferous vegetables (e.g. Brussels sprouts, cabbage and cauliflower) [4]. A mean daily intake of indolylmethyl glucosinolate has been estimated to be 7 mg/person from cooked cruciferous vegetables [5]. The hydrolysis products are formed during storage and processing of the vegetables by the plant enzyme myrosinase [4]. One of the major hydrolysis products is indole-3-carbinol (I3C‖). A high consumption of cruciferous vegetables has been related to a decreased risk of carcinogenesis in man [3, 4], and decreased chemically induced tumorigenesis in laboratory animals [4, 6]. In contrast, other studies indicated an enhanced promoter effect of I3C on tumorigenesis in rats [7].

Since many carcinogens are subjected to metabolic activation and inactivation, changing the biotransformation enzyme activities may be an effective means of affecting the carcinogenic potential of these xenobiotics. Dietary exposure to cruciferous vegetables or I3C is known to enhance several cytochrome P450 (P450) enzymes and other drug-metabolizing enzymes [4, 6, 8]. Recently, we described the induction of four different P450 forms (P450, 1A1, 1A2, 2B1 and 3A) in the liver of rats fed I3C for as short a period as 2 days [9]. Accordingly, the dealkylation of ethoxy- and pentoxyresorufin (EROD and PROD), and the 6β-hydroxylation of testosterone were enhanced. Longer exposure to I3C also enhanced several phase II enzymes such as glucuronyl transferase (GT), DT-diaphorase (DTD) (= NAD(P)H-quinone reductase) and glutathione *S*-transferase (GST) [9–11].

In an earlier study, Bradfield and Bjeldanes [12] also reported induction of EROD activity in the liver of rats orally exposed to I3C. However, when I3C was given i.p. no enhancement of EROD activity could be measured. The researchers suggested that the conversion of I3C in the acid environment of the

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‖ Abbreviations: BII, 2,3-bis [3-indolylmethyl] indole; CTI, 5,6,11,12,17,18-hexahydrocyclonona [1,2-*b*:4,5-*b'*:7,8-*b''*] tri-indole; DIM, 3,3'-diindolylmethane; DMSO, dimethyl sulfoxide; DTD, DT-diaphorase (= NAD(P)H-quinone oxidoreductase); EROD, 7-ethoxyresorufin O-deethylation (7-ethoxyphenoxazone O-deethylation); GST, glutathione *S*-transferase; GT, glucuronyl transferase; I3C, indole-3-carbinol; P450, cytochrome P450; OHT, hydroxytestosterone; LDH, lactate dehydrogenase.

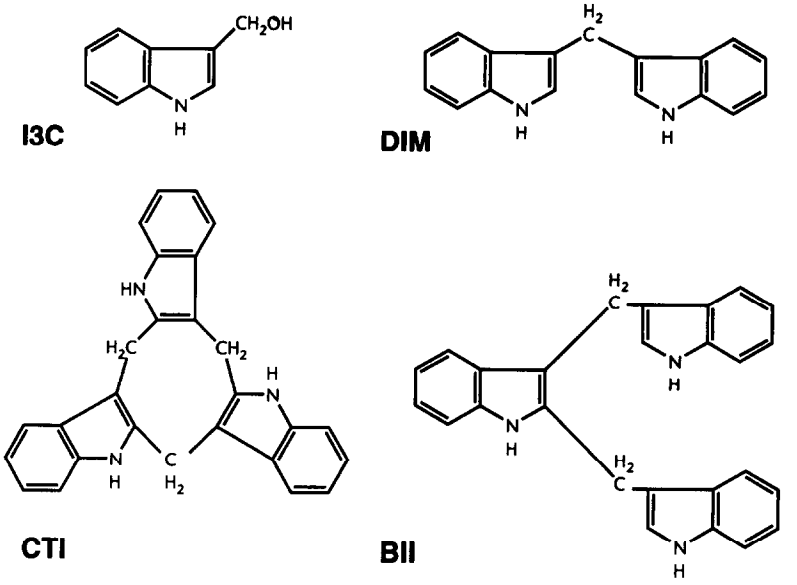

Fig. 1. Chemical structures of I3C, DIM, BII and CTI.

stomach was essential for its inducing effects. In a study at our own laboratory treatment of hepatocytes with I3C itself had only minor effects on biotransformation enzymes whereas the dimer 3,3'-diindolylmethane (DIM) and a complete acid reaction mixture of I3C markedly enhanced the EROD activity [13]. Within this acid reaction mixture, we identified the chemical structures of two trimers 5,6,11,12,17,18-hexahydrocyclonona[1,2-*b*:4,5-*b'*:7,8-*b''*]tri-indole (CTI) and 2,3-bis [3-indolylmethyl] indole (BII), in addition to the already known dimer DIM [14] (Fig. 1).

All three indole derivatives induced cytochrome P450 1A1 and its associated EROD activity [15] in rat hepatocytes [14]. Induction of cytochrome P450 1A1, however, can be related to metabolic activation of pro-carcinogens to reactive intermediates, giving rise to toxicity and carcinogenicity [16]. Since dietary exposure to I3C generally decreases tumorigenesis *in vivo* we wanted to know whether the acid condensation products of I3C are capable in affecting other P450 forms and/or phase II enzymes.

Primary cultures of hepatocytes are a useful alternative in studying the changes in biotransformation activities catalysed by several P450 forms [17–19]. One of the advantages in comparison with *in vivo* studies is that only small amounts of purified compounds are needed to elucidate the inductive capacity of these compounds. Moreover, a comparison of the effects on rat and human hepatocytes can improve human risk evaluation. Human cells, however, are not easily available. Hepatocytes from monkey liver might be a good alternative for human hepatocytes [20]. In the present study we investigated the effects of three indole oligomers, DIM, CTI and BII, on P450 pattern and several phase II enzymes in rat hepatocytes in more detail. In comparison, the effects of I3C, DIM and the trimer BII on biotransformation activities were studied in hepatocytes derived from cynomolgus monkey (*Macaca fascicularis*).

MATERIALS AND METHODS

Materials. Newborn calf serum was obtained from Gibco Europe (Breda, The Netherlands). Indole-3-carbinol, Williams' E medium, androstenedione, 11β- and 16α-hydroxytestosterone (11β- and 16α-OHT) were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). 2α-OHT was a gift from Prof. D. N. Kirk (Queen Mary College, University of London). 15β-OHT was a gift from G. D. Searle and Co. (Skokie, IL, U.S.A.), 6β- 7α- and 16β-OHT were obtained from Steraloids (Wilton, NH, U.S.A.). DIM, CTI and BII were purified from an acid-reaction mixture of I3C as described before [14]. All other chemicals were of analytical grade. Monoclonal antibodies towards P450 1A1/2, P450 2B1/2 and P450 3A were kind gifts from Dr P. J. Kremers, Université de Liège, Belgium. Secondary antibodies were obtained from Dakopatts a/s (Glostrup, Denmark).

Animals and cell isolation. Male Wistar rats (Ico:WU, Iffa-Credo, Someren, The Netherlands), weighing 200–350 g, were fed *ad lib.* a semisynthetic diet as described previously [9]. The animals had free access to drinking water. Hepatocytes were isolated using a two-step collagenase perfusion technique as described before [18]. Cynomolgus monkeys were bred at the National Institute of Public Health and Environmental Protection (RIVM, Bilthoven, The Netherlands). The monkeys served as donors for kidney cells necessary for the production of the poliomyelitis vaccin. Liver cells were isolated as described by Mennes *et al.* [21].

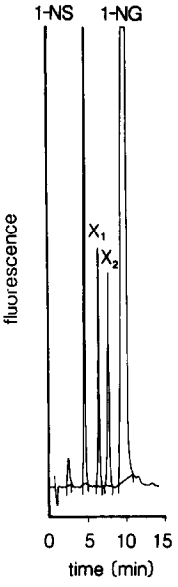

Fig. 2. HPLC elution profile of 1-naphthyl conjugates (1-NG,-glucuronide and 1-NS,-sulfate) in medium from intact monolayers of control rat hepatocytes incubated with 250 μM 1-naphthol for 50 min. X_1 and X_2 are unidentified products. No peaks interfering with the detection of the naphthyl conjugates were encountered in HPLC analyses of control media without naphthol.

Cell culture. The cells were plated on 6- or 9-cm tissue culture dishes (Sterilin) at a density of 4 or 8×10^6 cells/dish in 4 or 10 mL Williams' E medium, respectively. Media were supplemented with 3 (rats) or 5% (monkeys) newborn calf serum, 1 μM insulin, 10 μM hydrocortisone and 50 mg/L gentamycin. Additionally, media of monkey hepatocytes were supplemented with 4 mM $CaCl_2$ and 4 mM $MgCl_2$ during the first 4 hr of culture. Cells were incubated in a humidified atmosphere of air (95%) and CO_2 (5%) at 37°.

After 4 hr of culture, media were replaced. Thereafter, media were refreshed every 24 hr. After a total preincubation period of 24 hr, I3C, DIM, CTI and BII, dissolved in dimethyl sulfoxide (DMSO), were added to the culture media to give a final concentration range of 2.5 to 40 μM. An equal amount of DMSO was added to the control cultures (final DMSO concentration 0.1% v/v). Monkey hepatocytes were treated with only one concentration of either I3C, DIM or BII (25 μM).

Biochemical determinations. Leakage of lactate dehydrogenase (LDH, EC 1.1.1.27) was used as an indicator for cell viability. LDH activity was measured according to Bergmeyer *et al.* [22]. Microsomes and cytosol were prepared, and protein content determined, as described previously [23]. Determinations of EROD and hydroxylation of testosterone were performed directly in intact hepatocyte monolayers as described by Wortelboer *et al.* [23]. GST activity was measured in cytosol with 1-chloro-2,4-dinitrobenzene (5 μM) as a substrate using the spectrophotometric method of Habig *et al.* [24]. DTD activity was assayed fluorimetrically by the reduction of resorufin, as described previously [14].

GT and sulfotransferase activities were measured directly in intact hepatocyte monolayers. Cells were washed twice with Hank's balanced salt solution and incubated with 250 μM 1-naphthol in Hank's balanced salt solution at 37° in a humidified atmosphere of air (95%) and CO_2 (5%) at 37°. After 25 and 50 min, samples of 1 mL were taken and stored at −20°. Media were centrifuged (5 min, 300 *g*) and 1-naphthyl-glucuronides and 1-naphthyl-sulfates were analysed by HPLC according to Redegeld *et al.* [25] with a few modifications. A Chromsep C18 RP column (200 × 3 mm, length × i.d., Chrompack, Middelburg, The Netherlands) was used for separations. Products were eluted with a mixture of 17.5% acetonitrile in 10 mM

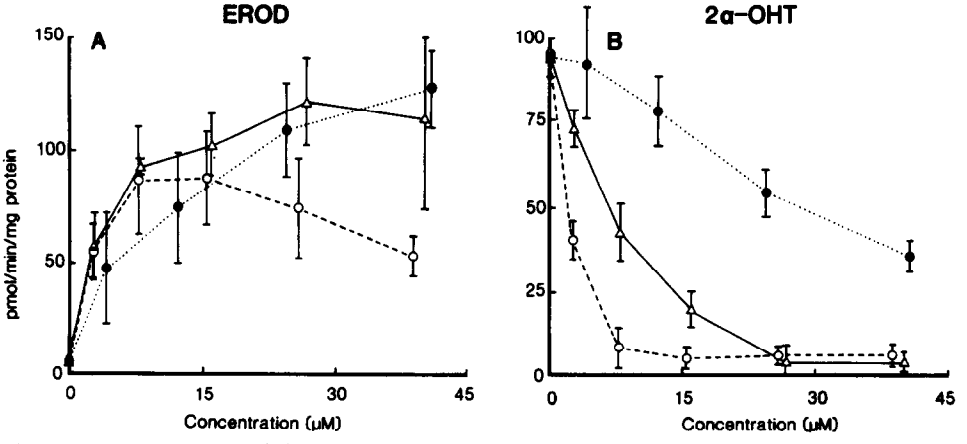

Fig. 3. The EROD activity (A) and testosterone 2α-hydroxylation (B) in intact monolayers of primary rat hepatocytes treated with DIM (●), CTI (○) or BII (△) for 48 hr. Data are means ± SD (N = 3 experiments).

Table 1. Testosterone hydroxylation in rat hepatocytes after exposure to different acid reaction products of indole-3-carbinol

Treatment	(μg/mL)	(μM)	15β-OHT	7α-OHT	16α-OHT	6β-OHT
			(pmol/min/mg cellular protein)			
Control			7.0 ± 0.5	6.1 ± 2.1	167.9 ± 5.9	33.3 ± 1.9
DIM	(1)	(4.1)	7.6 ± 1.6	11.5 ± 5.4	164.9 ± 5.9	36.5 ± 7.6
	(3)	(12.2)	8.5 ± 2.1	15.1 ± 6.7*	137.7 ± 9.9*	38.6 ± 7.9
	(6)	(24.4)	12.1 ± 0.5*	22.4 ± 9.9*	105.2 ± 5.1*	44.0 ± 5.3*
	(10)	(40.7)	11.9 ± 2.7*	28.2 ± 12.4*	77.1 ± 11.0*	53.3 ± 8.8*
CTI	(1)	(2.6)	10.6 ± 3.3	15.4 ± 7.4	67.9 ± 24.9*	37.4 ± 13.7
	(3)	(7.8)	7.3 ± 1.2	14.7 ± 5.9*	17.5 ± 4.8*	33.7 ± 11.5
	(6)	(15.5)	7.0 ± 2.5	17.3 ± 8.8*	8.6 ± 3.5*	27.9 ± 12.9
	(10)	(25.8)	4.3 ± 0.3*	15.5 ± 3.3*	6.2 ± 2.1*	18.0 ± 3.6*
	(15)	(38.7)	5.3 ± 2.6*	20.1 ± 4.0*	4.5 ± 1.6*	22.9 ± 4.8*
BII	(1)	(2.7)	9.7 ± 1.4*	12.4 ± 6.5	139.0 ± 19.0*	34.9 ± 6.7
	(3)	(8.0)	8.8 ± 2.1*	17.2 ± 8.1*	87.9 ± 25.2*	34.4 ± 12.2
	(6)	(16.0)	9.2 ± 1.5*	21.7 ± 7.5*	40.1 ± 11.1*	37.7 ± 7.9
	(10)	(26.7)	9.9 ± 2.8	25.4 ± 13.2	17.1 ± 3.5*	34.6 ± 6.5
	(15)	(40.0)	6.7 ± 1.9	20.4 ± 7.6*	6.4 ± 2.5*	22.6 ± 8.0*

Values represent means ± SD of three experiments.
* $P \leq 0.05$ when compared to control.
After a preincubation of 24 hr, hepatocytes were exposed for another 48 hr to DMSO alone (control), DIM, CTI or BII. Formation of 16β-OHT was below detection limits (2 pmol/min/mg cellular protein).

KH_2PO_4, pH 2.5 at a flow rate of 1.0 mL/min, giving a typical elution profile as shown in Fig. 2. Column effluents were monitored fluorimetrically using an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Metabolites were quantified by comparing peak areas with those of authentic standards.

Gel electrophoresis and immunoblotting. Microsomal proteins (2 μg protein) were electrophoretically separated using a Biorad mini Protean II cell by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE), and subsequently immunoblotted as described previously [18]. P450 1A1/2, P450 2B1/2 and P450 3A apoproteins were detected using monoclonal antibodies directed towards these isoenzymes.

Statistical analysis. Results have been expressed as means ± SD, where appropriate. Statistical analysis was performed by Student's *t*-test, for unpaired samples ($P \leq 0.05$).

RESULTS

Effects of indole derivatives on P450 enzymes in rat hepatocytes

Incubation of rat cells with the indole derivatives up to 40 μM did not result in significant loss of cell viability as measured by total LDH activity or LDH leakage (results not shown). All three acid condensation products of I3C enhanced the EROD activity dose-relatedly (Fig. 3A). At concentrations of only 3 μM and upwards a significant enhancement of EROD could be detected. At a concentration as high as 40 μM, however, the EROD enhancement due to CTI exposure was less than that due to that of DIM and BII (Fig. 3A). Both the trimers markedly decreased the 2α- and 16α-hydroxylation of testosterone to a mere 5% of the control cultures (Fig. 3B, Table 1). No competitive inhibition of the 2α- and 16α-hydroxylation of testosterone could be detected when microsomes of untreated rats were incubated with both testosterone and BII or CTI (results not shown). Treatment of cells with DIM diminished this hydroxylation of testosterone, but only to *ca.* 40% of the control cells. All three acid condensation products enhanced the 7α-hydroxylation of testosterone (to *ca.* 4-fold; Table 1), whereas the hydroxylation at the 15β- and 6β-sites were slightly enhanced in cells exposed to DIM and BII (1.5-fold), but decreased in cells exposed to CTI (0.6-fold). Western blotting revealed an enhancement of P450 1A1 [14], but not of 1A2, 2B1/2 and 3A apoprotein levels in cells treated with DIM, CTI or BII (results not shown).

Effects of indole derivatives on P450 enzymes in monkey hepatocytes

The basal P450-dependent activities in 72-hr-old monkey hepatocytes differed from the activities measured in rat hepatocytes. The basal EROD activity was about 4-fold higher than in rat hepatocytes. Also the hydroxylation at the 6β- site of testosterone was markedly higher (30-fold) in DMSO-treated monkey hepatocytes. In contrast, the hydroxylation at the 2α site was very low, and the formation of 7α-OHT could not be detected in these 72-hr-old monkey hepatocytes.

Exposure of monkey hepatocytes to I3C for 48 hr had no effect on EROD and testosterone hydroxylation (Fig. 4; Table 2). The dimer DIM enhanced the EROD activity to 6-fold the activity in cells treated with DMSO alone, but no changes were observed in the hydroxylation of testosterone. In contrast, exposure to BII enhanced the EROD activity to 9-fold in monkey cells (Fig. 4A), whereas

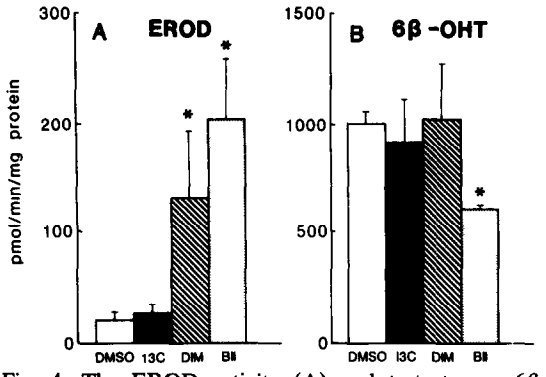

Fig. 4. The EROD activity (A) and testosterone 6β-hydroxylation (B) in intact monolayers of primary monkey hepatocytes treated with I3C, DIM or BII for 48 hr. Data are means ± SD. * $P \leq 0.05$ compared to cells treated with DMSO alone (N = 3 experiments).

the formation of several metabolites of testosterone (15β-, 6β-, 16β-, 2α-OHT and androstenedione) was reduced (Fig. 4B; Table 2).

Phase II *enzymes in rat and monkey hepatocytes*

In rat liver cells the indole derivatives enhanced the DTD activity dose-relatedly to 3.5-, 2.6- and 5.7-fold in DIM, CTI and BII treated cells, respectively (Fig. 5A). The glucuronidation of naphthol in intact rat cells was enhanced by treatment with all three indoles, although a higher concentration of CTI and BII diminished these effects (Fig. 6A). No effects of the acid condensation products of indole-3-carbinol were observed in rat cells on the GST activity (Fig. 5B) and sulfation of 1-naphthol (Fig. 6B).

In monkey hepatocytes treated with DMSO alone the glucuronidation and sulfation of 1-naphthol, and the DTD activity did not differ from those in similarly treated rat hepatocytes. In contrast, the basal GST activity was 2-fold higher in monkey hepatocytes compared with control rat hepatocytes. Treatment of monkey hepatocytes with 25 μM BII resulted in enhanced DTD activity (2-fold; Fig. 7A). No changes were observed in GST, and glucuronidation and sulfation of 1-naphthol after treatment of monkey cells with either I3C, DIM or BII (Fig. 7B; Fig. 8).

DISCUSSION

Treatment of rat hepatocytes with the acid condensation products of indole-3-carbinol resulted in a P450 pattern comparable to the pattern observed after treatment with the known inducer β-naphthoflavone [18]. Apart from a high induction of EROD, the indole derivatives DIM, CTI and BII markedly decreased the formation of 2α-OHT and 16α-OHT in rat hepatocytes. In the male rat at least five P450 enzymes hydroxylate testosterone at the 16α site, namely 2B1, 2C11, 2B2, 2C7 and 2C13, the latter three at a rate one-tenth of that observed with 2B1 and 2C11 [26, 27]. In untreated rats the levels of P450 2B1 apoproteins are very low and the testosterone 16α-hydroxylation is predominantly attributable to 2C11 [27]. The comparable effects of the indoles on the 2α-hydroxylation of testosterone, the latter specifically catalysed by P450 2C11 [26, 27], indicate that hydroxylation at both the 2α- and 16α-sites of testosterone can be attributed to the constitutive 2C11. A reduction of P450 2C11 was also observed after exposure to other inducers of the P450 1A subfamily ranging from 80 to 40% [16]. The trimers CTI and BII, however, decreased the formation of 2α- and 16α-OHT to barely detectable levels. Because no direct inhibition of enzyme activities could be measured in microsomal incubations, the reduction of these activities is probably due to down-regulation of this constitutive P450 form. A marked down-regulation of the constitutive P450s *in vivo* was also reported for the antioxidants butylhydroxytoluene and butylhydroxyanisole, although these compounds do not induce P450 1A1 [28]. The mechanism by which this down-regulation of constitutive P450 forms is initiated is not clear.

All three indole derivatives increased the 7α-hydroxylation of testosterone in rat hepatocytes, which is predominantly catalysed by P450 2A [29]. A similar enhancement of this activity is observed when rat hepatocytes are treated with β-naphthoflavone [18]. The unaffected 16β-hydroxylation of testosterone indicates no effect of the indole derivatives on P450 2B [27]. In rat liver 6β- and 15β-

Table 2. Testosterone hydroxylation in monkey hepatocytes after exposure to indole-3-carbinol and two acid condensation products of indole-3-carbinol

Treatment	(μM)	15β-OHT	16α-OHT	16β-OHT	2α-OHT
			(pmol/min/mg cellular protein)		
Control	—	40.0 ± 4.4	14.6 ± 5.3	39.1 ± 5.9	6.7 ± 0.6
I3C	(25)	35.0 ± 10.8	12.0 ± 1.7	33.9 ± 5.5	6.2 ± 1.8
DIM	(25)	40.8 ± 12.3	12.6 ± 4.7	37.9 ± 10.9	7.2 ± 2.0
BII	(25)	21.8 ± 2.0*	11.5 ± 5.0	23.8 ± 4.6*	5.1 ± 1.0*

Values represent means ± SD of three experiments.
* $P \leq 0.05$ when compared to control.
After a preincubation of 24 hr, hepatocytes were exposed for another 48 hr to DMSO alone (control), 25 μM, I3C, DIM or BII. Formation of 7α-OHT was below detection limits (2 pmol/min/mg cellular protein).

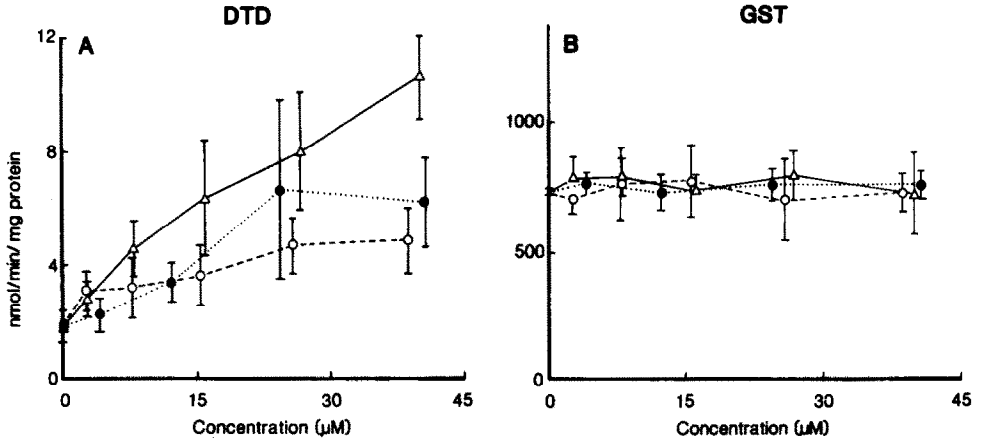

Fig. 5. The DTD (A) and GST activity (B) in cytosol of rat hepatocytes treated with DIM (●), CTI (○) or BII (△) for 48 hr. Data are means ± SD (N = 3 experiments).

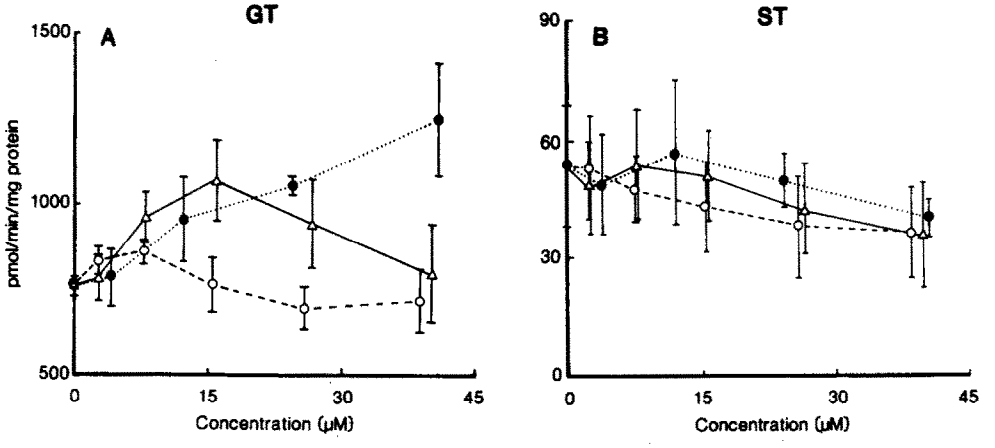

Fig. 6. The glucuronidation (A) and sulfation (B) of 1-naphthol in intact monolayers of primary rat hepatocytes treated with DIM (●), CTI (○) or BII (△) for 48 hr. Data are means ± SD (N = 3 experiments).

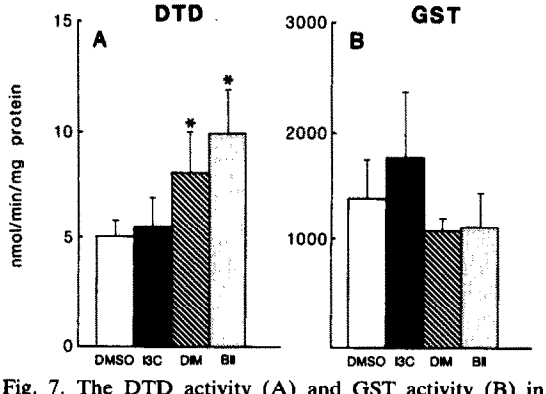

Fig. 7. The DTD activity (A) and GST activity (B) in cytosol of primary monkey hepatocytes treated with I3C, DIM or BII for 48 hr. * P ≤ 0.05 compared to cells treated with DMSO alone (N = 3 experiments).

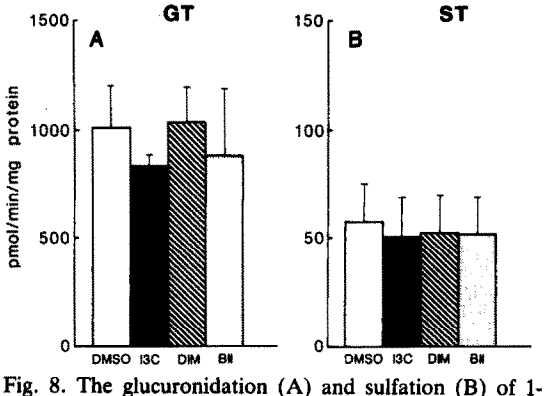

Fig. 8. The glucuronidation (A) and sulfation (B) of 1-naphthol in intact monolayers of primary monkey hepatocytes, treated with I3C, DIM or BII for 48 hr. * P ≤ 0.05 compared to cells treated with DMSO alone (N = 3 experiments).

hydroxylation of testosterone are both specifically catalysed by P450 3A [27]. However, in the present study no differences could be detected in P450 3A apoprotein levels and it is therefore not clear which P450(s) is (are) associated with the minor changes in testosterone 6β- and 15β-hydroxylation due to treatment with the indole derivatives.

Less data are available on the different P450 forms in monkey liver. So far, a few studies have shown the high similarity between monkey and human P450s [30, 31]. P450 enzymes in human liver belong to the subfamilies P450 1A, 2C, 2D, 2E, 3A and 4A [32]. P450 3A, but not 2C, is a major constitutive enzyme in human liver and has a high catalytic activity towards the 6β site of testosterone [33], which was also observed in the present study. The hydroxylation of other sites of testosterone was minimal and probably not specifically related to different P450 forms [33]. On the other hand, the dealkylation of ethoxyresorufin is catalysed by the P450 1A subfamily in both rat and human hepatocytes, although in human liver the P450 1A2 form is mainly detectable [34]. As found in rat hepatocytes [9], I3C itself had no effects on any of the measured biotransformation activities in monkey hepatocytes. The enhancement of EROD due to exposure to BII and an overall decrease in testosterone hydroxylation are in agreement with the effects observed in monkey hepatocytes treated with the P450 1A1 model inducer, β-naphthoflavone (Mennes, unpublished results).

Treatment of hepatocytes with the acid condensation products of I3C selectively affected phase II enzymes. In a previous study [14], we reported enhancement of DTD activity in rat cells after exposure to 25 μM of either DIM, CTI or BII. Here, we report a dose-related enhancement of DTD and GT activities in rat hepatocytes treated with BII and DIM. The selective inhibition of BII at concentrations above 15 μM may indicate competition between the substrate 1-naphthol and BII (or its metabolites) for the GT enzyme. A similar selective inhibition was measured for DIM and EROD activity at a concentration above 15 μM. The mechanisms behind these decreases in enzymatic activities are not clear.

Several studies suggest a concomitant induction of P450 1A1 and phase II enzymes, such as DTD and GT, by planar aromatic structures via binding of the inducer to a cytosolic receptor, the *Ah* receptor [35, 36]. In this respect, the mechanism by which the indole derivatives elicit their broad effects on biotransformation enzymes might be under control of this *Ah* receptor. Gillner *et al.* [37], however, reported a relatively low binding affinity of DIM to the *Ah* receptor, in contrast to the high binding affinity of β-naphthoflavone, and of a more planar indole, indolo[3,2-*b*]carbazole. Metabolism of DIM to a compound with a higher binding affinity to the *Ah* receptor has been suggested [37]. Despite its planar structure, CTI does not fit into the assumed rectangle which has been suggested to account for the binding of high-affinity ligands to the *Ah* receptor [38]. However, no *Ah* binding affinities of the trimers itself and/or possible metabolites are determined in this study. In addition, the differential induction of GT and DTD indicates that mechanisms other than the *Ah* receptor are probably also involved [35].

Exposure of rats to I3C *in vivo* is correlated with marked changes in biotransformation activities in the liver and small intestinal mucosa [4, 9–11]. As I3C exposure is associated with decreased tumor formation induced by a variety of chemically distinct compounds [2, 4], it has been postulated that an increased first-pass metabolism in the gastrointestinal tract could result in reduced absorption of the precarcinogen [9, 39]. Enhanced detoxication of precarcinogens by either phase I or phase II enzymes in the liver and/or other target organs has also been suggested [1, 2]. In this respect, the effects of indole derivatives on changes in P450 profile as well as on DTD strengthen the latter hypothesis. Many anticarcinogens such as phenolic antioxidants and 1,2-dithiol-3-thiones induce DTD activity [40]. Moreover, over the last few years the contribution of many individual P450s, including constitutive P450s, in the formation of the proximate carcinogen has been studied in more detail. For example, aflatoxin B_1 and dimethyl-benz[*a*]anthracene, two compounds often used to show the anticarcinogenic effects of cruciferous vegetables and I3C *in vivo* [2, 4, 39], are metabolically activated by the constitutive P450 forms of the subfamily P450 2C, whereas P450 1A1 enhances the formation of the non-mutagenic metabolite [41, 42]. The effects of indole derivatives on both cytochrome P450 profile and several phase II enzymes, makes it possible that conditions may exist in which enhancement of carcinogenesis may occur. Furthermore, the formation of tumors is a multi-stage process and apart from metabolic activation other factors such as species difference and tissue-specific expression of biotransformation enzymes in target organs can affect the ultimate risk of tumor formation.

In general, our study identifies the acid condensation products of I3C, namely DIM, CTI and BII, as potent modulators of several biotransformation activities. The fact that similar effects are observed in rat as well as in monkey hepatocytes indicates that these indole oligomers could be of importance for humans, too. However, the relevance of these changes in biotransformation on the modulation of carcinogenesis by indoles remains to be established.

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