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Role of cytochrome P450 1A2 in bilirubin degradation Studies in *Cyp1a2* (−/−) mutant mice

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Abstract

In congenital jaundice, which is due to defects of bilirubin gluruconidation, bilirubin is degraded by an alternative pathway into unidentified products. Previously, it was shown that plasma bilirubin levels can be decreased in rats with this defect by inducers of CYP1A enzymes. Here, liver microsomes from rats or mice treated with β-naphthoflavone (BNF) or 3-methylcholanthrene (3 MC) had increased activity for bilirubin degradation. The activity was further stimulated by addition of the coplanar molecule 3,4,3′,4′-tetrachlorobiphenyl (TCB). There was more stimulation of bilirubin degradation by TCB in microsomes from BNF-treated rats than in microsomes from BNF-treated mice. CYP1A1 to CYP1A2 ratios were greater in rats treated with BNF. In *Cyp1a2* (−/−) mutant mice, 3-MC treatment did not increase the rate of bilirubin degradation, but TCB increased this degradation severalfold. Between SWR and C57BL/6 inbred mouse strains that have a 2-fold difference in hepatic constitutive CYP1A2 levels, there was also a 2-fold difference in bilirubin degradation; TCB did not stimulate in either strain. We conclude that CYP1A2 is responsible for microsomal bilirubin degradation in the absence of TCB. TCB was required for bilirubin degradation by CYP1A1. Manipulation of CYP1A2 may be of therapeutic benefit in patients with these diseases of bilirubin conjugation.

Keywords: *Cyp1a2* knockout mice; CYP1A2; Bilirubin degradation; Congenital jaundice; Bilirubin oxidation by CYP1A enzymes; 3,4,3′,4′-tetrachlorobiphenyl

1. Introduction

The diseases of congenital jaundice (type I and II Crigler–Najjar syndrome and Gilbert's syndrome) are due to inherited defects of bilirubin glucuronidation [1]. The severity of these diseases is due to partial or complete loss of the ability to conjugate bilirubin with glucuronic acid [1]. There is an alternative pathway in which bilirubin is oxidized instead to bleached, diazo-negative products [2]. In congenitally jaundiced Gunn rats, which lack expression of UDP-glucuronyltransferase, this alternative pathway can be stimulated by inducers of CYP1A enzymes such as TCDD, as shown by the alleviation of jaundice [3]. Previous work has shown that treatment of chick embryos and rats with TCDD or other inducers of CYP1A enzymes caused a significant increase in the rate of NADPH-dependent bilirubin degradation by liver microsomes *in vitro*. This effect of TCDD is similar in both Gunn rats and in non-jaundiced Wistar-derived rats [4]. Liver microsomes from TCDD-treated rats showed a significant increase in the NADPH-dependent rate of bilirubin degradation compared to microsomes from uninduced animals. There was a further doubling in the rate of bilirubin degradation when a coplanar halogenated molecule, such as TCB, was added to the microsomal incubation together with NADPH [4,5]. Previously, evidence was presented for CYP1A1 catalyzing bilirubin degradation by induced rat liver microsomes in the presence of TCB [5]. The CYP form responsible for biliru-

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Abbreviations: BNF, β-naphthoflavone; CYP, cytochrome P450; EROD, 7-ethoxyresorufin 7-*O*-deethylase; 3-MC, 3-methylcholanthrene; MROD, 7-methoxyresorufin 7-*O*-demethylase; TCB, 3,4,3′,4′-tetrachlorobiphenyl; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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bin degradation in the absence of TCB was not identified. However, the oxidation of another target molecule, uroporphyrinogen, by liver microsomes from induced rodents has been shown to be specifically catalyzed by CYP1A2, rather than CYP1A1. In this case, no addition of TCB is apparently required [6,7].

The purpose of this work was twofold. First, we investigated the relative roles of CYP1A1 and CYP1A2 in bilirubin degradation. This was studied in hepatic microsomes from normal rats and mice, and from mice in which the *Cyp1a2* gene had been disrupted [8]. Secondly, we studied the bilirubin-lowering effect of TCDD, investigating the time-course of this effect and whether it could be potentiated by iron administration. The results show that CYP1A2 has a major role in bilirubin degradation catalyzed by hepatic microsomes. The data also suggest that CYP1A1 is mostly responsible for the additional activity in the presence of TCB. The physiological role of these findings was supported by the observation that administration of a single dose of TCDD to Gunn rats *in vivo* produced a marked lowering of plasma bilirubin for at least 1.5 months, with a new steady-state concentration of plasma bilirubin at only 40% of the values in the same animals before TCDD treatment. A preliminary communication of some of these findings has appeared in abstract form [9].

2. Materials and methods

2.1. Source of special chemicals

NADPH, bilirubin, and 7-methoxyresorufin were from Sigma-Aldrich; 7-ethoxyresorufin was from Pierce Chemical Co.; TCB was from Ultra Scientific; and ketoconazole was a gift from Janssen Pharmaceuticals Ltd. Imferon (iron–dextran) and dextran 5 were donated by Fisons Ltd.

2.2. Animals

Male Wistar rats (body weight, 175–200 g) and male C57BL/6J mice (body weight 22–24 g) were obtained from Charles River Italia and were allowed food and water *ad lib*. until they were killed by decapitation under CO_2 general anesthesia. Treatment with BNF involved two intraperitoneal injections of 80 mg/kg in corn oil, 48 hr and again 24 hr before killing. Liver microsomal fractions were isolated and washed as previously described [4], and stored at −80° resuspended in 0.1 M phosphate buffer, pH 7.4, containing EDTA (1 mM) and glycerol (20%, v/v).

In one experiment, female rats (body weight, 185–210 g) homozygous for the Gunn genetic trait [10] were used. They were given iron–dextran (Imferon), corresponding to 100 mg Fe/kg, or dextran alone by subcutaneous injection and, 3 days later, TCDD (10 μg/kg) by intraperitoneal injection; heparinized blood samples were obtained repeatedly from each animal, before and at various times after treatment, in order to measure plasma bilirubin. The dose of iron–dextran used in these rat experiments had previously been shown [11] to cause a 10-fold induction of hepatic heme oxygenase, a typical oxidative stress response [12], with other microsomal changes in the liver suggestive of increased free radical reactions [13].

Hepatic microsomes were also prepared from male mice of the C57BL/6 and SWR strains (obtained from Charles River and Jackson Laboratory, respectively) after homogenization of the livers in 0.25 M sucrose, containing: 25 mM Tris, pH 7.4; EDTA, 1 mM; butylated hydroxytoluene, 25 μM; and phenylmethylsulphonyl fluoride, 25 μM. They were then resuspended and stored, as described above.

Male mice of the *Cyp1a2* (−/−) line recently described were also used [8]. These mice had an 87% C57BL/6J genetic background, and were given a single i.p. injection of 3-MC (100 mg/kg) in corn oil (or corn oil alone) 48 hr before killing. Their liver microsomes were prepared and stored exactly as described above.

All procedures involving animal care or treatments were in agreement with institutional guidelines in compliance with national and international laws and policies.

2.3. Analytical techniques

Plasma bilirubin was measured by the colorimetric method of Michäelsson [14]. Cytochrome P450 content was measured according to the method of Omura and Sato [15] and protein by the method of Lowry *et al*. [16], using crystalline BSA as the standard. The loss of bilirubin in microsomal incubations was either monitored at 450 nm in a split-beam spectrophotometer or by the difference in absorbance between 450 and 500 nm (in a single-cell Beckman DU-640 spectrophotometer) using ϵmM values of 56.8 and 47.3, respectively, obtained experimentally in 0.1 M Tris–HCl buffer, pH 8.2.

2.4. Experiments with isolated microsomal fractions

The rate of metabolism of bilirubin by isolated microsomal fraction was studied at 28°, as previously described [4]. The incubation mixture contained the following components in a total volume of 2 mL, with final concentrations in parentheses: Tris–HCl buffer, pH 8.2 (90 mM); EDTA (2.5 mM); KCl (5 mM); phosphate/EDTA/glycerol mixture, at the composition given above (15 μL/mL) delivering the liver microsomes, so as to reach a final concentration of cytochrome P450 of 100 nM; NADPH (81.6 μM); bilirubin (12 μM); and DMSO (2.75 μL/mL) with or without TCB (final conc. 3.42 μM). This concentration of TCB had previously been found to afford maximum stimulation of bilirubin degradation [4]. In the experiments in which wild-type and *Cyp1a2* knockout lines or the C57BL/6 and SWR strains were compared, the microsomal protein, rather than cytochrome P450 concentration, was kept constant, at approx. 60 μg protein/mL.

Table 1
Rate of bilirubin degradation and 7-alkoxy 7-*O*-dealkylase activities of liver microsomes from control and BNF-treated rats and mice

Species	Pretreatment of animals *in vivo*	Rate of bilirubin degradation			7-alkoxyresorufin 7-*O*-dealkylase activity		
		A. In the absence of TCB	B. In the presence of TCB	C. Effect of TCB (B-A)	EROD	MROD	MROD/EROD ratio
		pmol/min/mg of protein					
Rat	None	470 ± 90 (5)	410 ± 90 (5)	−60 ± 40	300 ± 40 (5)	70 ± 10 (5)	0.25 ± 0.02
	BNF	720 ± 60 (5)**	1300 ± 140 (5)**·****	580 ± 90**	16,990 ± 2,210 (5)*	1380 ± 110 (5)**	0.09 ± 0.01**
Mouse	None	750 ± 60 (4)	760 ± 54 (4)	10 ± 22	210 ± 35 (4)	230 ± 40 (4)	1.10 ± 0.01***
	BNF	2590 ± 230 (4)**	2600 ± 145 (4)**	10 ± 17	10,040 ± 1,040 (4)*	5680 ± 280 (4)**·***	0.60 ± 0.08***

Animals were either untreated controls or were treated with BNF, as described in the Methods section. Hepatic microsomal fractions were isolated, and their 7-ethoxy and 7-methoxy 7-*O*-dealkylases (EROD and MROD, respectively) were assayed *in vitro*, as well as their bilirubin-degrading activity, the latter both in the absence and presence of a co-planar polyhalogenated biphenyl, 3,4,3′,4′-tetrachlorobiphenyl (TCB, final conc. 3.42 μM). Results are given as means ± SEM of the number of animals in parentheses.

* $P < 0.05$.

** $P < 0.01$, compared with corresponding value obtained with animals given no BNF.

*** $P < 0.01$, compared with corresponding value obtained in similarly treated rats.

**** $P < 0.01$, compared with corresponding value obtained without TCB.

EROD and MROD activities were measured by a slight modification of a previous method [17], by following the rate of increase in fluorescence in a cuvette incubated in the temperature-controlled compartment (28°) of a Perkin Elmer LS-5 luminescence spectrometer. The incubation mixture contained the following components in a total volume of 2.25 mL: Tris–HCl buffer, pH 7.5 (50 mM); $MgCl_2$ (25 mM); either 7-ethoxy- or 7-methoxyresorufin (2 μM) added in DMSO (4.4 μL/mL); and microsomes (4.4 μL/mL). The microsomes were added either as an undiluted suspension in phosphate/EDTA/glycerol (composition given above) or, in the case of induced microsomes, as a 10-fold dilution (with phosphate/EDTA/glycerol) carried out immediately before the assay. After a preliminary incubation of 1 min, the reaction was started by addition of NADPH to a final concentration of 80 μM. Under these conditions, the initial rates of both EROD and MROD activities were linearly related to the amount of microsomes taken.

When the effect of ketoconazole on the bilirubin-degrading and EROD/MROD activities was studied, the microsomes were preincubated for 1 min with the inhibitor (added in 1 μL DMSO/mL of incubation, to a final concentration of 10 μM) prior to addition of either bilirubin or the appropriate 7-alkoxyresorufin, and the assays were then carried out as described above.

3. Results

3.1. Rates of bilirubin degradation by hepatic microsomes from rats and mice in the presence and absence of TCB

BNF and 3-MC were used as inducers of CYP1A enzymes in the following experiments, since previous work had shown them to be capable of stimulating bilirubin oxidation as did TCDD [4,5]. Table 1 shows a comparison of the bilirubin degradation activities of hepatic microsomes from untreated and BNF-treated wild-type rats and mice. BNF induced a 2- to 3-fold increase in bilirubin-degrading activity in both species in the absence of TCB in the reaction mixture. However, in the presence of TCB, there was a further doubling of the activity catalyzed by rat microsomes, but only a small increase with mouse microsomes (Table 1).

We used EROD and MROD activities to measure the relative amounts of CYP1A1 and CYP1A2 in these microsomes. These activities have been taken [18–20] as indices of CYP1A1 and CYP1A2, respectively. After BNF treatment, CYP1A1 (EROD) predominated in rats, whereas in mice, there was a relatively greater contribution of CYP1A2 (MROD), as indicated by the change in the ratio of MROD to EROD (Table 1). A preponderance of CYP1A1, as compared to CYP1A2, was also described in rats induced with BNF by Levine *et al*. [21]. A similar species difference between rats and mice in MROD/EROD induction was reported by Nerurkar *et al*. [20], who also confirmed such a species difference in enzyme profiles by immunoblotting experiments. These data suggest that the stimulation of bilirubin degradation caused by TCB in rat, but not mouse, microsomes was due to CYP1A1 rather than CYP1A2. This hypothesis was next examined in *Cyp1a2* (−/−) mutant mice.

3.2. Rates of bilirubin degradation in Cyp1a2 (−/−) mutant mice

Cyp1a2 (−/−) mutant mice were used to investigate (a) the relative roles of CYP1A2 and CYP1A1 in bilirubin degradation and (b) whether CYP1A1 and CYP1A2 require TCB addition for bilirubin-degrading activity (Table 2). In wild-type mice, 3-MC treatment caused a 3-fold increase in the rate of bilirubin degradation. Addition of TCB to the reaction mixture caused a small but significant increase in bilirubin degradation. In *Cyp1a2* (−/−) mutant mice, there

Table 2
Effect of treating *Cyp1a2* (−/−) mutant and wild-type mice with 3-methylcholanthrene *in vivo*

Line of mice	Treatment *in vivo*	Rate of bilirubin degradation			7-alkoxyresorufin 7-*O*-dealkylase activities		
		A. In the absence of TCB	B. In the presence of TCB	C. Effect of 3,4-TCB (B-A)	EROD	MROD	MROD/EROD Ratio
		pmol/min/mg of protein					
Wild-type	None	850 ± 90 (5)	890 ± 110 (5)	40 ± 70	48 ± 6 (5)	85 ± 20 (5)	1.8 ± 0.05
	3-MC	2390 ± 90 (3)**	2820 ± 40 (3)**.***	430 ± 110*	5730 ± 190 (3)**	2530 ± 490 (3)**	0.44 ± 0.04**
Cyp1a2 (−/−) null mutant	None	690 ± 50 (5)	660 ± 35 (5)	−30 ± 56	40 ± 3 (5)	14 ± 1.8 (5)	0.35 ± 0.04******
	3-MC	740 ± 60 (3)******	2630 ± 3 (3)**.*****	1890 ± 60**.*****.******	5300 ± 310 (3)**.******	1290 ± 12 (3)**.******	0.24 ± 0.01******

The microsomal fractions from treated and corresponding control mice were tested *in vitro* for bilirubin-degrading activity (both in the absence and presence of 3.42 μM TCB). The rates of microsomal 7-alkoxy-7-*O*-dealkylase (EROD and MROD) activities were also determined. Results given are means ± SEM of observations obtained in the number of animals indicated in parenthesis.
* $P < 0.05$ and ** $P < 0.001$, compared to corresponding values in mice of the same genetic stock, but receiving no 3-MC treatment.
*** $P < 0.05$ and **** $P < 0.001$, compared to corresponding values obtained in the absence of TCB.
***** $P < 0.05$ and ****** $P < 0.001$, compared to corresponding values in wild-type strain.

was no MC-induced increase in microsomal bilirubin degradation in the absence of TCB. However, addition of TCB caused a 3- to 5-fold increase in bilirubin degradation in these mice.

Table 2 also shows that in wild-type and mutant mice, MC increased both EROD and MROD. EROD activities were about the same in each line, indicating that CYP1A1 was increased to about the same extent as confirmed elsewhere immunochemically [22]. As expected, MROD activities in MC-treated wild-type mice were about twice those in MC-treated mutant mice, reflecting the contribution of CYP1A2 to this activity. There was no CYP1A2 in the mutant mice as determined immunochemically [22]. These results are consistent with most EROD being catalyzed by CYP1A1 and most MROD by CYP1A2, the ratios being indicative of the relative CYP1A2/CYP1A1 contents, as discussed previously [23]. These results indicate that most of the bilirubin degradation activity in MC-treated wild-type mice was due to CYP1A2, even in the presence of TCB. However, in the MC-treated mutant mice, most of the activity in the presence of TCB appeared to be due to CYP1A1.

It is not clear why TCB caused only a small increase in activity in microsomes from MC-treated wild-type mice, since in the mutant mice addition of TCB caused a large increase in activity. We suggest that there is a maximal stimulated rate even when both CYP1A1 and CYP1A2 have been induced and contribute to the activity. This may be due to competition for reducing equivalents or conversely by one form inhibiting the bilirubin-degrading action of the other. An alternative explanation may be that CYP1A2 responds to addition of TCB with an inhibition (rather than stimulation) of bilirubin degradation, compensating in this way for a concurrent CYP1A1-dependent stimulation. Inhibition of bilirubin degradation was observed in some of our experiments after addition of TCB to microsomes from untreated mice, and a similar inhibition was observed in microsomal catalysis of uroporphyrinogen oxidation [24].

3.3. Bilirubin-degrading activity of liver microsomes from untreated mice of two strains with different constitutive levels of CYP1A2

An approximate twofold difference in the constitutive levels of CYP1A2 was reported between mice of the C57BL/6 strain and those of the SWR strain [23]. We therefore investigated whether similar differences between the two strains also existed in the bilirubin-degrading activity of their liver microsomes and whether this activity could be stimulated by TCB. The results showed about a 2-fold difference in the microsomal rate of bilirubin degradation between uninduced mice of the two inbred strains (Table 3). These differences correlated with similar differences in the rates of MROD activities. There was no increase in bilirubin degradation by addition of TCB in the reaction mixture. These findings suggest that CYP1A2, but not CYP1A1, is

Table 3
Differences between male mice of two inbred strains (C57 BL/6 and SWR) in the bilirubin-degrading and MROD activities of the liver microsomes

Strain	Rate of bilirubin degradation		MROD activity
	In the absence of TCB	In the presence of TCB	
	pmol/min/mg of protein		
C57BL/6	840 ± 75 (3)	745 ± 100 (3)	79 ± 6 (3)
SWR	1350 ± 90 (3)*	1130 ± 130 (3)	195 ± 12 (3)*

Results given are averages ± SEM of the number of observations in parenthesis.

* $P < 0.02$, compared to corresponding values obtained in C57 BL/6 mice.

responsible for these strain differences in constitutive rates of bilirubin oxidation; in agreement with this conclusion, CYP1A1 would be expected to be absent in these animals, since—unlike CYP1A2—CYP1A1 only appears in the liver after administration of the appropriate inducer [25].

Although CYP1A2 is shown here to contribute significantly to bilirubin degradation in microsomes from untreated mice, constitutive liver cytochrome P450s and microsomal oxidative systems other than CYP enzymes might also contribute to some extent to the constitutive rates of bilirubin degradation. This was suggested by the finding that uninduced *Cyp1a2* (−/−) mice, which lack both CYP1A1 and CYP1A2, still exhibited a significant microsomal rate of bilirubin oxidation (Table 2). We also found that 60% of the bilirubin-degrading activity of microsomes from untreated *Cyp1a2* (−/−) mice was inhibited by 10 μM ketoconazole (data not shown), which suggests the additional involvement of constitutive CYP enzymes other than CYP1A, but the identity of these additional CYP enzymes is at present unknown.

3.4. Effect of TCDD and iron–dextran on plasma bilirubin levels in Gunn rats

TCDD is known to be a powerful and persistent inducer of CYP1A enzymes in rats. Therefore, we investigated whether an injection of TCDD would cause a persistent decrease in plasma levels of bilirubin in Gunn rats. Bilirubin can also be oxidized by iron-catalyzed oxidative reactions *in vitro* [26,27]. Therefore, we also investigated whether administration of iron as iron–dextran *in vivo* causes further decreases in plasma levels of bilirubin. The main purpose of these experiments was to ascertain the relative physiological significance of CYP1A-dependent and iron-dependent bilirubin degradation in achieving a reduction in plasma bilirubin *in vivo*.

Jaundiced Gunn rats were first given an injection of either dextran (group 1) or iron–dextran (group 2); 3 days later, all rats received an i.p. injection of TCDD. Plasma bilirubin levels, all expressed in μmol/L, were as follows (means with range in parenthesis). Group 1: before treatment, 176 (137,215); 3 days after dextran, 173 (152,195); 1 day after TCDD, 148 (146,151); 4 days after TCDD, 66 (56,77). Group 2: before treatment, 184 (171,197); 3 days after iron–dextran, 178 (168,188); 1 day after TCDD, 160 (150,170); 4 days after TCDD, 75 (71,79). The bilirubin-lowering effect of TCDD was found to be maximal 4 days after injection, and to persist for at least 1.5 months (results not shown). A new steady-state concentration of plasma bilirubin was observed after TCDD treatment over this period, at concentrations only about 40% of those shown by untreated rats. As far as we are aware, this is the first time that a single injection of TCDD in rats has produced such a long-lasting effect on bilirubin plasma levels. In contrast, no evidence was obtained for a loss of plasma bilirubin caused by injection of iron alone, nor did iron loading appear to potentiate the bilirubin-lowering effect of TCDD.

These *in vivo* findings are compatible with the demonstrated role of CYP1A in bilirubin oxidation and also suggest that, even though bilirubin can be oxidized by iron-catalyzed oxidative reactions *in vitro* [26,27], the CYP1A system appears to be much more significant in lowering plasma bilirubin in congenitally jaundiced Gunn rat *in vivo*.

4. Discussion

In congenital jaundice, where the main pathway of glucuronidation is defective, an alternative pathway of oxidative degradation of bilirubin becomes activated [2]. In congenitally jaundiced Gunn rats that lack expression of UDP-glucuronyltransferase, this alternative pathway can be stimulated by inducers of CYP1A enzymes such as TCDD, as shown by the alleviation of jaundice [3]. The present *in vitro* results, obtained with microsomes from BNF- and MC-treated rats and mice, strongly implicate CYP1A2 as the major catalyst of bilirubin degradation. This conclusion is also supported by our studies with *Cyp1a2* (−/−) mice and with strains of mice with different constitutive levels of CYP1A2. CYP1A2 is present constitutively in the liver, but can also be markedly induced, together with CYP1A1, after exposure to the appropriate chemical inducer. The present findings confirm the previous findings of marked stimulation of rates of bilirubin degradation, both in the presence and absence of TCB, after treatment with CYP1A inducers [4,5]. Since CYP1A2 is constitutively present and did not require TCB for the oxidation of bilirubin, we conclude that this form contributes most to bilirubin homeostasis *in vivo*. In contrast, the stimulation of bilirubin degradation by TCB largely reflects the contribution of CYP1A1, a conclusion in line with previous, more direct findings [5] obtained *in vitro* with an inhibitory antibody. The physiological significance of this TCB-stimulated, CYP1A1-dependent bilirubin degradation is more difficult to assess. It is not yet clear whether

an endogenous chemical acting like TCB will also be required *in vivo*, and if so what its chemical nature might be.

The exact mechanism by which both enzymes catalyze bilirubin degradation also needs clarification. Since TCB itself can be metabolized by CYP1A1 [28,29], the possibility of a bilirubin-degrading metabolite, such as the reactive metabolite(s) shown to become bound to proteins [28] during metabolism of TCB by this enzyme, should be considered. However, the planar 3,4,5,3′,4′,5′-hexabromobiphenyl, which lacks the main structural features necessary for metabolism by cytochrome P450 (i.e. vicinal unsubstituted carbon atoms and unsubstituted meta positions [30]), was more active than TCB at stimulating bilirubin degradation by 3MC-induced rat liver microsomes [27]. This provides some support for a previously proposed alternative mechanism, involving binding of a planar biphenyl at the active site of CYP1A1, loss of monooxygenase activity, and uncoupled production of oxidizing species [4]. This hypothetical mechanism could result from an elicited transition of the spin state of the heme iron from low to high spin and an associated increase in redox potential, leading to facilitated reduction of oxygen and increased production of oxidizing species [31]. The hypothesis that TCB, on binding to CYP1A1, stimulates production of oxidative species has also been proposed by Schlezinger *et al*. [32]. In contrast, CYP1A2 intrinsically possesses a bilirubin-degrading potential. It is not yet clear whether it accepts bilirubin at its active site as a conventional substrate or whether bilirubin merely acts as an adventitious acceptor for oxidizing species produced by the enzyme.

As indicated earlier, there are similarities between microsomal bilirubin degradation and the oxidation of uroporphyrinogen, the suggested key reaction in development of uroporphyria [6,22]. In uroporphyrinogen oxidation, CYP1A2 is active in the absence of TCB. Interestingly, chick CYP1A5, the equivalent of mammalian CYP1A2, catalyzes uroporphyrinogen oxidation [33], but only in the presence of TCB [24].

It has been suggested [34] that CYP1A enzymes may initiate oxidative stress reactions in the cell, with several target molecules participating, among these—at least under certain conditions—DNA bases. The present work shows bilirubin to be one additional such target and lends support to this general view.

In summary, hepatic microsomal bilirubin degradation has been shown to be largely dependent on expression of CYP1A2, especially in microsomes from mice treated with inducers of CYP1As. TCB-stimulated bilirubin degradation is catalyzed by CYP1A1. We suggest that CYP1A2 activity contributes very significantly to the metabolism of bilirubin in Gunn rats, especially after induction, and that stimulation of this degrading activity may also be of therapeutic value in humans who lack UDP-glucuronidation activity. Non-toxic inducers of CYP1A such as indole-3 carbinol have been proposed and are effective in Gunn rats [35].

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