Biochemical Pharmacology, Vol. 40, No. 4, pp. 751–758, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

DRUG METABOLIZING CAPACITY *IN VITRO* AND *IN VIVO*—II

CORRELATIONS BETWEEN HEPATIC MICROSOMAL MONOOXYGENASE MARKERS IN PHENOBARBITAL-INDUCED RATS

DIANE E. MATTHEW* and J. BRIAN HOUSTON†
Department of Pharmacy, University of Manchester, Manchester M13 9PL, U.K.

(*Received* 10 *January* 1990; *accepted* 22 *March* 1990)

Abstract—Pretreatment with various doses of phenobarbital (PB) has been used to create a pool of rats with a wide range of hepatic microsomal monooxygenase activity to systematically examine relationships between and within *in vivo* and *in vitro* markers. The *in vivo* clearance of tolbutamide (TOL), theophylline (TH), antipyrine (AP) and its metabolites were determined in the same rats used for hepatic microsome preparation and assessment of P450 content and activities (via 7-ethoxycoumarin *O*-deethylase (ECOD), 7 ethoxyresorufin *O*-deethylase, 7-methoxycoumarin *O*-demethylase (MCOD) and aldrin expoxidase determinations). A graded dose–response relationship was found between PB treatment and most but not all parameters. The need for careful selection of *in vivo* and as well as *in vitro* markers is apparent from these studies. The most responsive parameters—TOL and AP clearances, MCOD and ECOD activities—were also those producing the strongest *in vivo–in vitro* correlations. Despite the diffuse nature of the PB induced response in P450 complement, good predictive relationships were apparent between ECOD and TOL clearance ($r^2 = 0.88$).

The hepatic microsomal monooxygenase system (cytochromes P450) is known to be influenced by numerous factors and considerable differences in activity have been documented under a variety of experimental conditions. The use of probe or marker substrates in both *in vitro* and *in vivo* investigations to quantify these differences in cytochromes P450 activity is routine yet little information is available on the relationships between *in vitro* and *in vivo* markers [1].

Early attempts to relate *in vitro* and *in vivo* cytochromes P450 activities centered on the use of biopsy liver samples from human subjects whose *in vivo* oxidative metabolizing capacity had been assessed using antipyrine (AP‡) [2–6]. In hindsight the inability of these investigators to establish useful correlates between *in vitro* and *in vivo* markers is not surprising. Many of the subjects used were suspected of liver disease (hence biopsy material was available) and the choice of markers was less than optimal given the current information on the degree of multiplicity of cytochrome P450. Analogous investigations in animals [7–9] also provided poor correlations due to the relatively narrow range of activities observed in these studies. In a previous investigation [1] we carried out a systematic examination of the relationships between a number of *in vitro* and *in vivo* markers of hepatic microsomal monooxygenase activity using a pool of rats with a wide range of cytochrome P450 activities due to prior exposure to various doses of BNF. The inducing properties of BNF are well known and isozyme specific, producing approximately a 70-fold increase in P450I [10]. As all other extrinsic factors were maintained constant differences between animals were largely a reflection of the different level of P450I resulting from a particular degree of induction. Excellent correlations were observed between TH clearance and ECOD and EROD activities. The latter microsomal probes were also predictive of AP disposition particularly when individual metabolite formation clearances were delineated; CL(4H) was better correlated with microsomal activity than CL(AP) or CL(N) while CL(3H) demonstrated no statistical relationship. This distinctive behaviour for the three AP metabolites are in accord with previous investigations in rat [11] and man [12].

The present investigations extend the previous studies on *in vitro* and *in vivo* markers using another classic inducer of hepatic microsomal monooxygenases PB. The major effect of PB induction is on P450IIB (a 40-fold increase being observed [13]) with lesser effects in members of the P450IIC subfamily [14]. Two studies were carried out with PB induced rats which differed in the *in vivo* probes examined. The first study used AP, its metabolites and TH, the same combination employed in the earlier BNF study. The second study used TOL

* Recipient of financial support from the Science and Engineering Research Council.

† To whom correspondence should be addressed.

‡ Abbreviations: ECOD, 7-ethoxycoumarin *O*-deethylase; EROD, 7-ethoxyresorufin *O*-deethylase; MCOD, 7-methoxycoumarin *O*-demethylase; ALE, aldrin epoxidase; 4H, 4-hydroxyantipyrine; N, norantipyrine; 3H, 3-hydroxymethylantipyrine; AP, antipyrine; TH, theophylline; TOL, tolbutamide; CL, clearance, *V*, volume of distribution; SRW, standard rat weight; PB, phenobarbital; BNF, β-naphthoflavone. The classifications of cytochromes P450 proposed by Nebert *et al.* (DNA **6**: 1–11, 1987) is used.

instead of TH to assess the suitability of the former probe and also to increase the sample size for the other probes. In both studies a range of P450 activities was achieved by varying the PB pretreatment dose and the microsomes used for the *in vitro* studies were obtained from the same rats who received the *in vivo* probes.

MATERIALS AND METHODS

Preliminary studies. Male Sprague–Dawley rats (N = 17, 230–280 g) were fitted with indwelling cannulae in the right carotid artery and jugular vein [15]. The day after surgery these rats were administered a bolus dose, via the jugular vein, of AP (50 mg/kg) and/or TOL (10 mg/kg). Blood samples were collected from the carotid artery (N = 7–9, 250 μL) over 6 hr and plasma assayed for drug as described. In addition four rats were pretreated with BNF (100 mg/kg) in corn oil for 3 days, cannulated on the day of the last dose and administered TOL (10 mg/kg) on the fourth day.

In vivo *studies.* Male Sprague–Dawley rats (N = 32, 230–280 g) were administered various daily i.p. doses of PB (0–100 mg/kg) in normal saline (2 mL/kg) for 3 consecutive days. Seven doses of PB (2.5, 5, 7.5, 10, 15, 20 and 80 mg/kg) were administered to groups of three to four animals and six rats received vehicle only. Rats were cannulated in the right carotid artery and jugular vein [15] under ether anaesthesia on the day of the third dose. On the fourth day they were placed in an approved restraining apparatus [16] allowing free movement and access to food and water while enabling facile sampling of blood from the carotid artery. A solution of [*N-methyl*-^{14}C]AP (50 mg/kg, 1 μCi/kg) and either TH (6.5 mg/kg) in normal saline (Group 1, N = 16) or TOL (10 mg/kg) in polyethylene glycol:polypropylene glycol, 9:1 (Group 2, N = 16) was administered as a bolus over 1 min, via the jugular vein.

Blood samples (N = 7–9, 250 μL) were collected over 3 hr (Group 1) or 6 hr (Group 2) and urine over 4 hr (Group 1 only). Following completion of sampling, the rats were immediately killed by cervical dislocation, their bladders drained and livers removed. Blood samples were assayed for AP [17], TH [18] or TOL [19] and urine samples for AP and its metabolites [20].

Clearance, volume of distribution and half-lives were calculated as previously described [1] and expressed per SRW of 250 g.

In vitro *studies.* Hepatic microsomes were prepared by standard differential centrifugation using a sucrose (0.25 M), Tris–HCl buffer (10 mM, pH 7.4). The final pellet from the 100,000 *g* centrifugations was resuspended in Tris–HCl buffer and stored at −70°.

The following microsomal parameters were determined using previously described methods [1]: total protein, cytochrome P450 content and ECOD, EROD, ALE and MCOD activities. It is assumed that if any residual inducer is present in the microsomes then its concentration will be in proportion to the dose administered and therefore contribute a constant percentage error.

Chemicals. PB sodium and TOL were obtained from BDH (Poole, U.K.) and Hoechst (Frankfurt, F.R.G.), respectively. Sources of all other chemicals were as previously described [1].

RESULTS AND DISCUSSION

Preliminary studies

Co-administration of *in vivo* probes is an integral part of the present experimental design. Previous investigations [1, 18] have established no pharmacokinetic interaction between AP and TH at doses of 50 mg/kg and 6.5 mg/kg, respectively. Table 1 summarizes the investigation into the feasibility of co-administration of AP with TOL. The clearance, volume of distribution and half-life of both compounds were not statistically different (by *t*-test) when administered alone or in combination. In addition Table 1 demonstrates that TOL kinetics are not influenced by BNF induction in contrast to the substantial increase in clearance previously reported following PB induction [21]. This selectivity shown by TOL contrasts this *in vivo* hepatic microsomal monooxygenase marker from AP [22, 23] and TH [24, 25].

Table 1. Pharmacokinetic parameters describing the disposition of antipyrine and tolbutamide following separate and co-administration in control and BNF induced rats

Treatment	Clearance (mL/min/SRW)	Volume of distribution (L/SRW)	Half-life (min)
Antipyrine 50 mg/kg (N = 6)	1.62 (32)	0.23 (0.02)	102.5 (29.9)
Antipyrine 50 mg/kg + Tolbutamide 10 mg/kg (N = 5)	1.48 (21)	0.23 (0.02)	109.7 (13.6)
	0.09 (0.02)	0.035 (0.002)	292.7 (64.9)
Tolbutamide 10 mg/kg (N = 6)	0.10 (0.02)	0.040 (0.008)	278.6 (46.1)
Tolbutamide* 10 mg/kg (N = 4)	0.12 (0.02)	0.052 (0.005)	292.8 (31.0)

Values are means with SD in parentheses.
* Rats induced with BNF.

Table 2. Effect of PB induction on various *in vivo* and *in vitro* markers of hepatic microsomal monooxygenase activity

Parameter	Control value	Induction with PB 20 mg/kg	Fold increase over entire PB dose range	N
CL(TH)*	0.83 (0.06)	0.95 (0.38)	1.2	15
V(TH)†	0.21 (0.02)	0.22 (0.05)	—	15
$T_{1/2}$(TH)‡	173.3 (3.3)	201.7 (138.3)	0.9	15
CL(AP)*	2.07 (0.21)	3.49 (1.29)	1.9	31
V(AP)†	0.24 (0.02)	0.25 (0.04)	—	31
$T_{1/2}$(AP)‡	80.3 (8.9)	54.9 (22.2)	0.5	31
CL(TOL)*	0.13 (0.03)	0.23 (0.03)	2.5	16
V(TOL)†	0.034 (0.004)	0.036 (0.002)	—	16
$T_{1/2}$(TOL)‡	180.8 (19.0)	112.0 (15.5)	0.4	16
CL(4H)*	0.23 (0.06)	0.39 (0.16)	1.8	15
CL(N)*	0.17 (0.06)	0.28 (0.17)	1.3	15
CL(3H)*	0.22 (0.05)	0.32 (0.18)	1.1	15
ECOD§	0.356 (0.089)	0.887 (0.130)	3.8	31
EROD§	0.042 (0.013)	0.067 (0.034)	1.5	15
P450‖	0.445 (0.117)	0.747 (0.130)	1.9	31
MCOD§	0.218 (0.072)	0.522 (0.084)	3.9	31
ALE§	1.781 (0.321)	2.516 (0.439)	1.6	31

Values are means with SD in parentheses.
* mL/min/SRW.
† L/SRW.
‡ min.
§ nmol/min/mg protein.
‖ nmol/mg protein.

Effect of PB administration

By administering various doses of PB (2.5–80 mg/kg) a wide range of hepatic microsomal monooxygenase activity was achieved. There was a hyperbolic relationsip between all clearance values, and most microsomal parameters, and PB dose. Maximal changes and those observed at 20 mg/kg PB (approximately half maximal) are shown in Table 2.

AP, TH and TOL plasma concentration–time profiles declined in a monoexponential fashion in all experiments. Neither compound showed an alteration in volume of distribution following pretreatment with PB, thus the changes in clearance resulting from induction were accompanied by a corresponding decrease in plasma half-life. The individual AP metabolite formation clearances showed similar trends to AP total clearance following PB induction. This behaviour is in accord with the previously documented lack of specificity of the three AP metabolic pathways towards PB induction [22, 26] although CL(4H) consistently demonstrated larger increases than CL(N) and CL(3H). The rank order of sensitivity of the *in vivo* markers to PB was tolbutamide > antipyrine > theophylline.

Four microsomal parameters (ECOD, MCOD, ALE and cytochrome P450) showed a graded PB dose–response relationship as has been reported previously for the N-demethylation of ethylmorphine [27] and aminopyrine [28] and P450 content [29]. There was no clear dose–response relationship evident for EROD. The largest changes noted in the present study were the four-fold increase in MCOD and ECOD. In general, the degree of change in these parameters was markedly less, and the variability between animals receiving similar PB doses was considerably greater, than was reported in the earlier BNF induction study using an identical experimental protocol [1]. However, the PB induction profile is known to be more diffuse than that resulting from other classic inducers like BNF [30]. Although P450IIB is induced by PB over 40-fold [13], several other subfamilies are also affected including members of P450IIC [14]. Furthermore, a recent study by Pei *et al.* [31] was unable to demonstrate a relationship between steady-state PB plasma concentration (achieved via intravenous infusion) and the degree of induction manifest in antipyrine clearance.

Relationships between and within in vitro *and* in vivo *markers*

Tables 3 and 4 summarize the relationships between the markers of hepatic microsomal monooxygenase activity in the two groups of PB induced rats. Although many of the correlation coefficients listed are statistically significant ($P < 0.001$, $r > 0.704$, $N = 15$), relatively few are of predictive value in Study 1 (Table 3) whereas Study 2 (Table 4) was more successful. It is the square of the correlation coefficient which is a measure of explained variance in y by x and hence provides assessment of the predictive value of x (e.g. microsomal data) for y (e.g. *in vivo* clearance).

In vivo *markers*

Strong correlations were observed between the clearances for the two *in vivo* marker drugs used in each study, and Fig. 1 depicts these relationships for AP–TOL ($r = 0.891$) and AP–TH ($r = 0.846$). The use of individual metabolite formation clearance did

Table 3. Relationship between and within *in vivo* and *in vitro* markers of hepatic microsomal monooxygenase activity in control and PB treated rats from Study 1

CL(TH)	CL(4H)	CL(N)	CL(3H)	EROD	ALE	P450	MCOD	ECOD	
0.846	0.873	0.816	0.691	0.399	0.759	0.806	0.765	0.782	CL(AP)
	0.812	0.623	0.617	0.407	0.635	0.715	0.653	0.639	CL(TH)
		0.853	0.831	0.533	0.582	0.807	0.741	0.732	CL(4H)
			0.949	0.298	0.398	0.533	0.465	0.445	CL(N)
				0.281	0.237	0.430	0.331	0.314	CL(3H)
					0.301	0.650	0.478	0.571	EROD
						0.722	0.722	0.827	ALE
							0.862	0.903	P450
								0.964	MCOD

Correlation coefficients are shown. Critical value for statistical significance ($P < 0.001$, $N = 15$) is 0.704.

Table 4. Relationship between and within *in vivo* and *in vitro* markers of hepatic microsomal monooxygenase activity in control and PB treated rats from Study 2

CL(AP)	ALE	P450	MCOD	ECOD	
0.891	0.671	0.839	0.894	0.939	CL(TOL)
	0.719	0.720	0.845	0.858	CL(AP)
		0.564	0.720	0.765	ALE
			0.847	0.887	P450
				0.952	MCOD

Correlation coefficients are shown. Critical value for statistical significance ($P < 0.001$, $N = 16$) is 0.725.

not improve the strength of the correlation between AP and TH. Of the three formation clearances determined for AP, CL(4H) correlated better with TH as has been previously reported in BNF induced rats [1]. The high degree of covariance between the three AP metabolite formation clearances ($r = 0.831$–0.949) is in accord with the lack of specificity of PB induction discussed above. Due to the limited additional information obtained from the AP formation clearances in the first PB induction study, no analysis of AP metabolites was carried out in the second study.

In vitro *markers*

Both MCOD and ECOD activities were very sensitive to induction by PB, therefore it was not surprising that these parameters correlated highly with each other ($r = 0.96$). The correlations between P450 content and both MCOD ($r = 0.86$) and ECOD activities ($r = 0.90$) were also high. The insensitivity of EROD activity to PB induction is well known [30] and in the present investigations correlations

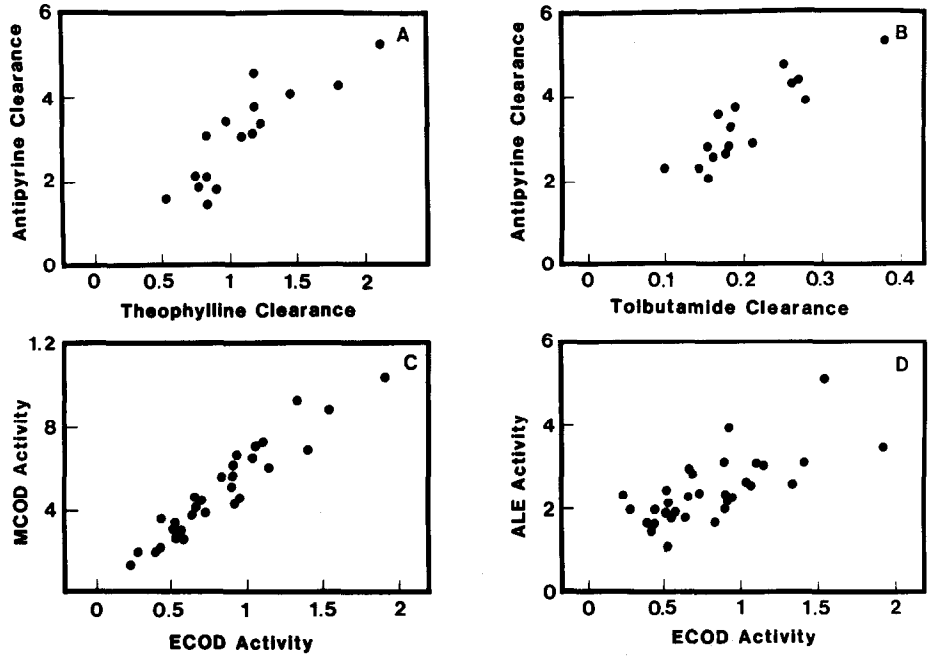

Fig. 1. Relationships between various indices of hepatic microsomal monooxygenase activity. (A) CL(AP) and CL(TH) from Study 1; (B) CL(AP) and CL(TOL) from Study 2; (C) MCOD and ECOD activities from Studies 1 and 2; (D) ALE and ECOD activities from Studies 1 and 2.

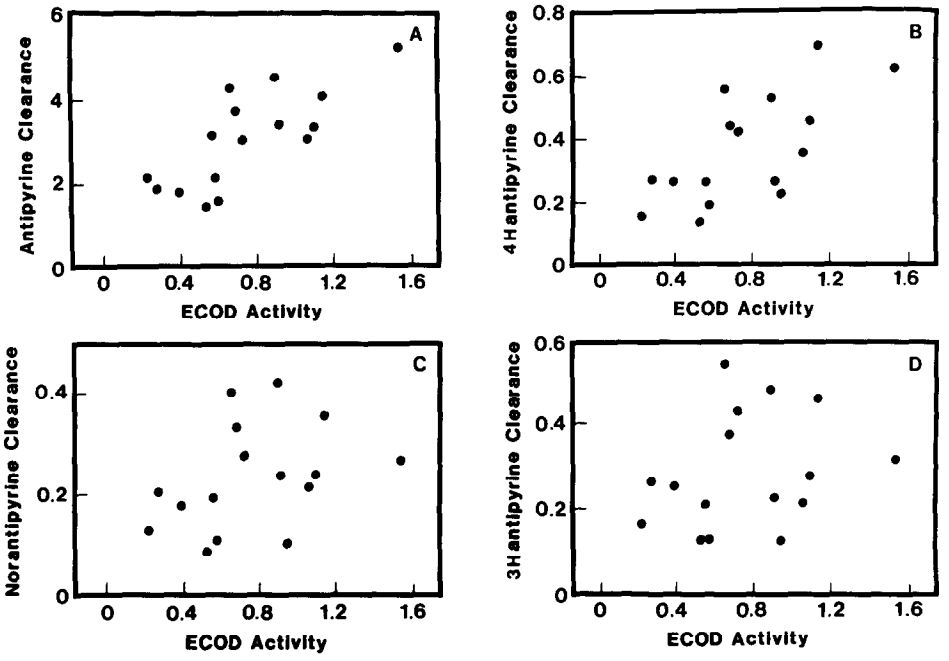

Fig. 2. Relationship between AP and its individual metabolite formation CLs and ECOD activity. (A) CL(AP) and ECOD activity; (B) CL(4H) and ECOD activity; (C) CL(N) and ECOD activity; (D) CL(3H) and ECOD activity. All data from Study 1.

between EROD and the other *in vitro* parameters proved to be poor. The correlations observed with ALE activity were also too low to be of a predictive value. Similar trends were observed for the *in vitro* data generated in both studies. Figure 1 depicts the correlations between ECOD and MCOD activities and ECOD and ALE activities, using the data from both studies (N = 31).

In vitro–in vivo *markers*

Consideration of the data in Tables 3 and 4 shows that AP clearance may be predicted from either ECOD, MCOD, ALE activities or P450 content with varying degrees of success (r^2 ranging from 0.52 to 0.74). The resolution of AP clearance into individual metabolite formation clearances did not improve the correlation coefficients and hence the prediction of these clearance parameters from microsomal data (see Fig. 2) were either comparable to AP clearance (in the case of 4H) or worse (in the case of 3H and N).

Previous investigtions [32, 33] have shown good correlations between cytochrome P450 content and AP clearance in PB induced rats. The present studies confirm and extend these observations to a wider range of *in vitro* and *in vivo* markers. As would be anticipated markers displaying narrower selectivity towards PB inducible members of the P450 family provide better predictions of *in vivo* oxidative capacity (see Fig. 3). Both MCOD and ECOD are more sensitive markers of PB induction than total cytochrome P450, however only in Study 2 (Fig. 4) did these activities produce better correlations with *in vivo* probes. MCOD would appear to be a relatively specific marker of PB inducible P450 activity [34] whereas ECOD activity has been demonstrated for a wide range of P450s including the polycyclic aromatic hydrocarbon inducible forms [30]. ALE was less useful in the present studies and this substrate may be a better marker of P450III activity rather than P450IIB [35].

The relationships between TH clearance and microsomal parameters were in general poor; only P450 content correlated to any moderately useful degree ($r > 0.7$). In contrast TOL clearance correlated substantially better than AP clearance with ECOD and MCOD activities and P450 content (see Tables 3 and 4). The former two enzyme activities provided useful predictions of TOL clearance (r^2 = 0.88 and 0.80, respectively). TOL is metabolized via a single oxidative pathway [36] and it is proposed that a single form of P450 mediates this reaction [37]. The identity of this P450 has still to be elucidated but it would appear to be distinct from P450I (the lack of effect of BNF pretreatment reported herewith would support this conclusion) and P450IID [37]. Initial evidence supporting the role of P450IIC9 [38] based on inhibitory experiments has not been substantiated [37–39].

Conclusions

In the previous communication in this series [1] it was demonstrated that under the well-controlled conditions which may be imposed in animal studies, excellent *in vitro–in vivo* relationships may be established. BNF was used as an inducer to broaden the range of hepatic microsomal monooxygenase activities for experimental purposes. In the present study an identical approach was adopted using PB induction to produce a range of activities associated with a different cytochrome P450 complement.

The second study using PB induced rats reported

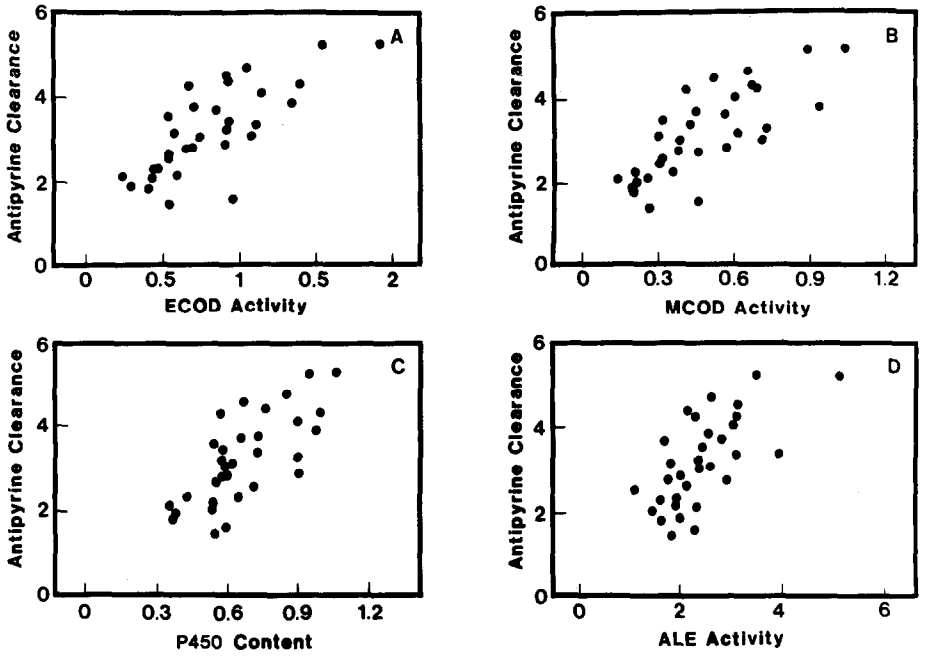

Fig. 3. Relationship between CL(AP) and four markers of *in vitro* activity. (A) CL(AP) and ECOD activity; (B) CL(AP) and MCOD activity; (C) CL(AP) and P450 content; (D) CL(AP) and ALE activity. All data from Studies 1 and 2.

herewith employed TOL as an *in vivo* probe and resulted in strong correlations between *in vivo* clearance and certain microsomal parameters. The predictive nature of the ECOD–CL(TOL) relationship is comparable with the previous reported ECOD–CL(TH) relationships using BNF induced rats [1]. The utility of ECOD under conditions of both BNF and PB induction highlights the known overlapping specificity of this substrate. Hence the predictive value of ECOD is limited to that of an empirical index reflecting gross changes in P450 activity. However, it is of interest that correlations involving more

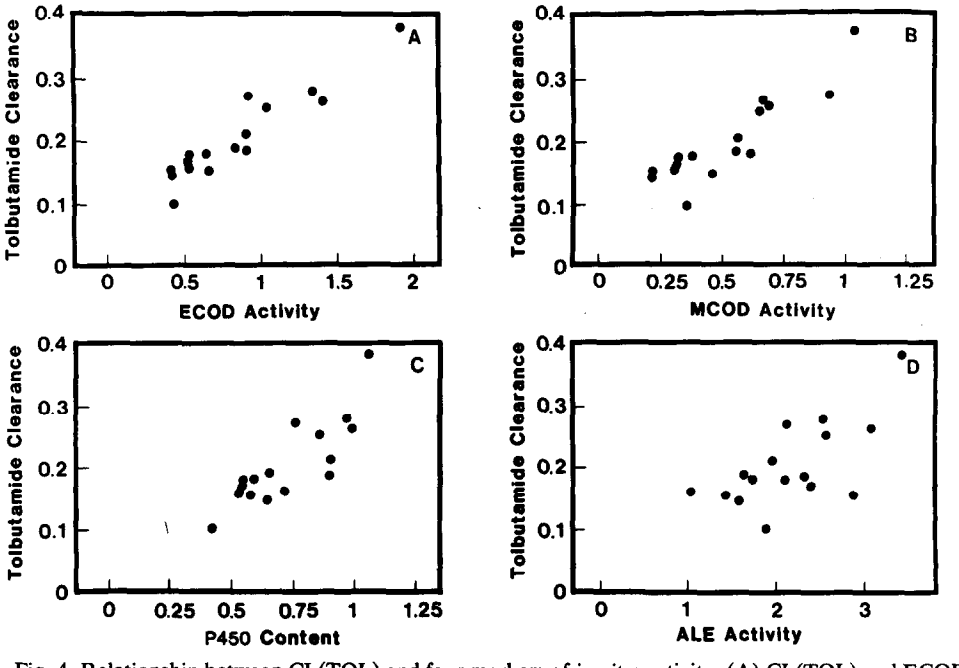

Fig. 4. Relationship between CL(TOL) and four markers of *in vitro* activity. (A) CL(TOL) and ECOD activity; (B) CL(TOL) and MCOD activity; (C) CL(TOL) and P450 content; (D) CL(TOL) and ALE activity. All data from Study 2.

specific substrates—MCOD–CL(TOL) and EROD–CL(TH) for PB and BNF studies, respectively—are only marginally weaker (2–4%) than the ECOD correlations discussed above.

Thus the present investigations extend and complement the previous communication [1]. When either PB or BNF are used to produce a range of P450 activities, strong and predictively useful correlations can be demonstrated between *in vitro* and *in vivo* markers of the hepatic microsomal monooxygenase system. It is also evident from these studies that a judicious choice of markers is required. When the history of exposure to P450 modifying agents is unknown, such as in human studies, this situation is obviously problematic. This is particularly so *in vivo* where there is no clear single recommendation of probe. The traditional choice of antipryine [11, 12] reflects most investigators' preference for a permissive probe with the sacrifice of any selectivity towards P450 isozymes.

REFERENCES

1. Matthew DE and Houston JB, Drug metabolizing capacity *in vitro* and *in vivo*—I. Correlations between hepatic microsomal monooxygenase markers in β-naphthoflavone-induced rats. *Biochem Pharmacol* **40**: 743–749, 1990.
2. Farrell GC, Cooksley WGE and Powell LW, Drug metabolism in liver disease: activity of hepatic microsomal metabolizing enzymes. *Clin Pharmacol Ther* **26**: 483–492, 1979.
3. Vuitton D, Miguet JP, Camelot G, Delafin C, Bechtel CJP, Gillet M and Carayon P, Relationship between metabolic clearance rate of antipyrine and hepatic microsomal drug oxidising enzyme activities in humans without liver disease. *Gastrenterology* **80**: 112–118, 1981.
4. Sotaniemi EA, Pelkonen RO, Ahokas JT, Pirttiaho HI and Alhqvist J, Relationship between *in vivo* and *in vitro* drug metabolism in man. *Eur J Drug Metab Pharmacokinet* **1**: 39–45, 1978.
5. Kalameghan R, Krishnaswary K, Krichnamorthy S and Bhargara RNK, Metabolism of drugs and carcinogens in man: antipyrine eliminators as an indicator. *Clin Pharmacol Ther* **25**: 25–73, 1979.
6. Sotaniemi EA, Pelkonen RO and Puukka M, Measurement of hepatic drug metabolizing enzyme activity in man. *Eur J Clin Pharmacol* **17**: 267–274, 1980.
7. Vesell ES, Lee CJ, Passananti GT and Shively CA, Relationship between plasma antipyrine half-lives and hepatic microsomal drug metabolism in dogs. *Pharmacology* **10**: 317–328, 1973.
8. Miller JL, Clark CR, Gee SJ and Kreiger RI, Antipyrine plasma half-life. *In vivo* indicator of oxidative metabolic capability in Rhesus monkey. *Pharmacology* **16**: 279–286, 1978.
9. Wedlund PJ, Nelson SD, Nickelson S and Levy RH, Linear relationshp between cytochrome P450 and carbamazepine clearance in Rhesus monkey. *Drug Metab Dispos* **10**: 480–485, 1982.
10. Thomas PE, Reik LM, Ryan DE and Levin W, Regulation of three forms of cytochrome P450 and epoxide hydrolase in rat liver microsomes. Effects of age, sex and induction. *J Biol Chem* **256**: 1044–1052, 1981.
11. Poulsen HE and Loft S, Antipyrine as a model drug to study hepatic drug-metabolizing capacity. *Hepatology* **6**: 374–382, 1988.
12. Breimer DD, Vermeulen NPE, Danhof M, Teunissen MWE, Joeres RP and van der Graaff M, Assessment and prediction of *in vivo* oxidative drug metabolising activity. In: *Pharmacokinetics. A Modern View* (Eds. Benet LZ, Levy G and Ferraiolo BL), pp. 191–216. Plenum Press, New York, 1984.
13. Phillips IR, Shephard EA, Bayney RM, Pike SF, Rabin BR, Heath R and Carter, N, Induction and repression of the major phenobarbital-induced cytochrome P450 measured by radioimmunoassay. *Biochem J* **212**: 55–64, 1983.
14. Wolf CR, Seilman S, Oesch F, Mayer RT and Burke MD, Multiple forms of cytochrome P450 related to forms induced marginally by phenobarbital. *Biochem J* **240**: 27–33, 1986.
15. Harms PG and Ojeda SR, A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J Appl Physiol* **36**: 391–392, 1974.
16. Toon S and Rowland M, A simple restraining device for chronic pharmacokinetic and metabolism studies in rats. *J Pharmacol Methods* **5**: 321–323, 1981.
17. Rhodes JC and Houston JB, Antipyrine metabolite kinetics: dose and time dependence studies in rats. *Biopharm Drug Dispos* **4**: 124–135, 1983.
18. Teunissen MWE, Brorens ION, De Langen HJ, Geerlings AM and Breimer DD, Correlation between *in vivo* antipyrine metabolite formation and theophylline metabolism in rats. *Pharmaceut Res* **3**: 156–161, 1986.
19. Nation RL, Peng GW and Chiou WL, Simple, rapid and micro high performance liquid chromatographic method for the simultaneous determination of tolbutamide and carboxytolbutamide in plasma. *J Chromatogr* **146**: 121–131, 1978.
20. Houston JB and Rhodes JC, Quantification of metabolites of aminopyrine and antipyrine in plasma and urine. In: *Drug Metabolite Isolation and Determination* (Ed. Reid E), pp. 207–214. Plenum Press, New York, 1983.
21. Back D, Sutcliffe F and Tjia JF, Tolbutamide as a model drug for the study of enzyme induction and enzyme inhibition in the rat. *Br J Pharmacol* **81**: 557–562, 1984.
22. Rhodes JC and Houston JB, Antipyrine metabolite kinetics in phenobarbital and β-naphthoflavone induced rats. *Drug Metab Dispos* **11**: 131–136, 1983.
23. Teunissen MWE, Joeres RP, Vermeulen NPE and Breimer DD, Influence of 9-hydroxyellipticine and 3-methylcholanthrene treatment on antipyrine metabolite formation in rats *in vivo*. *Xenobiotica* **13**: 223–231, 1983.
24. McManus ME, Miners JO, Gregor D, Stupans I and Birkett DJ, Theophylline metabolism by human, rabbit and rat liver microsomes and by purified forms of cytochrome P450. *J Pharm Pharmacol* **40**: 388–391, 1988.
25. Williams JF, Lowitt S and Szentivanyi A, Effects of phenobarbital and 3-methylcholanthrene on plasma half-life and urinary excretion profile on theophylline and its metabolites in rats. *Biochem Pharmacol* **28**: 2935–2940, 1979.
26. Teunissen MWE, van der Graaff M, Vermeulen NPE and Breimer DD, Influence of allylisopropylacetamide and phenobarbital treatment on *in vivo* antipyrine metabolite formation in rats. *Xenobiotica* **13**: 497–502, 1983.
27. Breckenridge A, Orme ML'E, Davies L, Thorgeirsson SS and Davies DS, Dose dependent enzyme induction. *Clin Pharmacol Ther* **14**: 514–520, 1973.
28. Poland A, Mak I, Grover E, Boatman RJ, Ebetino FH and Kende AS, 1,4-Bis[2-(3,5-dichloropyridyloxy)]-benzene, a potent phenobarbital-like inducer of microsomal monooxygenase activity. *Mol Pharmacol* **18**: 571–580, 1980.
29. Tavoloni N, Jones MJT and Berk PD, Dose-related effects of phenobarbital on hepatic microsomal enzymes. *Proc Soc Exp Biol Med* **174**: 20–27, 1983.

30. Burke MD and Wolf CR, Substrates and inhibitors as probes of individual forms of drug metabolising systems. In: *Drug Metabolism—from Molecules to Man* (Eds. Benford DJ, Bridges JW and Gibson GG, pp. 219–243. Taylor and Francis, London, 1987.
31. Pei Y-Y, Bialer M and Levy RH, Effects of phenobarbital steady state levels on antipyrine clearance and distribution in the rat. *Biopharm Drug Dispos* **7**: 11–19, 1986.
32. Chang S-L, Nelson SD and Levy RH, Correlation between antipyrine clearance and cytochrome P450 level after phenobarbital induction in rat. *Drug Metab Dispos* **12**: 139–141, 1984.
33. Rowse Brouwer KL, Kostenbauder PJ, McNamara PJ and Blouin RA, Phenobarbital in the genetically obese Zucker Rat—II. *In vivo* and *in vitro* assessments of microsomal enzyme induction. *J Pharmacol Exp Ther* **231**: 654–659, 1984.
34. Matsubara T, Yoshimura E, Iwata T, Tochino Y and Hachino Y, Biotransformation of coumarin derivatives—I. 7-Alkoxycoumarin *O*-dealkylase in liver microsomes. *Jap J Pharmacol* **32**: 9–21, 1982.
35. Newman SL and Guzelian PS, Identification of the cyanopregnenolone-inducible form of hepatic cytochrome P450 as a catalyst of aldrin epoxidation. *Biochem Pharmacol* **32**: 1529–1531, 1983.
36. Thomas RC and Ikeda GJ, The metabolic fate of tolbutamide in man and in the rat. *J Med Chem* **9**: 507–510, 1966.
37. Miners JO, Smith KJ, Robson RA, McManus ME, Veronese ME and Birkett D, Tolbutamide hydroxylation by human liver microsomes. Kinetic characterization and relationship to other cytochrome P450 dependent xenobiotic oxidations. *Biochem Pharmacol* **37**: 1137–1144, 1988.
38. Knodell RG, Hall SD, Wilkinson GR and Guengerich FP, Hepatic metabolism of tolbutamide. Characterization of the form of cytochrome P450 involved in methyl hydroxylation and relationship to *in vivo* disposition. *J Pharmacol Exp Ther* **241**: 1112–1119, 1987.
39. Purba HS, Back DJ and Orme ML'E, Tolbutamide 4-hydroxylase activity of human liver microsomes: effect of inhibitors. *Br J Clin Pharmacol* **24**: 230–234, 1987.