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Isozyme Selectivity of the Inhibition of Rat Liver Cytochromes P-450 by Chloramphenicol *in Vivo*

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SUMMARY

The isozyme-selectivity of chloramphenicol as an inhibitor of rat liver cytochromes P-450 has been investigated. Untreated rats and rats treated with the inducers phenobarbital, β-naphthoflavone, pregnenolone 16α-carbonitrile, and clofibrate have been injected intraperitoneally with chloramphenicol, and inhibition of specific cytochrome P-450 isozymes has been assessed by monitoring the metabolism of warfarin, testosterone, isosafrole, or lauric acid in subsequently prepared hepatic microsomal preparations. Of eight major cytochrome P-450 isozymes which could be monitored in this fashion, three were inhibited by more than 50% by a dose of chloramphenicol of 300 mg/kg, whereas no evidence of inhibition of the remaining isozymes was obtained. P-450$_{PB-C}$, an isozyme which is present in significant amounts in untreated rats and which is induced approximately 2-fold by phenobarbital, was the most susceptible cytochrome P-450 to inhibition by chloramphenicol both *in vivo* and *in vitro*. P-450$_{PB-B}$, the major phenobarbital-inducible isozyme, and P-450$_{UT-A}$, a male-specific testosterone 2α- and 16α-hydroxylase, were intermediate in their susceptibility to chloramphenicol. In contrast, the major isozymes induced by β-naphthoflavone, pregnenolone 16α-carbonitrile, and clofibrate, as well as a constitutive testosterone 7α-hydroxylase, were not inhibited by chloramphenicol.

INTRODUCTION

The inhibition of hepatic microsomal cytochrome P-450-dependent monooxygenases by the antibiotic chloramphenicol is well documented in a number of species including humans (1–7). *In vitro*, chloramphenicol causes both reversible and irreversible inhibition. The reversible inhibition is competitive in nature (2, 4), whereas the irreversible inhibition results from the covalent modification of the protein moiety of the enzyme by reactive metabolites of chloramphenicol and can thus be classified as a suicidal process (5, 6, 8, 9). Such covalent modification and enzyme inactivation has also been demonstrated *in vivo* in rats treated with radiolabeled chloramphenicol, using either intact liver microsomes or purified isozymes (5, 10).

Since suicide substrates, also known as mechanism-based inactivators, rely not only on binding but also on catalytic specificity, they are among the most selective enzyme inhibitors (11). A number of investigators have commented on the potential utility of such compounds as specific inhibitors of different isozymes of cytochrome P-450 *in vivo* (12, 13), yet few systematic studies of the isozyme selectivity of known suicide substrates of cytochromes P-450 have actually been performed (14, 15). Very recently, we obtained evidence that chloramphenicol acts as an isozyme-selective inhibitor of cytochromes P-450 in rat lung and liver involved in the metabolism of *n*-hexane (6). In the present investigation we extend our previous work by using a battery of different assays to monitor the inhibition by chloramphenicol of eight major isozymes of rat liver cytochrome P-450 *in vivo*.

EXPERIMENTAL PROCEDURES

Materials. Warfarin was purchased from Calbiochem. Resolution of the racemic warfarin into the optically pure *R*- and *S*-sodium salts (16) as well as synthesis of metabolite standards (17) have been described previously. [1,2-^{14}C]Chloramphenicol and [4-^{14}C] testosterone were purchased from New England Nuclear. [1-^{14}C]Lauric acid was obtained from Amersham Corp. Unlabeled chloramphenicol and lauric acid, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, NADP, NADPH, BNF,[1] and clofibrate were purchased from Sigma. Isosafrole

[1] The abbreviations used are: BNF, β-naphthoflavone; UT, untreated; PB, phenobarbital; PCN, pregnenolone 16α-carbonitrile.

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was obtained from ICN (Plainview, NY) and PCN was a gift from The Upjohn Co.

Treatment of animals and preparation of microsomes. Male Sprague-Dawley rats (200 g) in groups of four were pretreated with PB, BNF, PCN, or clofibrate. PB was given for 5 days in the drinking water as a 0.1% solution (w/v). BNF (40 mg/kg) and clofibrate (400 mg/kg) were given for 3 days as single daily i.p. injections in 0.5 and 0.25 ml of corn oil, respectively. PCN (100 mg/kg) was administered by gastric intubation once daily for 3 days in 1 ml of 0.9% NaCl, 0.01% Tween 80 (w/v). Controls received vehicle only. On the last day of pretreatment, food was withheld, and in the case of the PB-treated rats, the PB solution was replaced with water. The next day, animals were injected with 100 or 300 mg/kg chloramphenicol given i.p. in 0.5 ml of propylene glycol. Controls received vehicle only. After 1 hr, the animals were killed by cervical dislocation, and individual liver microsomes were prepared essentially as described previously (5). The microsomes were stored at −70° in 10 mM Tris acetate buffer (pH 7.4) containing 20% glycerol, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride.

Assay procedures. Cytochrome P-450 contents were determined by the method of Omura and Sato (18) and protein by the method of Lowry *et al.* (19) using bovine serum albumin as the standard. Incubations with warfarin and identification and quantification of metabolites by high performance liquid chromatography were performed as previously described (17). Assays of testosterone metabolism have also been described previously (20). Formation of a metabolite complex of isosafrole was assayed spectrally as described by Ryan *et al.* (21). Metabolism of lauric acid was assayed by the TLC method of Orton and Parker (22), which measures the sum of 12- and 11-hydroxylated metabolites. All of the above assays were linear with respect to time and protein concentration under the conditions used. Covalent binding of radiolabeled chloramphenicol to microsomal protein was determined as described previously (5, 6).

Statistical analyses. Results of the *in vivo* experiments were analyzed by Student's *t* test. All results reported in the text as percentage of inhibition were statistically significant ($p < 0.05$) compared to the controls.

RESULTS

Effect of chloramphenicol on warfarin hydroxylase activity. In a series of previously reported studies we demonstrated that the regio- and stereoselective metabolism of warfarin by intact rat liver microsomes can be used to monitor the levels and activities of five distinct isozymes of rat liver cytochrome P-450 (23–26). In brief, the supporting evidence has included the profiles of warfarin metabolites produced by the purified enzymes in reconstituted systems (23) and inhibition of the formation of specific metabolites by antibodies raised to the purified cytochrome (25, 26). We have also demonstrated that the regio- and stereoselectivities of warfarin hydroxylase activities of the five purified isozymes are equivalent to those in microsomes (24). In the latter case, the conclusion was based on immunochemical quantitation of the hepatic cytochrome P-450 isozymes in microsomes from rats treated with various inducers (23). Based on these previous studies, the formation of the following metabolites of warfarin has been used in the present investigation to monitor inhibition of specific isozymes of cytochrome P-450 by chloramphenicol: *R*-6- and *S*-4′-hydroxylase activity in uninduced rats (isozyme UT-A)[2]; *R*-4′-hydroxylase activity in PB-treated rats (isozyme PB-B); *R*-10-hydroxylase activity in PB- and PCN-treated rats (isozyme PB/PCN-E); *R*-7-hydroxylase activity in uninduced and PB-treated rats (isozyme PB-C); and *R*-6-, *R*-8-, and *S*-6-hydroxylase activity in BNF-treated rats (isozyme BNF-B).

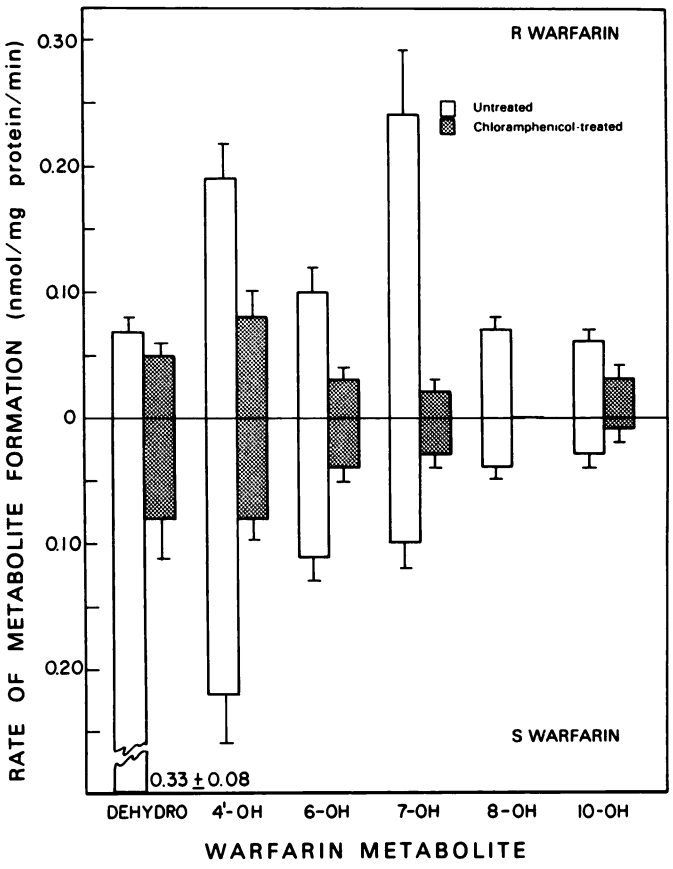

FIG. 1. *Effect of chloramphenicol administration (300 mg/kg) to untreated rats on in vitro rates of hepatic microsomal metabolism of R- and S-warfarin to dehydrowarfarin (dehydro), 4′-hydroxywarfarin (4′-OH), 6-hydroxywarfarin (6-OH), 7-hydroxywarfarin (7-OH), 8-hydroxywarfarin (8-OH), and 10-hydroxywarfarin (10-OH)*

Values above the line represent rates with *R*-warfarin and values below the line represent rates with *S*-warfarin. Bars represent the mean and error bars represent the standard deviation of triplicate analyses of rates from four individual rats. Chloramphenicol was administered to rats 1 hr prior to death. Metabolites were analyzed by high performance liquid chromatography.

The effect of 300 mg/kg chloramphenicol on *R*- and *S*-warfarin metabolism by liver microsomes from uninduced, PB-, PCN-, and BNF-treated rats is shown in Figs. 1–4. There was no evidence for any inhibition of isozyme PB/PCN-E in either the PB- (Fig. 2) or PCN-treated (Fig. 4) rats as evidenced by the absence of significant inhibition of *R*-10-hydroxylase activity, nor for inhibition of isozyme BNF-B in the BNF-treated rats (Fig. 3) as evidenced by the absence of significant inhibition of *R*-6-, *R*-8-, and *S*-6-hydroxylase activities. In contrast, isozyme UT-A in untreated rats (Fig. 1) was inhibited by approximately 60% as evidenced by inhibition of *R*-6- and *S*-4′-hydroxylase activities, and isozyme PB-B in PB-treated rats (Fig. 2) was also inhibited by 60% as evidenced by inhibition of *R*-4′-hydroxylase ac-

[2] The individual cytochrome P-450 isozymes examined are designated UT-A, PB-B, BNF-B, PB-C, PB-D, PB/PCN-E, UT-F, and ISF-G according to the nomenclature of Guengerich *et al.* (23). The prefix refers to the most effective inducer(s) of the particular isozyme, whereas the letters A through G are arbitrary designations based on the fractionation scheme. Constitutive isozymes are prefixed UT (untreated).

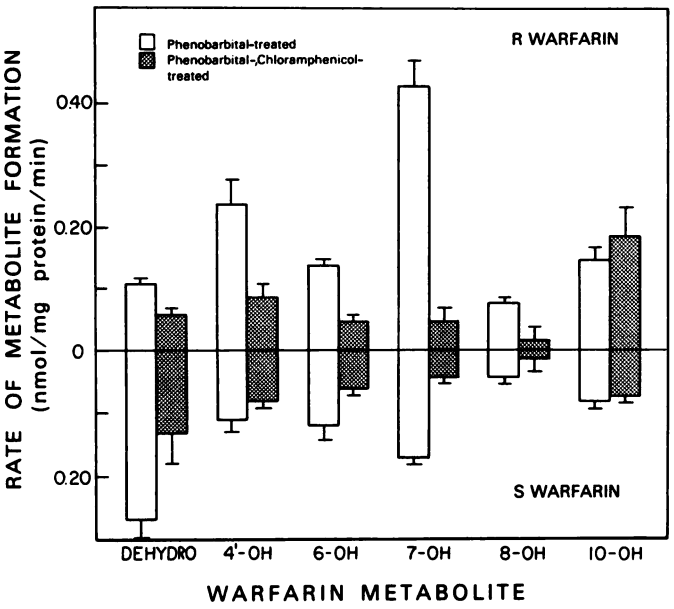

FIG. 2. *Effect of chloramphenicol administration to phenobarbital-induced rats on in vitro rates of hepatic microsomal metabolism of R- and S-warfarin*

Other details were as in Fig. 1.

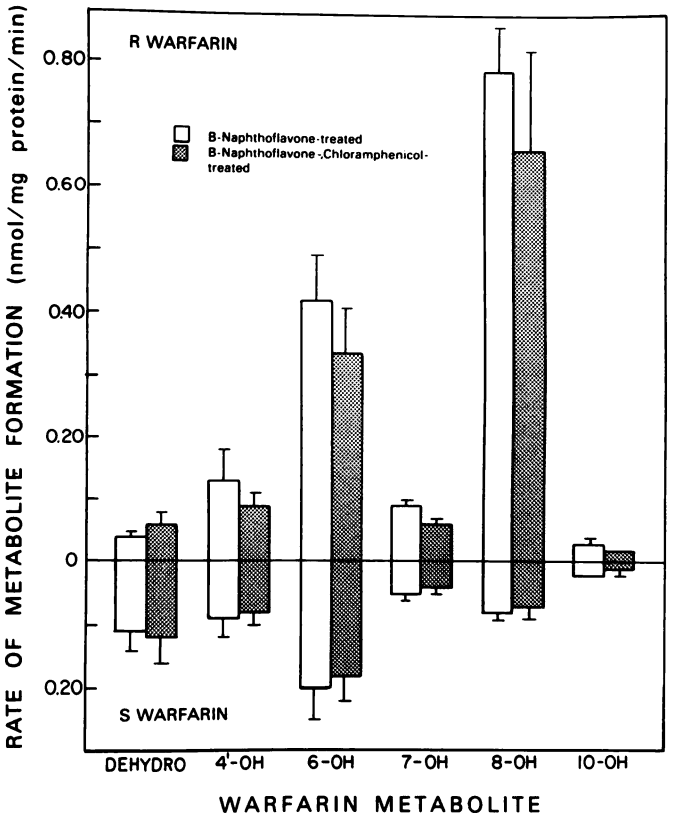

FIG. 3. *Effect of chloramphenicol administration to β-naphthoflavone-induced rats on in vitro rates of hepatic microsomal metabolism of R- and S-warfarin*

Other details were as in Fig. 1.

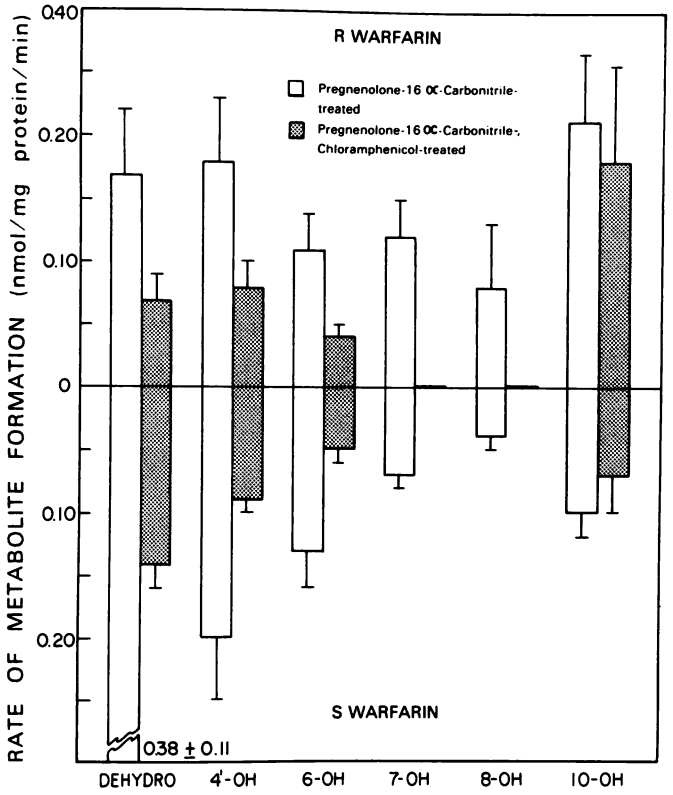

FIG. 4. *Effect of chloramphenicol administration to pregnenolone 16α-carbonitrile-induced rats on in vitro rates of hepatic microsomal metabolism of R- and S-warfarin*

Other details were as in Fig. 1.

tivity. The isozyme which was most markedly affected by chloramphenicol was PB-C in untreated (Fig. 1) and PB-treated (Fig. 2) rats as evidenced by the approximately 90% inhibition of *R*-7-hydroxylase activity. At a dose of 100 mg/kg administered to uninduced rats, only 40% inhibition of UT-A but 90% inhibition of PB-C was observed, whereas the same dose administered to PB-treated rats caused 20% inhibition of PB-B and 75% inhibition of PB-C (data not shown). All of these assessments are based on statistical evaluations of the data. The greater susceptibility to inhibition by chloramphenicol of PB-C compared to PB-B, as well as the lack of inhibition of PB/PCN-E, was also found after *in vitro* preincubation with chloramphenicol of microsomes from PB-induced rats, prior to assay of warfarin hydroxylase activity (Fig. 5).

An interesting result was the virtually complete inhibition by chloramphenicol of *R*-8-hydroxylase activity in untreated (Fig. 1) and PCN-treated (Fig. 4) rats. Warfarin *R*-8-hydroxylation is associated with isozyme BNF-B in the BNF-treated rats, but the concentration of BNF-B in untreated and PCN-treated rats is too low to account for the observed activity (23, 24). Furthermore, antibodies to BNF-B do not inhibit *R*-8-hydroxylase activity in control microsomes (25). Therefore, the chloramphenicol-mediated decrease in *R*-8-hydroxylase activity in the liver microsomes from the untreated and PCN-treated rats must be due to inhibition of some other isozymes than BNF-B.

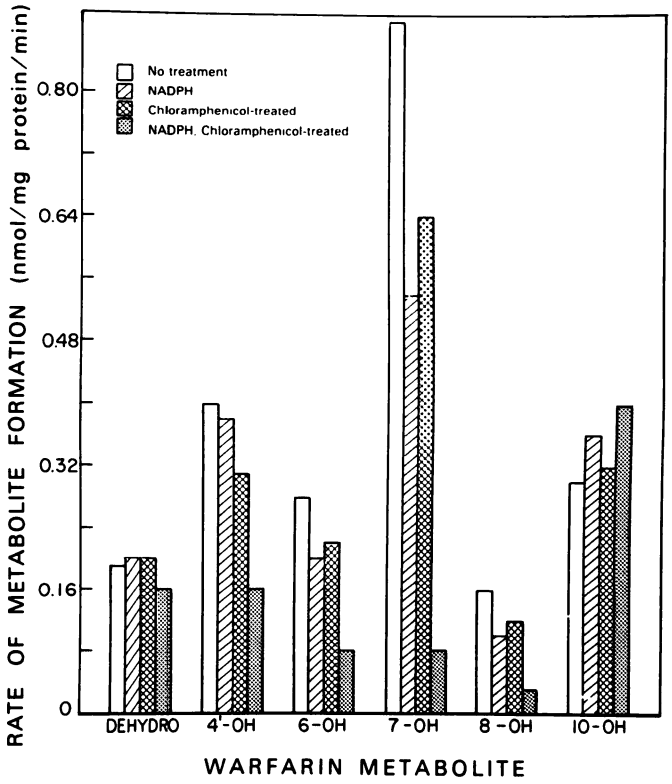

FIG. 5. *The effect of chloramphenicol in vitro on the rates of metabolite formation from R-warfarin catalyzed by hepatic microsomes from phenobarbital-induced rats.*

Microsomes (1 mg of protein/ml) were incubated with and without chloramphenicol (50 μM) and with and without NADPH (2 mM) for 10 min at 37°. Microsomes were centrifuged at 110,000 × *g*, and the pellet was washed with Tris-HCl buffer, pH 7.4, and recentrifuged. The pellet was resuspended and assayed for warfarin hydroxylase activity. The bars represent the averages of duplicate experiments.

Effect of chloramphenicol on testosterone metabolism. As a complement to the assays of warfarin metabolism, the effects of chloramphenicol administration to uninduced rats on testosterone metabolism by subsequently isolated liver microsomes were examined. In uninduced rats, both testosterone 2α and 16α-hydroxylation have been attributed to a cytochrome P-450 isozyme identical to UT-A (27), whereas testosterone 7α-hydroxylation (27, 28) is catalyzed by an isozyme corresponding to UT-F. Thus, the use of testosterone allowed us to confirm our results regarding the inhibition of UT-A and to examine the possible inhibition of UT-F, which forms no specific warfarin metabolites (23). As seen in Table 1, 300 mg/kg chloramphenicol caused approximately 50% inhibition of UT-A, in good agreement with the inhibition of warfarin metabolism, but caused no inhibition of UT-F.

Effect of chloramphenicol on isosafrole-metabolite complex formation. Studies by Guengerich *et al.* (23) and Thomas *et al.* (29) have shown that BNF induces not only isozyme BNF-B (P-450$_c$) but also isozyme ISF-G (P-450$_d$). Both of these isozymes are approximately equally efficient in converting isosafrole to a metabolite which forms a complex with the enzyme, characterized by a peak at 455 nm (21). Since the ratio of BNF-B to ISF-G in BNF-induced microsomes is approximately 3:1 (23, 29), one would expect the relative contributions of these two isozymes to product complex formation by BNF microsomes also to be approximately 3:1. As a preliminary means of assessing inhibition by chloramphenicol of ISF-G and to confirm the results regarding the lack of inhibition of BNF-B obtained with the warfarin system, we examined the effect of chloramphenicol administered *in vivo* on product complex formation from isosafrole by microsomes from BNF-induced rats. (Uninduced microsomes produced only 3% as much product complex as BNF-induced, in accordance with the low concentrations of BNF-B and ISF-G in control microsomes.) Product complex formation measured as $\Delta A_{455-480\ nm}$/20 min/nmol of cytochrome P-450 at 25° was 0.032 ± 0.002 for the microsomes from the BNF-treated rats and 0.031 ± 0.003 for the BNF- and chloramphenicol-treated rats. Although these results do not rigorously preclude some inhibition by chloramphenicol of isozyme ISF-G, they do suggest that neither BNF-induced isozyme is inhibited to any major extent by chloramphenicol.

Effect of chloramphenicol on lauric acid metabolism in clofibrate-treated rats. Recently it has been reported that the antilipidemic agent clofibrate induces a cytochrome P-450 isozyme which is highly efficient in the metabolism of lauric acid and which does not appear to correspond to any of the isozymes investigated so far in this study (30). The purified enzyme produces 12-hydroxy- and 11-hydroxylauric acid in a ratio of approximately 5:1, as do intact liver microsomes from clofibrate-treated rats (22). Antibody inhibition studies have confirmed a major role for this enzyme in metabolizing lauric acid in clofibrate-induced microsomes (31). In order to assess the possible inhibition of this isozyme by chloramphenicol, we administered the compound to clofibrate-treated rats and examined the total production of hydroxylated metabolites of lauric acid. As seen in Table 2, the clofibrate treatment gave an approximately 7-fold induction of lauric acid metabolism, whereas no significant inhibition by chloramphenicol was observed. In support of this latter finding, microsomes from clofibrate-induced rats preincubated with chloramphenicol essentially as described for the PB-induced microsomes in Fig. 5, exhibited identical activity to microsomes preincubated in the absence of NADPH and of chloramphenicol (9.0 nmol of lauric acid metabolized/min/mg of protein).

Effect of chloramphenicol on cytochrome P-450 content. We have previously reported that chloramphenicol administered to PB-induced rats has no effect on the level of hepatic cytochrome P-450 measured as the reduced carbon monoxide complex (5, 6). In the present study there were no significant effects of chloramphenicol on P-450 levels with microsomes from PB-, BNF-, and clofibrate-induced rats, whereas in control and PCN-induced animals, significant decreases were observed (Table 3). The latter finding was puzzling in view of the lack of inhibition of the major known PCN-induced isozyme, PB/PCN-E. In order to determine whether PCN might be inducing some other isozyme susceptible to inhibition by chloramphenicol, we examined the effect

TABLE 1

Effect of chloramphenicol in vivo on testosterone metabolism by liver microsomes from uninduced male rats

Adult male Sprague-Dawley rats were fasted overnight and then injected i.p. with 100 or 300 mg/kg chloramphenicol in 0.5 ml of propylene glycol or with vehicle only. One hour later, the animals were killed and individual liver microsomes were prepared. Assays of testosterone metabolism were performed as described previously (20). Results represent the mean ± SD of assays of 3–4 microsomal samples/treatment group.

Treatment	Steroid metabolite				
	2α-OH	6β-OH	7α-OH	16α-OH	Androstenedione
	nmol/min/mg protein				
Control	2.05 ± 0.30	1.39 ± 0.34	0.22 ± 0.04	2.38 ± 0.35	1.16 ± 0.28
100 mg/kg	1.72 ± 0.34	1.18 ± 0.12	0.19 ± 0.02	2.02 ± 0.35	0.97 ± 0.31
300 mg/kg	0.96 ± 0.50[a]	0.89 ± 0.56	0.26 ± 0.06	1.27 ± 0.42[a]	0.67 ± 0.22[b]

[a] $p < 0.01$ compared to the control group.
[b] $p < 0.05$ compared to the control group.

TABLE 2

Effect of chloramphenicol in vivo on lauric acid metabolism by liver microsomes from clofibrate-treated rats

Twelve adult male Sprague-Dawley rats were divided into three groups of four. Two of the groups were pretreated with clofibrate for 3 days as described under Experimental Procedures. The control group received vehicle only. Twenty-four hours after the last pretreatment, the animals in one of the clofibrate-treated groups were injected i.p. with 300 mg/kg chloramphenicol, whereas the animals in the remaining two groups received vehicle only. After 1 hr, the animals were killed and individual liver microsomes were prepared. Assays of lauric acid metabolism were performed as described by Orton and Parker (22). Results represent the mean ± SD of duplicate assays on four samples/group.

Clofibrate	Chloramphenicol	Lauric acid metabolized
		nmol/min/mg protein
−	−	0.9 ± 0.3
+	−	6.8 ± 1.2[a]
+	+	5.1 ± 2.2

[a] $p < 0.001$ compared to the minus clofibrate group.

TABLE 3

Effect of chloramphenicol in vivo on cytochrome P-450 contents in liver microsomes from uninduced and induced rats

Adult male Sprague-Dawley rats in groups of four were treated with inducers and/or with 300 mg/kg chloramphenicol as described under Experimental Procedures. The experimental design was essentially as described in the legend to Table 2, except in Experiment 1, where four rather than three groups of animals were used. Results represent the mean ± SD of duplicate determinations on four samples/group. All the inducers caused a significant increase in cytochrome P-450 content compared to the uninduced animals ($p < 0.05$).

Inducer	Chloramphenicol	Cytochrome P-450
		nmol/mg protein
Experiment 1		
−	−	0.85 ± 0.05
−	+	0.60 ± 0.04[a]
PB	−	2.03 ± 0.36
PB	+	1.80 ± 0.07
Experiment 2		
−	−	1.02 ± 0.11
PCN	−	1.50 ± 0.36
PCN	+	0.78 ± 0.05[b]
Experiment 3		
−	−	0.88 ± 0.06
BNF	−	1.38 ± 0.21
BNF	+	1.30 ± 0.24
Experiment 4		
−	−	0.63 ± 0.10
Clofibrate	−	1.01 ± 0.23
Clofibrate	+	0.83 ± 0.15

[a] $p < 0.001$ compared to the minus chloramphenicol group.
[b] $p < 0.01$ compared to the plus PCN, minus chloramphenicol group.

of *in vivo* administered PCN on the covalent binding of chloramphenicol to microsomes *in vitro*. Such covalent binding is thought to be the cause of the inactivation of isozyme PB-B by chloramphenicol. Indeed, we found a 3-fold increase in covalent binding to microsomal protein following induction with PCN.[3] In contrast, BNF caused a 50% decrease in covalent binding, which is consistent with the lack of inhibition of BNF-B and ISF-G and with the repression by BNF of UT-A (23, 27). Incubation of PCN microsomes with 50 μM chloramphenicol for 30 min in the presence of NADPH was found to cause a 25% decrease in cytochrome P-450 content compared to controls lacking the NADPH or the chloramphenicol, thus confirming the *in vivo* results.

DISCUSSION

Although the concept of overlapping substrate selectivities of microsomal cytochromes P-450 is well ingrained, in recent years it has become evident that potential specificity exists toward certain substrates, especially when the formation of particular metabolites rather than total metabolism is monitored. In the present investigation we have utilized the regio- and stereoselective metabolism of certain substrates as well as the overall metabolism of others as probes of the inhibition of specific cytochrome P-450 isozymes in liver microsomes from rats treated *in vivo* with the suicide substrate chloramphenicol. Using *S*- and *R*-warfarin as substrates, we were able to monitor the activities of five different cytochrome P-450 isozymes from rat liver, and we obtained clear-cut evidence for inhibition by chloramphenicol of three of them (UT-A, PB-B, and PB-C) and for lack of inhibition of PB/PCN-E and BNF-B. Of the three inhibited isozymes, PB-C was affected to the great-

[3] PCN microsomes incubated with 50 μM [^{14}C]chloramphenicol formed 0.52 ± 0.03 nmol of covalently bound metabolites/30 min/mg of protein compared to a value of 0.16 ± 0.03 for the corresponding controls.

est extent by chloramphenicol both after *in vivo* administration of the compound and after preincubation of PB microsomes *in vitro*. The lack of inhibition of PB/PCN-E was also confirmed by the *in vitro* incubations.

As a complement to the studies of warfarin metabolism we also examined the metabolism of testosterone, isosafrole, and lauric acid using microsomes from uninduced, BNF-induced, and clofibrate-induced rats, respectively. The testosterone assays confirmed the inhibition of UT-A and demonstrated a lack of inhibition of UT-F, a testosterone 7α-hydroxylase. The isosafrole assays confirmed the lack of inhibition of BNF-B and indicated no appreciable inhibition of ISF-G. Finally, the assays of lauric acid metabolism indicated no inhibition of the major clofibrate-induced isozyme of rat liver cytochrome P-450. Since isozyme PB-D, a close homolog of PB-B, could not be monitored by any of the above methods, studies were performed using two preparations of the protein which we had previously purified from rats treated *in vivo* with the same dose of chloramphenicol used in the present study. These experiments indicated a minor (15%) inhibition of PB-D compared to 55% inhibition of PB-B.[4]

Although the above results indicate that chloramphenicol inhibits four of the nine rat liver cytochrome P-450 isozymes monitored in this study, this is not meant to imply that we have examined all possible isozymes nor that we have identified all those isozymes inhibited by chloramphenicol. For example, the P-450 isozyme responsible for the formation of dehydrowarfarin is unknown, and we have obtained definite inhibition of the formation of this metabolite from both *R*- and *S*-warfarin after administration of chloramphenicol to untreated and to PB- and PCN-treated rats. Interestingly, antibodies to PB/PCN-E inhibit both the formation of dehydro- and 10-hydroxywarfarin from *R*-warfarin (25) in microsomal preparations, whereas chloramphenicol inhibits only dehydrowarfarin formation. These results suggest that dehydrowarfarin and 10-hydroxywarfarin formation are catalyzed by two different enzymes. In fact, very recently it was reported that antibodies to PB/PCN-E recognize two proteins in PB rat liver microsomes, only one of which corresponds to PB/PCN-E (32). Whether the second protein is also induced by PCN is unknown at this time. However, such a finding might account for the fact that we observed a significant decrease in cytochrome P-450 content in PCN microsomes from chloramphenicol-treated rats, despite the lack of inhibition of the only known major isozyme induced by PCN.

Our primary goal was to assess the isozyme selectivity of chloramphenicol as an inhibitor of rat liver cytochromes P-450, but the possible clinical and physiological implications of the inhibition of warfarin and testosterone metabolism have not escaped our attention. Although we have expressed our results per milligram of microsomal protein, in none of the cases where inhibition of cytochromes P-450 by chloramphenicol was observed was there any compensatory increase in liver weight or in the amount of total microsomal protein (data not shown). Therefore, the decreased activities we have observed in microsomes should be indicative of a decreased intrinsic capacity of the whole liver to metabolize warfarin and testosterone. In fact, very recently Yacobi *et al.* found that chloramphenicol appreciably increases the half-life of both *R*- and *S*-warfarin *in vivo*, and they suggested that this might be due to inhibition of warfarin metabolism (33). Our present results provide firm experimental support for the above suggestion.

In summary, we have documented that chloramphenicol is a partially but not completely specific inhibitor of rat liver cytochromes P-450. Although the experimental approach utilized was straightforward, the present study represents to our knowledge the first systematic study of the isozyme selectivity of a suicide substrate of cytochromes P-450 *in vivo*. Our present research is directed toward enhancing the selectivity by synthesizing structural analogs of chloramphenicol.

[4] With PB-B we have found an excellent correlation between a loss of monooxygenase activity and an inability of the cytochrome to undergo reduction by NADPH-cytochrome P-450 reductase under both aerobic and anaerobic conditions. In agreement with our previous results with isozyme PB-B, control preparations of PB-D were found to be 90% reducible by NADPH-cytochrome P-450 reductase under aerobic conditions, whereas only 75% of the PB-D and only 40% of the PB-B from chloramphenicol-treated rats was reducible under the same conditions.

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