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THE EFFECTS OF INDUCING AGENTS ON CYTOCHROME P450 AND UDP-GLUCURONYLTRANSFERASE ACTIVITIES IN HUMAN HEPG2 HEPATOMA CELLS

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Abstract—Selective induction *in vitro* of cytochrome P450-dependent mixed-function oxidase (MFO) and UDP-glucuronyltransferase (GT) activities was observed in the human HepG2 hepatoma cell line. 1,2-Benzanthracene (BA) induced MFO O-dealkylation activities for ethoxyresorufin, methoxyresorufin and benzyloxyresorufin, whereas phenobarbitone (PB) selectively induced pentoxyresorufin O-dealkylation and rifampicin (RIF) selectively induced benzyloxyresorufin O-dealkylation. Antibody inhibition experiments indicated that ethoxyresorufin and methoxyresorufin O-dealkylations were catalysed mainly by the P450 1A subfamily in untreated and BA-induced HepG2 cells, that additional unidentified P450 forms were considerably involved in methoxyresorufin and benzyloxyresorufin O-dealkylations and that the P450 2B subfamily was partially responsible for pentoxyresorufin O-dealkylation in PB-induced cells. Bilirubin GT activity was induced by PB, BA, RIF and dexamethasone, but 1-naphthol, morphine and testosterone GT activities were not induced by any of these treatments.

The induction of xenobiotic metabolism by drugs and other chemicals in man is of clinical and toxicological importance. It has, however, proven difficult to study the effects of inducing agents on xenobiotic-metabolizing enzymes in man directly, although this has been researched extensively in experimental animals [1]. Most of the information available in man is indirect, obtained from pharmacokinetic studies *in vivo* [e.g. 2], although some direct, retrospective measurements of the effects of inducing agents on human enzyme levels and activities have been carried out *in vitro*, using biopsies and liver samples from surgical patients or kidney donors who had been treated previously, *in vivo*, with inducing drugs. Thus, cigarette smoking and clinical treatment with anticonvulsants (including barbiturates and hydantoins) or macrocyclic antibiotics (e.g. erythromycin and rifampicin) have been shown to increase both cytochrome P450-dependent mixed-function oxidase (MFO**) and UDP-glucuronosyltransferase (GT) activities in man [3–10]. Primary cultures of human hepatocytes have been used for prospective studies of induction in man [11–13]. However, primary hepatocyte cultures are phenotypically unstable, losing many of their differentiated functions, including MFO activities, within 48–72 hr [11, 14] and, furthermore, being non-proliferating they have a limited lifespan in culture. The HepG2 human hepatoma cell line is an immortal cell culture system which we are evaluating as a model for studying hepatic drug metabolism and its induction in man. These cells carry out both MFO and conjugation reactions [15–19] and activate xenobiotics to cytotoxic and/or mutagenic metabolites [20–22]. The activities of drug-metabolizing enzymes in HepG2 cells can be manipulated by altering the composition of the growth medium [23] and both the MFO activities and bilirubin GT activity can be regulated by intracellular haem levels [24]. In this paper we report the effects of four chemicals which are well-established inducing agents in experimental animals, namely phenobarbitone (PB), 1,2-benzanthracene (BA), rifampicin (RIF) and dexamethasone (DEX), on MFO and GT activities in HepG2 cells, in order to ascertain whether these cells might be a suitable model in which to carry out studies of infection by xenobiotics in man.

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** Abbreviations: MFO, cytochrome P450-dependent mixed-function oxidase; GT, UDP-glucuronyltransferase; DMSO, dimethyl sulphoxide; EROD, ethoxyresorufin O-dealkylation; MROD, methoxyresorufin O-dealkylation; PROD, pentoxyresorufin O-dealkylation; BROD, benzyloxyresorufin O-dealkylation; AROD, alkyloxyresorufin O-dealkylation; BA, 1,2-benzanthracene; PB, phenobarbitone; RIF, rifampicin; DEX, dexamethasone; PAH, polycyclic aromatic hydrocarbon; 3MC, 3-methylcholanthrene; ANF, α-naphthoflavone.

MATERIALS AND METHODS

Williams' E medium was from ICN Flow Laboratories (Irvine, U.K.) and foetal calf serum was from Gibco (Paisley, U.K.). 1-Naphthol, 1-naphthol glucuronide, testosterone, bilirubin, UDP-glucuronic acid, BA, RIF, dimethyl sulphoxide

(DMSO), DEX-21-phosphate and NADPH were from the Sigma Chemical Co. (Poole, U.K.). Phenobarbitone (sodium salt) was from BDH (Poole, U.K.). [*N-methyl*-^{14}C]Morphine and [4-^{14}C]-testosterone were obtained from Amersham International (Amersham, U.K.). Ethoxyresorufin, methoxyresorufin, benzyloxyresorufin and pentoxyresorufin were synthesized from resorufin as described by Burke and Mayer [25]. The HepG2 cell line was kindly provided by Prof. C. N. Hales, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, U.K. Polyclonal antibodies to purified rat P450 1A1, P450 1A2 and P450 2B1 were raised in rabbits and characterized as described elsewhere [26], and both antibodies and pre-immune serum (controls) were used as lyophilized 50% ammonium sulphate precipitates.

Cell culture. HepG2 cells were grown as monolayer or multilayer cultures at 37° in a humidified atmosphere of 5% CO_2 in air in 75-cm^2 flasks in Williams' E medium supplemented with 10% (v/v) foetal calf serum, penicillin (100 U/mL) and streptomycin (100 mg/L) [17]. The timing of cell manipulations was calculated from the day of passage = day 0. Cells were subcultured on day 7 (i.e. at confluence) at a 1:3 split ratio (seeding density 3.5×10^6 cells in a 75-cm^2 flask). The medium was renewed routinely on days 3 and 6. Cultures were treated on days 7, 8 and 9 (i.e. for 3 days) with one of the following: 25 μM BA, 2 mM PB, 1 μM DEX, 50 μM RIF or 0.0625% (v/v) DMSO. Stock solutions of BA and RIF were dissolved in DMSO and added to the culture medium so that the concentration of DMSO in the medium was 0.0625% (v/v). PB and DEX were dissolved in water and added to the medium. The culture medium was replaced every day during the 3-day treatment period with fresh medium containing the appropriate inducer. Cells were removed for analysis on day 10 as follows: cultures were washed twice with ice-cold Krebs–Henseleit buffer containing HEPES (10 mM, pH 7.4) and the cells were then scraped off into ice-cold 0.1 M sodium phosphate buffer (pH 7.6) using a "rubber policeman" and homogenized using eight strokes of a motor-driven teflon-glass Potter–Elvejem type homogenizer on ice [15].

Analytical methods. Enzyme activities were measured in cell homogenates prepared in 0.1 M sodium phosphate buffer, pH 7.6, as described previously [15]. Four MFO activities were measured, namely the O-dealkylation of ethoxyresorufin (EROD), methoxyresorufin (MROD), benzyloxyresorufin (BROD) and pentoxyresorufin (PROD), using 5 μM substrate as described previously [15]. Antibody inhibition of these alkoxyresorufin O-dealkylation (AROD) activities was studied by initially mixing antibody or pre-immune serum preparation with 0.1 mL HepG2 cell homogenate at a ratio of 20 mg antibody or pre-immune protein/mg cell homogenate protein, leaving the mixture on ice for 20 min, then adding the remainder of the reaction mixture and carrying out the reaction as above. Four GT activities were measured, using 1-naphthol (50 μM), bilirubin (0.4 mM), morphine (2.4 mM) or testosterone (1 mM) as substrate. 1-Naphthol GT activity was measured in the presence of 0.5 mM UDP-glucuronic acid and 5 mM $MgCl_2$ by continuous fluorescence detection of the glucuronide formed [27]. Bilirubin, morphine and testosterone GT activities were measured in the presence of 4 mM UDP-glucuronic acid and 10 mM $MgCl_2$; a diazotization assay was used for measuring bilirubin conjugation, while a radiometric assay employing ^{14}C-labelled substrate was used for morphine and testosterone, as described previously [28, 29]. The total protein content of adhered HepG2 cells was measured by the method of Lowry *et al.* [30] using bovine serum albumin as the standard, following washing of the cells and then their removal from the culture as described above. Lactate dehydrogenase activity in the culture medium was measured as described elsewhere [22]. The statistical significance of data differences were calculated using Student's *t*-test for pair-wise comparisons and ANOVA with Dunnett's *post-hoc* test for multiple comparisons against a common control set, using $P \leq 0.05$ as the criterion of significance in all cases.

RESULTS

The individual concentrations of inducers used did not appear to decrease the gross viability of the cells after treatment for 3 days, as judged from the protein content of adhered HepG2 cells in the flasks (dead HepG2 cells detach from the culture flask), from microscopic examination of the cells (cell size and morphology remained unchanged) and from lactate dehydrogenase leakage into the culture medium (there was no increase in leakage).

In the following results the extents of induction by DMSO, PB and DEX were calculated relative to untreated cells, whereas the extents of induction by BA and RIF were calculated relative to 0.0625% DMSO-treated cells.

MFO activities

The effects of the inducing agents on the MFO activities of HepG2 cells and the percentages by which these activities were increased are shown in Table 1 and Fig. 1, respectively. DMSO (0.0625%) did not induce any of the AROD activities. Exposure of HepG2 cells to BA for 3 days in culture resulted in a marked, significant increase (11-fold) in EROD activity, smaller but significant increases (5–6-fold) in MROD and BROD activities, but no significant effect on PROD activity. PB treatment caused a 6-fold (albeit non-significant, $P > 0.05$) increase in PROD activity but no change in EROD, MROD or BROD activities. DEX caused a small (2–3-fold, non-significant) increase in all four MFO activities. RIF induced a large, significant increase in BROD activity (10-fold) and smaller increases in MROD (5-fold), EROD (4-fold) and PROD (3-fold, but non-significant).

The question of which forms of P450 were responsible for the MFO AROD activities in HepG2 cells was addressed by inhibiting the reactions with specific antibodies against individual forms of P450 (Fig. 2). Pre-immune serum had no notable effect (i.e. caused less than 5% change) on any of the AROD activities in any of the cells (data not shown).

Table 1. The effects of inducer treatments on MFO and GT activities in HepG2 cells

Activity	Untreated	DMSO	PB	DEX	RIF	BA
MFO (nmol/min/mg cell protein)						
MROD	8.3 ± 1.6	6.5 ± 0.9	5.8 ± 0.5	13.4 ± 0.3	30.8 ± 2.4*	36.4 ± 9.1*
EROD	16.4 ± 4.5	14.3 ± 3.5	11.0 ± 0.3	26.2 ± 2.9	61.0 ± 2.3*	153.1 ± 26.7*
PROD	0.5 ± 0.03	0.4 ± 0.02	2.8 ± 1.2	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.1
BROD	1.7 ± 0.5	1.3 ± 0.4	1.9 ± 0.2	4.9 ± 0.7	12.9 ± 2.6*	8.5 ± 3.1*
GT (nmol/min/mg cell protein)						
NAP	1.47 ± 0.20	0.74 ± 0.03	0.95 ± 0.27	1.33 ± 0.07	0.94 ± 0.06	1.17 ± 0.34
GT (pmol/min/mg cell protein)						
BIL	5.7 ± 2.1	5.7 ± 0.5	29.9 ± 0.6*	16.8 ± 2.1*	27.9 ± 2.8*	33.0 ± 0.8*
MOR	33.5 ± 1.3	26.6 ± 3.6	24.8 ± 5.9	31.5 ± 12.9	30.7 ± 10.3	15.5 ± 0.8
TES	16.8 ± 1.8	19.9 ± 3.3	15.2 ± 3.9	ND	13.2 ± 1.3	9.8 ± 0.5*

HepG2 cells were treated with 0.0625% (v/v) DMSO, 25 μM BA, 2 mM PB, 1 μM DEX or 50 μM RIF, then MFO and GT activities measured as described in Materials and Methods.

NAP, BIL, MOR and TES, 1-naphthol, bilirubin, morphine and testosterone glucuronidation, respectively.

Results are means ± SD of three to five experiments.

* Activity significantly different ($P \leq 0.05$) from the same reaction in either untreated cells (for DMSO-, PB- and DEX-treated cells) or DMSO-treated cells (for BA- and RIF-treated cells).

ND, not determined.

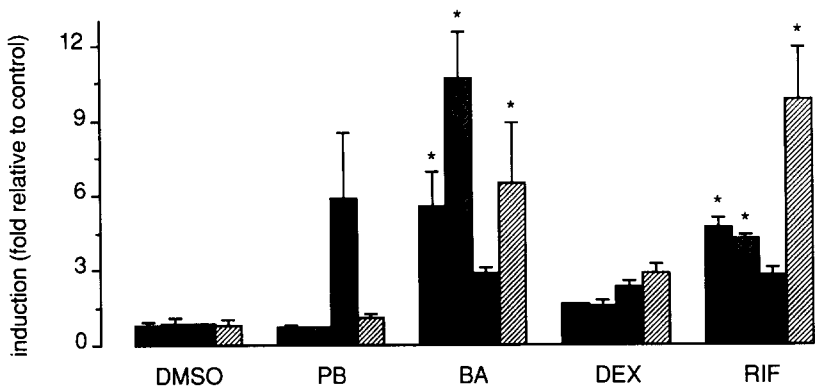

Fig. 1. Induction of AROD activities in HepG2 cells. HepG2 cells were treated for 3 days with 0.0625% (v/v) DMSO, 25 μM BA, 2 mM PB, 1 μM DEX or 50 μM RIF as described in Materials and Methods. The figure shows the fold increases in MROD (■), EROD (▨), PROD (▩) and BROD (▨) activities in DMSO-, PB-, BA-, DEX- and RIF-treated cells, calculated relative to the mean values for the respective reactions in either untreated (UT) cells (for DMSO, PB and DEX treatments) or DMSO-treated cells (for BA and RIF treatments). Data are means ± SD for three to five experiments. *The mean activity in the treated cells was significantly different from the mean activity for the respective reaction in UT or DMSO control cells ($P \leq 0.05$ by ANOVA and Dunnett's *post-hoc* test).

The effects of each individual antibody were generally similar in untreated and DMSO-treated cells, but different in PB- or BA-treated cells. In both untreated (UT) and DMSO-treated cells MROD and EROD activities were strongly inhibited (by 78–81%) by anti-P450 1A1 and moderately inhibited (52–59%) by anti-P450 1A2, whereas BROD was generally less strongly inhibited (29–54%) by these antibodies. If antibody inhibition levels lower than 25% are considered as signifying no effect, then PROD in DMSO-treated cells was weakly inhibited (31%) by anti-P450 1A1, but not by anti-P450 1A2, and was not inhibited by either of these antibodies in UT cells. None of the reactions were inhibited by anti-P450 2B1 in UT, DMSO-treated, BA-treated or, with a single exception (see below), PB-treated cells. Anti-P450 1A1 and anti-P450 1A2 antibodies were much less inhibitory towards MROD, EROD and BROD in PB-treated cells than in UT cells. In contrast, the inhibition of PROD by anti-P450 2B1, although remaining weak, was greater in PB-treated cells (27% inhibition) than in UT cells (8% inhibition). In BA-treated cells the inhibition of EROD and BROD by anti-P450 1A1 remained

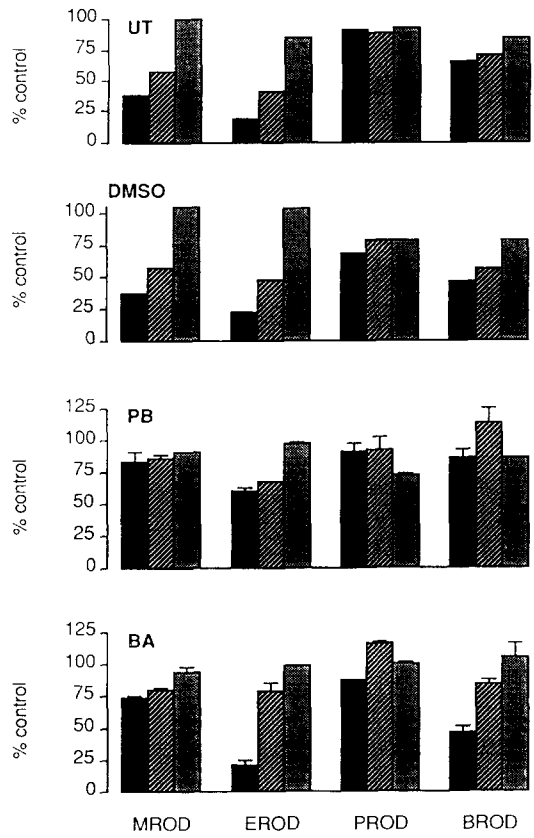

Fig. 2. Inhibition of AROD activities by anti-P450 antibodies in HepG2 cells. HepG2 cells were treated for 3 days with 0.0625% (v/v) DMSO, 25 μM BA or 2 mM PB as described in Materials and Methods. The figure shows the MROD, EROD, PROD or BROD activities remaining in untreated (UT) or DMSO-, PB- or BA-treated cells after the addition of antibody to rat P450 1A1 (■), P450 1A2 (▨) or P450 2B1 (▩) (20 mg antibody/mg cell protein) as described in Materials and Methods. Activity remaining is shown as a percentage of the activity of the respective reaction measured in the presence of a pre-immune serum preparation instead of antibody, but at the same concentration. Results are either means of duplicates (UT and DMSO) or means ± SD for three experiments (PB and BA).

unchanged (79% and 54% inhibition, respectively) compared to DMSO-treated control cells, but the inhibition of MROD by anti-P450 1A1 was greatly decreased (26% inhibition), and the inhibition of EROD, BROD and MROD by anti-P450 1A2 was eliminated (<25% inhibition). In the BA-treated cells there was no effect of anti-P450 2B1 on these reactions and PROD was not inhibited by any of the antibodies.

GT activities

Table 1 and Fig. 3 show the responses of GT activities to the treatment of HepG2 cells with inducing agents in culture. Bilirubin GT activity increased significantly 3–6-fold in response to BA, PB, DEX or RIF treatment. BA increased 1-naphthol GT activity slightly (1.6-fold), but significantly decreased testosterone and morphine GT activities by approximately 50%. PB, DEX and RIF treatments did not increase 1-naphthol, testosterone or morphine GT activities.

DISCUSSION

The effect of BA on MFO activities in HepG2 cells was a selective 11-fold induction of EROD activity (compared with 5–6-fold increases in MROD and BROD activities and no increase in PROD). BA and other polycyclic aromatic hydrocarbons (PAHs) selectively induce the hepatic P450 1A subfamily and P450 1A-catalysed EROD and MROD activities in rats *in vivo* [31, 32], and the effect of BA treatment on the four AROD activities in HepG2 cells is consistent with a selective induction of the P450 1A subfamily in these cells. PB selectively induces hepatic P450 2B1 and P450 2B-catalysed PROD activity in rats *in vivo* [32], but in cultured hepatocytes and liver cell lines PB has historically appeared to act aberrantly, like a PAH inducer, selectively inducing the P450 1A subfamily (P448) instead [33, 34]. However, typical PB-like induction of P450 2B1 has more recently been reported in cultured rat hepatocytes [14, 35]. Although the effects of PB on monooxygenase reactions in HepG2 cells were not statistically significant, the mean 6-fold increase observed in PROD activity without any accompanying increase in EROD, MROD or BROD activities would be consistent with a selective induction of the P450 2B subfamily. There is no convincing evidence in the literature that PB induces P450 2B in man *in vivo*, despite the presence of the CYP2B6 gene in human liver, but it is possible that the therapeutic dose of PB in man might be insufficient to induce P450 2B. DEX and RIF *in vivo* selectively induce members of the P450 3A subfamily in experimental animals and humans [10, 36, 37] and RIF induces the metabolism of steroids, antipyrine, diazepam, hexobarbitone and tolbutamide in man [6, 38–40]. BROD activity in rats is induced by PAHs, PB and the P450 3A-inducer, pregnenolone 16α-carbonitrile [32, 41], but BROD activity in human liver microsomes correlates mainly with P450 3A [42]. The selective induction by RIF of BROD activity in HepG2 cells is consistent with an induction of the P450 3A subfamily in these cells. Our results on the induction of P450 MFO activities are in accord with a report that the treatment of HepG2 cells with 3-methylcholanthrene (3MC; a PAH inducer of P450 1A1 and P450 1A2 in rats) increases the levels of mRNA for P450 1A1 and P450 1A2, but not P450 3A3, whilst treatment of HepG2 cells with DEX increases mRNA levels for P450 3A3 but not for the P450 1A forms [43].

The inhibitory effects of anti-P450 antibodies on the HepG2 AROD activities in several instances prove that these were catalysed by P450. The fact that the extent of antibody inhibition was in several cases altered in PB- or BA-treated cells compared to the UT or DMSO-treated controls suggests that the inducers caused changes in the forms of P450 expressed in the HepG2 cells. The effects of the antibodies on EROD are consistent with this activity

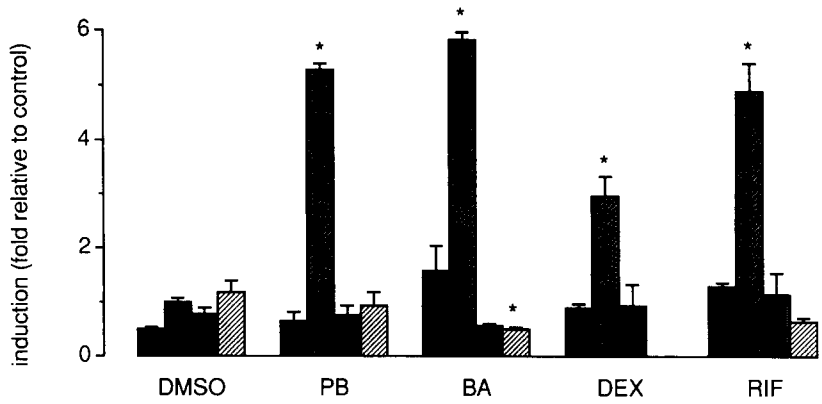

Fig. 3. Induction of GT activities in HepG2 cells. HepG2 cells were treated for 3 days with 0.0625% (v/v) DMSO, 25 μM BA, 2 mM PB, 1 μM DEX or 50 μM RIF as described in Materials and Methods. The figure shows the fold increases in naphthol (■), bilirubin (▨), morphine (▩) and testosterone (▨) GT activities in DMSO-, PB-, BA-, DEX- and RIF-treated cells, calculated relative to the mean values for the respective reactions in either untreated (UT) cells (for DMSO, PB and DEX treatments) or DMSO-treated cells (for BA and RIF treatments). Data are means ± SD for three to five experiments. *The mean activity in the treated cells was significantly different from the mean activity for the respective reaction in UT or DMSO control cells ($P \leq 0.05$ by ANOVA and Dunnett's *post-hoc* test).

being catalysed by P450 1A1 in UT, DMSO-treated and BA-treated HepG2 cells, but with the possible involvement of additional, unidentified forms of P450 in PB-treated cells. A major role for P450 1A1 in EROD activity in both BA- and PB-treated HepG2 cells (albeit cultured in a different medium, Dulbecco's minimum essential medium) was previously indicated by a strong inhibition of EROD in both cases by the selective P450 1A inhibitor, α-naphthoflavone (ANF) [15]. ANF selectively inhibits rat and human P450 1A1 and P450 1A2 but not human P450 3A [44, 45]. The inhibitory effect of anti-P450 1A2 on EROD in UT and DMSO-treated cells and its lack of effect in BA-treated cells might indicate that P450 1A2 also is involved in EROD in the control cells but is supplanted by induced P450 1A1 in the BA-treated cells. Alternatively, inhibition by anti-P450 1A2 might merely be due to cross-reactivity of the antibody with P450 1A1, but it then becomes difficult to explain why the inhibitory potency for EROD of anti-P450 1A2 relative to anti-P450 1A1 should be so different in BA-treated cells compared to UT and DMSO-treated cells. (Immunoblot studies using anti-P450 1A1 and anti-P450 1A2 to stain purified rat P450 1A1 and P450 1A2, and 3MC-induced and isosafrole-induced rat liver microsomes separated by SDS–PAGE show that each antibody cross-reacts only very weakly with the converse antigen—data not shown). The literature on P450 1A forms in HepG2 cells is inconsistent, in that McManus *et al.* [45] stated that HepG2 cells express P450 1A1 but not P450 1A2, whereas Fukuda *et al.* [43] reported the presence of mRNA for both P450 1A1 and P450 1A2 in 3MC-treated HepG2 cells, but mRNA for only P450 1A1 in control cells. The antibody data presented here also indicate that P450 1A1 and additional, unidentified forms of P450 are probably together involved in BROD in control and BA-treated cells, but that only unidentified forms (not, however, P450 2B) are probably involved in PB-treated cells. This is consistent with our previous observation that ANF caused approximately 50% inhibition of BROD in BA-treated HepG2 cells (cultured in Dulbecco's medium) but did not inhibit this reaction in PB-treated HepG2 cells [15]. The apparent lack of involvement of P450 2B forms in BROD in PB-treated cells is also consistent with the lack of induction of this activity by PB. The main P450 subfamily induced in rats by PB *in vivo* is P450 2B [1], which is responsible for virtually all the PROD activity in PB-induced rat liver microsomes [32]. The weak inhibition of PROD in PB-treated HepG2 cells might indicate that a PB-induced P450 2B form was involved in catalysing PROD activity in these cells, although it seems necessary to apportion the major role to other, unidentified forms. Since PB induces the P450 3A subfamily in man [3], and both rat and human P450 3A are associated with BROD activity [41, 42], it may be that P450 3A forms were responsible for much of the PROD and BROD activity in PB-treated HepG2 cells, a possibility that will be investigated in a forthcoming study.

The increases in AROD MFO activities observed in the HepG2 cells in response to inducer treatments *in vitro* were in general many times lower than those found in rodent liver in response to inducer treatments *in vivo*. A likely explanation is that the inducing agents act synergistically *in vivo* with endogenous factors, missing from our HepG2 cultures, to produce their maximal effect. In this context DEX has been shown to act synergistically with PB to induce P450 2B mRNA in adult rat liver *in vivo* [46] and to increase P450 2B activity in cultured rat hepatocytes [35].

BA, PB, DEX and RIF treatments of HepG2 cells in culture each increased bilirubin GT activity by 3–6-fold. The non-selective nature of this response

of bilirubin GT activity is in contrast to the highly selective nature of the effects of these inducers on P450 forms, both in culture and *in vivo*. Since bilirubin is the product of haem catabolism and is excreted mainly as the glucuronide [24], the increase seen in bilirubin GT may possibly be a consequence of a common, unspecified effect of the inducers on haem synthesis in HepG2 cells. We have reported previously that the haem synthesis inducers, DMSO (2%) and δ-aminolaevulinic acid, increase both bilirubin and bilirubin GT in HepG2 cells [24]. Our observation of a small (1.6-fold) increase in 1-naphthol GT activity in response to BA treatment of HepG2 cells reflects an earlier report that 1-naphthol GT activity is increased 2-fold by 3MC treatment of HepG2 cells in culture [19]. We have previously demonstrated PB induction of phenolphthalein GT activity in HepG2 cells [15] and of bilirubin GT activities in cultured human hepatocytes [47]. However, an increase in testosterone GT activity in response to PB treatment *in vitro*, which had been observed in primary human hepatocyte cultures [47], was not mirrored in HepG2 cells in the present study, which may reflect the absence of a specific, inducible GT form in HepG2 cells. Very little is known about the response of human liver GT isoenzymes to inducing agents *in vivo*, but human liver microsomal bilirubin GT shows an approximately 3-fold increase in activity following treatment of patients with barbiturates *in vivo* [19].

This paper has demonstrated that the MFO and GT isoenzymes of human HepG2 hepatoma cells are capable of responding differentially to known inducing agents. A characteristic response to PB induction has been suggested as an indicator of differentiated liver function [48], and the fact that PB treatment of HepG2 cells caused a selective increase in PROD activity and an increase, although non-selective, in bilirubin GT activity suggests that the cell line retains this differentiated hepatocyte characteristic. The availability of a suitable, stable cell line derived from human liver would greatly facilitate direct studies of the induction of xenobiotic-metabolizing enzymes in man. The ability to study induction in cell culture would have the added benefit of enabling investigation of the effects of low-level exposure to chemicals over a long period, which is typical of chronic drug therapy and of human exposure to inducing agents present in the environment and very different from the usual short-term, high-dose induction regimens used in experimental animals *in vivo*. These results have shown that HepG2 cells may be such a suitable *in vitro* model system for induction studies.

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