Biochemical Pharmacology, Vol. 36, No. 8, pp. 1321–1323, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

CORRELATION OF APPARENT INTRINSIC CLEARANCES OF SIMULTANEOUSLY ADMINISTERED *S* (+) AND d_3 *R* (−) HEXOBARBITAL IN THE RAT

M. VAN DER GRAAFF, N. P. E. VERMEULEN,* P. H. HOFMAN and D. D. BREIMER†

Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden, The Netherlands

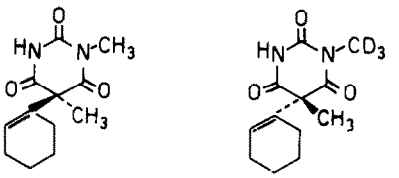
(*Received* 28 *August* 1986; *accepted* 27 *October* 1986)

Abstract—Pseudoracemic hexobarbital (HB), consisting of equal molar fractions of *S* (+) HB and deuterium-labeled *R* (−) HB, d_3 *R* (−) HB, was administered orally to rats in a dose of 50 mg/kg. Concentrations of both enantiomers in blood were measured by an enantioselective mass fragmentographic assay. Clearance data of *S* (+) HB and d_3 *R* (−) HB were correlated in untreated rats, and in rats pretreated with 3-methylcholanthrene (MC), carbon tetrachloride (CCl_4), and different doses of phenobarbital (PB). Although in the different groups some variation in the clearance ratio of *S* (+) HB over d_3 *R* (−) HB was found, the clearance of *S* (+) HB was generally up to a factor of five higher than the clearance of d_3 *R* (−) HB, except for the CCl_4-treated rats. From the present data it can be tentatively concluded that *S* (+) HB and *R* (−) HB are metabolized by similar (PB-inducible) cytochrome P-450s in control and PB- and MC-pretreated rats and that clearance data obtained with racemic HB following different pretreatments may be employed as a reflection of (PB-inducible) cytochrome P-450 activity.

Hexobarbital (HB) is oxidatively metabolized, and a well-known model substrate for the assessment of changes in the oxidative activity of hepatic cytochrome P-450 in animals and man [1, 2]. From a pharmacokinetic point of view, and as far as its employment as a model substrate is concerned, HB has mostly been treated as a single chemical entity and not as a racemic mixture of two enantiomers. Recently, however, it has been shown that *S* (+) HB has a sevenfold higher clearance than *R* (−) HB [3]. Although this can be a complicating factor, it is not detrimental to the utility of racemic HB as a model substrate, since it has been shown that there is no significant reciprocal influence on *S* (+) HB and *R* (−) HB pharmacokinetics or metabolite formation *in vivo* [2, 4].

However, it still must be demonstrated that the (iso-) enzymes oxidizing *S* (+) HB and *R* (−) HB are the same or at least rather similar. Evidence for this has been presented *in vitro*, where racemic HB was preferentially oxidized by cytochrome P-450b [5, 6]. If a comparable situation did not exist *in vivo*, pretreatment with agents selectively inducing certain cytochrome P-450 subspecies might result in differential effects on oxidative activity towards the enantiomers, and hence on the pharmacokinetic parameters used to reflect enzyme activity.

In the present study, an attempt was made to establish whether the enantiomers of HB are indeed metabolized by the same or similar cytochrome P-450 subspecies. For this purpose, the extent of correlation of total metabolic clearance (CL) of the enantiomers of HB was assessed in the rat. Pseudoracemic HB, consisting of equimolar quantities of *S* (+) HB and *R* (−) HB containing a trideuterated N_1-methyl group [d_3 *R* (−) HB] (fig. 1) was administered orally to untreated rats and to rats pretreated with phenobarbital, 3-methylcholanthrene or carbon tetrachloride.



S-(+)-HB d_3-R-(-)-HB

Fig. 1. Structural formulae of *S* (+) HB and d_3 *R* (−) HB.

MATERIALS AND METHODS

Materials. *S* (+) Hexobarbital [HB; 1,5-dimethyl-5-(1′-cyclohexenyl) barbituric acid] was a kind gift from Prof. J. Knabe, Universität des Saarlandes (Saarbrücken, F.R.G.). The synthesis of d_3 *R* (−) HB, as well as the combination of *S* (+) HB and d_3 *R* (−) HB to a pseudoracemic mixture were described recently [4]. $[\alpha]_D$ of *S* (+) HB was +10.8° ($c = 0.48$; absolute ethanol) and $[\alpha]_D$ of d_3 *R* (−) HB was −10.8° (same conditions).

Sodium phenobarbital (PB) and sesame oil were obtained from Brocacef (Maarssen, The Netherlands); 3-methylcholanthrene (MC) from Sigma Chemical Co. (St Louis, MO); and carbon tetrachloride (CCl_4) from J. T. Baker (Deventer, The Netherlands).

Animals. Male Wistar rats from the laboratory breed, weighing 180–220 g, were used in all experiments. Descriptions of their maintenance, surgery

* Present address: Department of Pharmacochemistry (Molecular Toxicology), Free University, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

† To whom correspondence should be addressed.

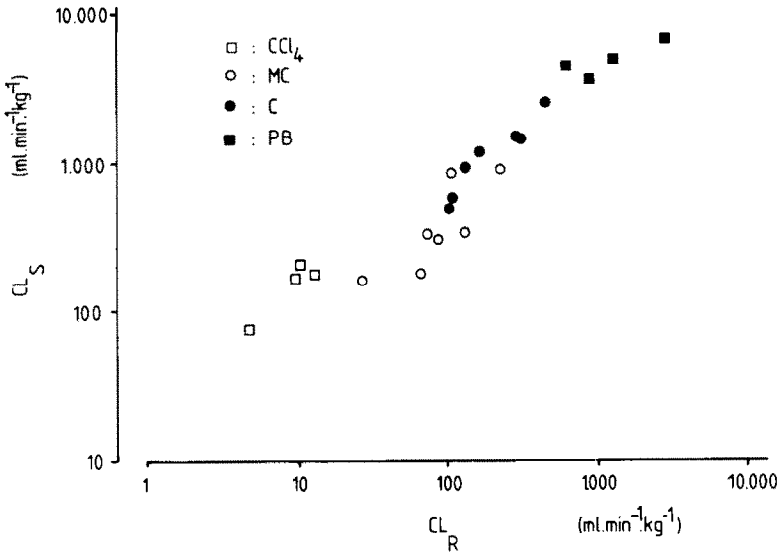


Fig. 2. Correlation of *CL* values of *S* (+) HB and d_3 *R* (−) HB in untreated rats (●) and in rats pretreated with phenobarbital (PB; ■), 3-methylcholanthrene (MC; ○), carbon tetrachloride (CCl_4; □). Log–log presentation.

and blood sampling have been reported elsewhere [3].

Pretreatments. Except for the untreated animals (N = 7), the rats were injected intraperitoneally with three different agents in order to create broadly ranging clearance data.

Carbon tetrachloride (CCl_4), 0.8 ml/kg was administered as a 1:3 v/v solution in sesame oil, once 24 hr before the experiments (N = 4). Sodium phenobarbital (PB), 75 mg/kg, was dissolved in water. The animals received either one or two daily doses, and the experiments were carried out 24 hr afterwards (N = 4). PB was administered only once or twice since the increase in hepatic drug-metabolizing activity after more injections would result in such a large extraction ratio that the resulting HB blood concentrations would defy detection. In addition, clustering of data points around a maximal clearance value following a 5-day pretreatment regimen would hardly contribute to a realistic statistical evaluation of data. 3-Methylcholanthrene (MC: 18 mg/kg) was dissolved in sesame oil, and administered daily for three consecutive days, the experiments being performed 24 hr after the last dose (N = 7). This type of pretreatment was carried out in order to maximally induce cytochrome P-450 subspecies which are practically inactive in the metabolic degradation of HB.

Drug administration. Pseudoracemic HB, in a dose of 50 mg/kg, was administered orally via gastric intubation as described previously [4].

Analysis of S (+) *HB and* d_3 R (−) *HB in blood.* The simultaneous analysis of *S* (+) HB and d_3 *R* (−) HB was carried out by mass fragmentography as described previously [4].

Data analysis. Areas under the blood concentration vs time curves (AUC) were calculated using the logarithmic trapezoidal rule method. In analogy to a previous paper [7] metabolic clearance (CL) was calculated as D/AUC, where *D* is the oral dose which is assumed to be completely absorbed; this clearance parameter is called the apparent intrinsic clearance. For *S* (+) HB and d_3 *R* (−) HB, clearance was designated as CL_S and CL_R, respectively.

In every pretreatment group, the mean of individual clearance ratios, CL_S/CL_R, was calculated. Correlation of all clearance points was performed using non-weighted least-squares regression analysis.

RESULTS AND DISCUSSION

The aim of this study was to show whether pretreatment of rats with different agents influences CL_S and CL_R, in the same direction and also to the same extent upon administration of HB as a pseudoracemic mixture. This would imply metabolism of the enantiomers of HB by similar or even common types of cytochrome P-450 isozymes.

CL_S and CL_R in untreated rats (N = 7) varied by a factor of five. Similar differences have also been observed in correlation studies of racemic HB with heptabarbital [7], antipyrine [8] and aminopyrine [2]. By and large, the CL_S/CL_R ratio corresponds satisfactorily to a previously observed mean ratio of about seven following separate administration of the enantiomers [3]. For both enantiomers, the mean CL values following MC pretreatment (N = 7) were somewhat lower than those of untreated animals. This is in agreement with data on the racemate obtained *in vitro* [5] and *in vivo* [7–9] and also with data on the enantiomers *in vitro* [5, 10]. Apparently, induction of cytochrome P-450 is taking place at the expense of those cytochrome P-450 isozymes which catalyse the degradation of both enantiomers of HB. Following CCl_4 pretreatment (N = 4), a strongly decreased cytochrome P-450 activity was observed with a concomitant and consistent increase in the

Table 1. Determinants of correlation lines based on CL_S and CL_R data of pseudoracemic HB in four differentially pretreated groups of rats

	Controls (N = 7)	3-Methylcholanthrene (N = 7)	Phenobarbital (N = 4)	Carbon tetrachloride (N = 4)
Intercept*	162 ± 414	26.8 ± 373	3024 ± 1787	7.9 ± 153
Slope†	4.70 ± 0.65	3.71 ± 1.31	1.33 ± 0.32	15.3 ± 4.65
Correlation coefficient	0.95 P < 0.01	0.75 P = 0.05	0.95 P = 0.05	0.94 P < 0.10
Mean clearance ratio CL_S/CL_R†	5.62 ± 1.27	4.13 ± 1.95	4.20 ± 1.96	16.0‡ ± 2.06

* With 95% confidence limits.
† With SD.
‡ Significantly different from CL_S/CL_R in control rats (P < 0.01).

CL_S/CL_R ratio up to 15. An explanation for this cannot easily be furnished. Treatment with CCl_4 is supposed to cause hepatotoxic effects, including non-selective destruction of cytochrome P-450s.

The observed increase of CL_S and CL_R up to a factor of five following PB-pretreatment (N = 4) was in agreement with previously reported increases in rates of metabolism of enantiomers in rat liver preparations [5, 10] and also with pharmacokinetic *in vivo* data on racemic HB in rats [7]. Apparently, PB-inducible cytochrome P-450s are involved in the metabolism of both enantiomers. In Fig. 2, CL_S and CL_R in each individual rat are plotted against each other on a log–log scale. This presentation was chosen to accommodate data ranging over a four-order clearance interval in one figure; however, the equations of the correlation lines that follow are all based on non-transformed data. In Table 1, the determinants of the linear correlation lines based on CL_S and CL_R data of pseudoracemic HB in four differently pretreated groups of rats are given. The corresponding equations are of the general form:

$$CL_S = \text{slope} \times CL_R + \text{intercept}.$$

Also, the mean clearance ratios CL_S/CL_R are included. By comparing intercept, slope and correlation coefficient for the different groups, it is clear that the MC data can be considered to belong to the same statistical population as the control group. This is not the case for the CCl_4 group, which has a quite different slope. The PB data also do not seem to fit well to the control (and MC) data. However, although the intercept is large, differences in slope with control and MC data are less pronounced than in the CCl_4 group. The mean of the clearance ratios was considered as an additional parameter to compare the different groups (Table 1). On this basis, the PB data are likely to belong to the same population as the control and MC data, whereas the deviation of CCl_4 data is confirmed by an extremely high CL_S/CL_R ratio.

The resulting equation for the correlation line consisting of controls, and PB- and MC-pretreated rats is:

$$CL_S = 2.57\ CL_R + 567\ (r = 0.92;\ P < 0.001).$$

The slope of the equation reflects the higher clearance of CL_S as compared to CL_R. The intercept is rather high, but should be viewed in relation to the four-order interval of clearances. The correlation coefficient obtained is as strong as correlation coefficients found in other studies with an identical experimental set-up [7, 8].

For the present study it can tentatively be concluded that *S* (+) HB and *R* (−) HB are metabolized *in vivo* by the same or rather similar (PB-inducible) cytochrome P-450s under control conditions, and that the CL_S/CL_R ratio is not differentially affected by pretreatment of the rats with PB or MC. The situation may be different after pretreatment with CCl_4. Clearance data obtained with racemic HB following pretreatment with enzyme inducers may be regarded as a reflection of PB-inducible cytochrome P-450 activity.

REFERENCES

1. M. T. Bush and W. L. Weller, *Drug. Metab. Rev.* **1**, 249 (1972).
2. M. van der Graaff, Ph.D. thesis, University of Leiden (1985).
3. M. van der Graaff, N. P. E. Vermeulen, R. P. Joeres and D. D. Breimer, *Drug metab. Dispos.* **11**, 489 (1983).
4. M. van der Graaff, N. P. E. Vermeulen, P. H. Hofman, D. D. Breimer, J. Knabe and L. Schamber, *Biomed. Mass Spectrom.* **12**, 464 (1985).
5. K. Miyano, Y. Fujii and S. Toki, *Drug Metab. Dispos.* **8**, 104 (1980).
6. D. E. Ryan, P. E. Thomas, L. M. Reik and W. Levin, *Xenobiotica* **12**, 727 (1982).
7. M. van der Graaff, N. P. E. Vermeulen, P. N. J. Langendijk and D. D. Breimer, *J. Pharmac. exp. Ther.* **225**, 747 (1983).
8. M. van der Graaff, N. P. E. Vermeulen, R. P. Joeres, T. Vlietstra and D. D. Breimer, *J. Pharmac. exp. Ther.* **227**, 459 (1983).
9. N. P. E. Vermeulen, Ph.D. thesis, University of Leiden (1980).
10. J. McCarthy and R. E. Stitzel, *J. Pharmac. exp. Ther.* **176**, 772 (1969).