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Research Article

Correlation Between *In Vivo* Antipyrine Metabolite Formation and Theophylline Metabolism in Rats

Michael W. E. Teunissen,[1] Irinka O. N. Brorens,[1] H. Joke De Langen,[1] Anne M. Geerlings,[1] and Douwe D. Breimer[1,2]

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Two model substrates for oxidative hepatic enzyme activity, *viz.* antipyrine (A) and theophylline (T), were given simultaneously to rats by iv administration. Blood concentrations of A and T were measured by a high-performance liquid chromatographic (HPLC) method. Urinary excretions of A, T, and the major metabolites arising from A—4-hydroxyantipyrine (OHA), norantipyrine (NORA), 3-hydroxymethylantipyrine (HMA), and 4,4′-dihydroxyantipyrine (DOHA)—and from T—1-methyluric acid (1-MU) and 1,3-dimethyluric acid (1,3-DMU)—were also determined by HPLC. It was found that the pharmacokinetic parameters obtained after the simultaneous administration of A and T at relatively low dose levels (A, 5.0 mg; and T, 1.3 mg) were not different from those obtained after the separate administration of A or T at the same dose level. In order to investigate whether the metabolic pathways of A and T are mediated by the same or closely related forms of the cytochrome *P*-450 system, metabolic clearances of A ($CL_{A,M}$) and T ($CL_{T,M}$) and the clearances for production of their various metabolites, obtained in untreated rats and in rats pretreated with 3-methylcholanthrene (MC) or with MC followed by 9-hydroxyellipticine (E), were correlated. These two compounds are a selective cytochrome *P*-448 inducer and inhibitor, respectively. Strong correlations were found between $CL_{T,M}$ and the clearances for production of OHA, NORA, and DOHA but not HMA. The best correlation, however, was observed between $CL_{T,M}$ and CL_{OHA}, not only when all data points were taken into account ($r = 0.99$), but also in separate pretreatment groups (r ranging from 0.87 to 0.92). Moreover, the slopes of these correlation lines varied only slightly among groups, while the intercepts were not significantly different from zero. In the separate pretreatment groups, the correlation coefficients for the correlations between $CL_{T,M}$ and the clearance for production of the other metabolites of A were considerably lower, while the slopes of the correlation lines varied substantially. Clearances for production of the metabolites of T were strongly correlated with each other ($r = 0.99$) and with CL_{OHA} ($r = 0.95$). It can be concluded that theophylline metabolism and formation of OHA are mediated by the same or very similar forms of cytochrome *P*-450, whereas formation of the other major metabolites of A is not or only partly. The study of the various pathways of metabolism after simultaneous administration of drugs is a powerful tool in the study of correlations in drug metabolism *in vivo*.

KEY WORDS: antipyrine; theophylline; cytochrome *P*-450; induction; inhibition; drug metabolizing enzyme activity; metabolic clearance correlations; rats.

INTRODUCTION

Antipyrine is frequently used to assess the activity of hepatic oxidative drug-metabolizing enzyme activity, in particular in studies on the effect of environmental factors upon it (1,2). Several attempts have been made to predict the metabolic clearance of other oxidatively metabolized drugs with the aid of antipyrine clearance. Except for some benzodiazepines, however (3), correlations found in studies where antipyrine and other drugs were given to the same subjects were rather poor (4–8). There may be several reasons for this including intraindividual variability of enzyme activities in a longitudinal crossover study and neglect of the existence of different forms of cytochrome *P*-450, with different substrate and product selectivity (9,10).

Recently, studies in rats were performed in which these factors were taken into account. Upon the simultaneous administration of hexobarbital and heptabarbital, their clearance was found to correlate strongly ($r = 0.96$) (11).

In a subsequent study high correlation coefficients were obtained between the rate of 3-hydroxymethylantipyrine formation and the intrinsic clearance of hexobarbital, whereas the total clearances of hexobarbital and antipyrine correlated rather poorly (12). The underlying reason for this discrepancy is likely the involvement of different forms of the cytochrome *P*-450 system in antipyrine metabolism. The

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

[2] To whom correspondence should be addressed.

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formation of 3-hydroxymethylantipyrine (HMA)[3] is mediated by a different subspecies of cytochrome *P*-450 than the formation of 4-hydroxyantipyrine (OHA) (13–15). The formation of norantipyrine (NORA) and 4,4'-dihydroxyantipyrine (DOHA) is probably mediated by still other forms of cytochrome *P*-450 (15,16).

In the present study an attempt was made to investigate the existence of correlations between the clearances for production of antipyrine metabolites and the clearance of theophylline to its metabolites. The choice of antipyrine may be obvious from the foregoing, while theophylline was chosen as a model drug because it is also extensively metabolized and its metabolism is easily influenced by environmental factors (6,24,25).

MATERIALS AND METHODS

The materials used and the assay methods of A and T in blood and A and T and their metabolites in urine by HPLC have been published in detail in a separate paper (17).

Animals and Treatments. Male SPF Wistar rats (180–220 g) from a laboratory breed were used throughout the study and were allowed free access to water and food (Hope Farms, Woerden, The Netherlands). From 16 hr before the experiment until 24 hr afterward, only water containing 20 g sucrose/liter was allowed. MC (18 mg/kg, dissolved in sesame oil) was administered ip 40 and 16 hr before the experiment. E (20 mg/kg, dissolved in 0.5 *M* acetate buffer, pH 4.5) was administered ip 1 hr (E1) or 16 hr (E2) before the experiment. Theophylline (6.5 mg/kg) and antipyrine (25 mg/kg) were given together or separately as a freshly prepared solution of aminophylline (1.27 g aminophylline = 1 g theophylline) and/or antipyrine in 0.5 ml saline. They were administered by means of a cannula that was inserted into the left common carotid artery. After drug administration the cannula was flushed with 0.5 ml of saline.

Blood Sampling. The rats were anaesthesized with diethyl ether and subsequently polyvinylchloride cannulas (length, 60 cm; inside diameter, 0.1 mm; filled with heparinized saline, 50 IU/ml) were inserted into the left common carotid artery for drug administration and blood sampling. The cannula was threaded subcutaneously to the back of the neck. The rats were placed in metabolism cages allowing free movement during blood sampling and were left to recover from the operation for 2 hr until drug administration. Serial blood samples (100 μl) were collected at regular intervals and were hemolyzed immediately in 0.25 ml of distilled water. The samples were stored at −20°C until analysis.

Urine Collection. Urine was collected for 24 hr, a time interval sufficient for complete excretion of all measured metabolites of antipyrine (18) and theophylline (19).

Data Analysis. Blood elimination half-lives of antipyrine and theophylline were determined for each rat by least-squares linear regression of the terminal part of the log blood concentration versus time curves. The area under the blood concentration versus time curve (AUC) was calculated according to the log-linear trapezoidal rule. Total blood clearance (CL) was calculated as D/AUC, where D is the dose. The apparent volume of distribution (V_d) was calculated as V_d-area. Rates of formation of the metabolites were expressed as clearances for production (CL_m) and were calculated as the product of the fraction of the dose excreted as a particular metabolite m and the total blood clearance of the parent compound: $CL_m = fm \times CL$. Likewise, renal clearance was calculated as the product of the fraction of the dose excreted unchanged and the total clearance. Metabolic clearance of the parent compound ($CL_{P,M}$) was calculated by subtracting renal clearance from total clearance. Statistical analysis was performed by analysis of variance (in combination with the Studentized Newman–Keuls test). Correlation analysis was performed by calculating the correlation coefficient of the least-squares correlation line through the data points.

RESULTS

Comparison of Separate Administration of Antipyrine and Theophylline with Simultaneous Administration. In order to check whether A and T might influence each other's metabolism, the two drugs were also administered separately. In Tables I–III it is shown that none of the blood kinetics or excretion data obtained after simultaneous administration without pretreatment (controls) were different from the results obtained after separate administration of A or T.

Pharmacokinetics in Blood. Typical time courses of antipyrine and theophylline concentrations in blood of a control rat and MC-pretreated rat are shown in Fig. 1. From the curves obtained first-order elimination kinetics can be inferred as reflected by the straight lines of log concentrations vs time. MC treatment resulted in similar decreases in elimination half-lives of A and T from 51 ± 14 to 16 ± 3 min and from 71 ± 18 to 15 ± 4 min, respectively (Table I).

The relative increase in clearance of T was considerably larger than that of A: from 5.7 ± 1.3 to 40.7 ± 9.4 ml/min/kg and from 14.4 ± 4.1 to 48.3 ± 15.9 ml/min/kg, respectively.

After MC treatment the V_d of T was increased from 0.51 ± 0.05 to 0.88 ± 0.27 liters/kg, whereas no change was observed in the V_d of A. Treatment with 9-hydroxyellipticine after MC induction (MCE-1) resulted in a marked inhibition of the metabolism of both A and T, with even longer half-lives and lower clearances than in control rats. After MCE-2 pretreatment (administration of E 16 hr prior to the experiment) a milder inhibition occurred. The only significant difference with control rats was the increased V_d of both drugs. All kinetic data for A and T in blood are summarized in Table I.

Cumulative Excretion of Metabolites in Urine. The total excretion of antipyrine plus its major metabolites, OHA, NORA, HMA, and DOHA, was about the same in all

[3] Abbreviations used: A, antipyrine; T, theophylline; OHA, 4-hydroxyantipyrine; NORA, norantipyrine; HMA, 3-hydroxymethylantipyrine; DOHA, 4,4'-dihydroxyantipyrine; 1-MU, 1-methyluric acid; 1,3-DMU, 1,3-dimethyluric acid; CL, total blood clearance; CL_M, metabolic clearance; CL_m, clearance for production of metabolites; $CL_{P,M}$, metabolic clearance of the parent compound; $CL_{A,M}$, metabolic clearance of antipyrine; $CL_{T,M}$, metabolic clearance of theophylline; MC, 3-methylcholanthrene; E, 9-hydroxyellipticine; MCE-1, induction followed by E, 1 hr before substrate administration; MCE-2, MC induction followed by E, 16 hr before substrate administration; HPLC, high-performance liquid chromatography.

Table I. Pharmacokinetics of Antipyrine and Theophylline in Blood After Separate Administration and After Simultaneous Administration to Four Groups of Rats with Different Pretreatment (Mean Values ± SD)

	Antipyrine alone (N = 6)	Theophylline alone (N = 6)	Antipyrine administered simultaneously with theophylline			
			Controls (N = 8)	MC (N = 12)	MCE-1 (N = 13)	MCE-2 (N = 9)
Antipyrine						
$t_{1/2}$ (min)	45 ± 6	—	51 ± 14	16 ± 3[a]	111 ± 34[a,b]	55 ± 16[b]
V_d (liters/kg)	0.92 ± 0.10	—	0.98 ± 0.12	1.16 ± 0.33	1.10 ± 0.19	1.29 ± 0.17[a]
CL (ml/min/kg)	14.5 ± 2.5	—	14.4 ± 4.1	48.3 ± 15.9[a]	7.4 ± 2.1[a]	22.8 ± 9.8[b]
CL_M (ml/min/kg)	13.9 ± 2.4	—	13.8 ± 2.4	47.8 ± 15.7[a]	7.1 ± 2.0[a,b]	21.3 ± 9.7[b]
Theophylline						
$t_{1/2}$ (min)	—	72 ± 17	71 ± 18	15 ± 4[a]	183 ± 74[a,b]	88 ± 26[b]
V_d (liters/kg)	—	0.54 ± 0.07	0.51 ± 0.05	0.88 ± 0.27[a]	0.94 ± 0.13[a]	1.66 ± 0.06[a,b]
CL (ml/min/kg)	—	5.7 ± 1.3	5.4 ± 1.4	40.7 ± 9.4[a]	4.3 ± 2.3[b]	7.0 ± 2.1[b]
CL_M (ml/min/kg)	—	4.7 ± 1.3	4.5 ± 1.4	40.3 ± 9.2[a]	3.1 ± 1.5[b]	6.0 ± 1.9[b]

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from MC ($P < 0.05$).

groups studied with the exception of MCE-2-pretreated rats, in which a lower total recovery was found. The most conspicuous changes were observed after MC pretreatment, which resulted in a near-doubling of the excretion of OHA, whereas HMA excretion amounted to only 12% of control values. OHA excretion was also increased after MCE-1 pretreatment, but in this group HMA excretion was the same as in controls, while the excretion of DOHA was lower. In the MCE-2 group, antipyrine metabolite excretion was intermediate to the values obtained after MC and MCE-1 pretreatment, except for OHA and total excretion, which had significantly decreased.

Only minor changes in the excretion of theophylline and its metabolites were observed. MC pretreatment resulted in a reduction in the excretion of unchanged theophylline, whereas metabolite excretions were slightly elevated. The reverse was observed after MCE-1 pretreatment. All urinary excretion data are presented in Table II.

Rates of Formation of Metabolites. Table III gives clearance values for production of metabolites. Since it has been suggested that DOHA is the oxidation product of OHA (20), the sum of OHA and DOHA was also included to reflect the total activity of OHA formation. MC pretreatment resulted in differential effects on the rates of formation of the metabolites of A and T. Six- to ninefold increases were observed for CL_{OHA}, $CL_{1\text{-}MU}$, and $CL_{1,3\text{-}DMU}$, and two- to fourfold increases were found for CL_{NORA} and CL_{DOHA}, whereas CL_{HMA} was decreased in comparison with no pretreatment. After MCE-1 treatment, clearances for the production of OHA, DOHA, 1-MU, and 1,3-DMU were reduced to about 10% of the values obtained after MC pretreatment, and CL_{NORA} had decreased to a smaller extent, whereas CL_{HMA} was unaffected by E1. When compared with controls, the inhibiting effect of MCE-1 was most striking for CL_{DOHA}. Clearance for production of antipyrine metabolites was the same after MCE-2 pretreatment as in controls. Clearance of both theophylline metabolites, however, was slightly increased.

Correlation of Clearance Data. In Table IV the results are summarized for the correlations of total antipyrine clearance (CL_A), metabolic clearance of antipyrine ($CL_{A,M}$), CL_{OHA}, CL_{NORA}, CL_{HMA}, CL_{DOHA}, and $CL_{OHA+DOHA}$ with total theophylline clearance (CL_T), $CL_{T,M}$, $CL_{1\text{-}MU}$, $CL_{1,3\text{-}DMU}$, and $CL_{1\text{-}MU+1,3\text{-}DMU}$, respectively, including data for all pretreatment groups. CL_{HMA} correlated negatively with theophylline clearance data. Among all other clearance data

Table II. Urinary Recovery of Antipyrine, Theophylline, and Selected Metabolites of These Drugs (Mean ± SD Percentage of Dose)

	Antipyrine alone (N = 6)	Theophylline alone (N = 6)	Antipyrine administered simultaneously with theophylline			
			Controls (N = 8)	MC (N = 12)	MCE-1 (N = 13)	MCE-2 (N = 9)
Antipyrine						
Antipyrine	3.9 ± 0.9	—	4.0 ± 1.0	1.2 ± 0.8[a]	3.8 ± 1.9[b]	2.3 ± 1.3[a]
OHA	20.9 ± 4.2	—	19.7 ± 3.8	38.6 ± 6.9[a]	25.5 ± 4.3[a,b]	13.6 ± 2.4[a,b]
NORA	15.8 ± 3.8	—	17.4 ± 5.4	18.6 ± 3.3	16.6 ± 3.8	15.9 ± 4.5
HMA	23.0 ± 4.0	—	26.4 ± 3.8	3.2 ± 1.2[a]	26.5 ± 4.6[b]	14.3 ± 3.6[a,b]
DOHA	11.8 ± 1.5	—	12.0 ± 1.4	9.5 ± 1.8[a]	5.0 ± 1.5[a,b]	7.3 ± 2.5[a]
Total	75.4 ± 9.8	—	79.5 ± 10.9	71.0 ± 7.9	79.5 ± 9.7	53.4 ± 6.8[a,b]
Theophylline						
Theophylline	—	16.9 ± 3.5	18.6 ± 4.3	2.9 ± 1.7[a]	25.0 ± 9.2[b]	15.3 ± 3.5[b]
1-MU	—	26.8 ± 4.2	27.2 ± 5.8	30.4 ± 7.5	20.9 ± 4.8[a,b]	30.0 ± 4.8
1,3-DMU	—	26.0 ± 4.6	25.6 ± 5.5	30.9 ± 5.7	22.1 ± 4.7[b]	30.7 ± 4.5
Total	—	69.7 ± 9.8	71.4 ± 11.0	64.3 ± 12.7	68.0 ± 4.9	76.0 ± 7.2

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from MC ($P < 0.05$).

Table III. Clearance for Production of the Major Metabolites of Antipyrine and Theophylline (Mean ± SD ml/min/kg)

	Antipyrine alone (N = 6)	Theophylline alone (N = 6)	Antipyrine administered simultaneously with theophylline			
			Controls (N = 8)	MC (N = 12)	MCE-1 (N = 13)	MCE-2 (N = 9)
Antipyrine						
CL_{OHA}	2.9 ± 0.9	—	2.8 ± 0.9	18.3 ± 5.7[a]	1.9 ± 0.8[a,b]	3.0 ± 1.5[b]
CL_{NORA}	2.2 ± 0.8	—	2.5 ± 1.0	8.6 ± 2.1[a]	1.3 ± 0.6[a,b]	3.4 ± 1.8[b]
CL_{HMA}	3.4 ± 0.9	—	3.7 ± 1.0	1.4 ± 0.6[a]	1.9 ± 0.5[a]	3.0 ± 1.3[b]
CL_{DOHA}	1.6 ± 0.6	—	1.8 ± 0.6	4.5 ± 1.6[a]	0.4 ± 0.2[a,b]	1.5 ± 0.7[b]
$CL_{OHA+DOHA}$	4.5 ± 1.3	—	4.6 ± 1.3	22.8 ± 7.0[a]	2.3 ± 1.0[a,b]	4.5 ± 2.1[b]
Theophylline						
$CL_{1\text{-}MU}$	—	1.5 ± 0.5	1.5 ± 0.5	12.5 ± 3.6[a]	0.9 ± 0.6[a,b]	2.1 ± 0.7[a,b]
$CL_{1,3\text{-}DMU}$	—	1.5 ± 0.5	1.4 ± 0.5	12.7 ± 3.0[a]	0.9 ± 0.5[a,b]	2.1 ± 0.6[a,b]

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from MC ($P < 0.05$).

originating from A and T, positive correlations were found, with coefficients ranging from 0.80 to 0.99.

Although a strong correlation (r = 0.99) was found between the rate of formation of 1-MU and that of 1,3-DMU (Fig. 2), neither $CL_{1\text{-}MU}$, $CL_{1,3\text{-}DMU}$, nor $CL_{1\text{-}MU+1,3\text{-}DMU}$ gave better correlations with clearance data of antipyrine and/or its metabolites than CL_T or $CL_{T,M}$. However, $CL_{T,M}$ was considered to be a better parameter than CL_T, since this study was undertaken to correlate metabolic pathways. The strongest correlation with $CL_{T,M}$ (r = 0.99) was observed for CL_{OHA} (Fig. 3). Results of the correlations between $CL_{T,M}$ and metabolic clearance data of antipyrine and its metabolites for individual pretreatment groups and all groups combined are shown in Table V together with the slopes (± SE) of the correlation lines obtained. Except for the correlation between $CL_{T,M}$ and CL_{OHA}, the slopes of the correlation lines were found to vary markedly among different pretreatment groups. Summation of CL_{OHA} and CL_{DOHA} did not result in better correlations with theophylline clearance data than CL_{OHA} alone.

DISCUSSION

Previous efforts to assess the *in vivo* activities of hepatic drug-metabolizing enzymes, with the aim of predicting pharmacokinetic variables of drugs from corresponding data on model compounds, have not been very successful. Since many drugs, including model compounds such as antipyrine, are transformed to several metabolites which may be mediated by different forms of the cytochrome *P*-450 system (13,15,21), correlating the enzyme activities of such single pathways (clearance to metabolites) may prove to be a better approach than correlating total clearance (12). If the metabolism of a drug is not subject to such product selectivity, it should be considered that enzyme activities are reflected by metabolic clearances and hence total clearance should be corrected for renal clearance. Another prerequisite for a successful approach may be the simultaneous administration of drugs (9,11,12). Thus, time-dependent intraindividual variability in liver blood flow and oxygenase activity is circumvented. However, two simultaneously administered drugs that are metabolized by the same enzyme (system) should be given at a sufficiently low dose to prevent competitive inhibition at the enzyme level (10,11). This is especially important for theophylline metabolism, which has been shown to display dose-dependent characteristics in humans (22) as well as in rats (23) at relatively low doses.

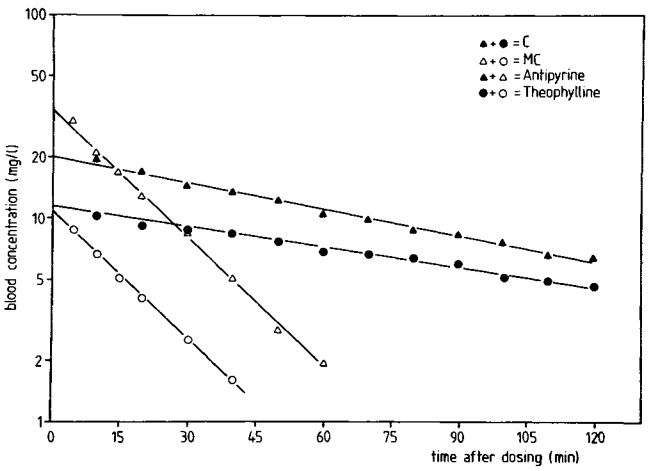

Fig. 1. Antipyrine and theophylline blood concentration–time curves in a control rat (filled symbols) and a MC-pretreated rat (open symbols) after simultaneous administration of the two drugs.

In the present study the results of all pharmacokinetic parameters obtained after simultaneous administration of 5.0 mg antipyrine and 1.3 mg theophylline were not found to differ from those after separate administration (Tables I–III).

The objective of the present investigation was to search for correlations between the clearances of different metabolic pathways of antipyrine and the metabolic clearance of theophylline, taking into account the above considerations. Although it has been shown that theophylline metabolism is mediated by the cytochrome *P*-450 system (6,24,25), it would be unrealistic to presuppose that the enzymes involved in the metabolism of T would not exhibit multiplicity. Therefore, clearances for the production of the major metabolites of T in rats, 1-MU and 1,3-DMU, were also measured. From Fig. 2 it is evident that the rates of formation of these metabolites are highly correlated irrespective of pretreatment, strongly suggesting that their metabolism is indeed mediated by the same enzyme system. This is rather surprising since 1,3-DMU is oxidized only at the 8 position, whereas the rate-limiting step in 1-MU formation is demethylation to 1-MX (which hardly appears in rat urine).

Pharmacokinetic data of antipyrine and metabolites

Table IV. Coefficients of Correlation Among Total Clearance, Metabolic Clearance, and Individual Metabolite Formation Clearances of Antipyrine and Theophylline in Rats (N = 42)

	CL_T	$CL_{T,M}$	$CL_{1\text{-}MU}$	$CL_{1,3\text{-}DMU}$	$CL_{1\text{-}MU+1,3\text{-}DMU}$
CL_A	0.92	0.92	0.82	0.84	0.83
$CL_{A,M}$	0.92	0.92	0.82	0.84	0.83
CL_{OHA}	0.98	0.99	0.94	0.95	0.95
CL_{NORA}	0.92	0.91	0.86	0.88	0.87
CL_{HMA}	−0.41	−0.41	−0.42	−0.43	−0.42
CL_{DOHA}	0.90	0.90	0.80	0.81	0.81
$CL_{OHA+DOHA}$	0.98	0.98	0.93	0.94	0.94

after different pretreatments were similar to those reported previously (13–15,21), except that in the present study the inhibiting effect of E after MC induction was found to be more pronounced and showed less enzyme selectivity. These differences may be explained by the considerably shorter duration of MC pretreatment (2 vs 5 days). The MC-pretreated group was used together with an untreated control group in order to obtain broad ranges of CL, $CL_{P,M}$, and CL_m for both substrates to be correlated (11). This indeed resulted in enhanced clearances of T and its metabolites and of CL_{OHA}, indicating that the formation of OHA and the metabolism of T are mediated by the same or very similar enzymes. As 9-hydroxy-ellipticine (E) has been shown to inhibit selectively the rate of formation of OHA (15), combined MC and E treatment was studied to fill the gap between the clearance data of controls and those of MC-pretreated rats. However, this approach was not entirely successful, because the inhibition caused by E was considerably stronger than expected, which resulted in values around the control data (Figs. 2 and 3). Because of this cluster formation of data points, coefficients of correlation through all those points may be falsely elevated (Table IV).

For a more appropriate statistical evaluation of the data, coefficients of correlation and slopes of the correlation lines between $CL_{T,M}$ and metabolic clearance data of A were also calculated for each pretreatment group (Table V). Again, the best correlations were found between $CL_{T,M}$ and CL_{OHA}, ranging from 0.87 to 0.92. Of equal importance for the existence of a good correlation is that the slopes of the lines for the different groups differed only slightly, while the intercepts were not significantly different from zero for all pretreatment groups (latter data not shown). These results indicate that metabolic elimination of T and formation of OHA are probably mediated by the same or highly related subspecies of cytochrome P-450. Correlations found between $CL_{T,M}$ and CL_{NORA} or CL_{DOHA} were considerably lower and coefficients of correlation as well as slopes varied markedly among pretreatment groups. The observed correlations between $CL_{T,M}$ and both $CL_{A,M}$ and $CL_{OHA+DOHA}$ were also fairly good, but it should be remembered that CL_{OHA} is a major constituent of $CL_{A,M}$ as well as of $CL_{OHA+DOHA}$. Although summation of CL_{OHA} and CL_{DOHA} did not result in better correlations with $CL_{T,M}$ and CL_{OHA} alone, the observed differences are too small to conclude that DOHA is not an oxidation product of OHA (20). Rather poor and even negative correlations were obtained between $CL_{T,M}$ and CL_{HMA}, whereas CL_{HMA} was found to correlate well with the clearance of hexobarbital, another cytochrome P-450 marker substrate (11).

As a conclusion to the present study it can be stated that investigating correlations between oxidative drug-metabolizing enzyme activities of single metabolic pathways after the simultaneous administration of model substrates is a useful and promising approach to elucidate which pathways of metabolism are mediated by the same or very similar forms of cytochrome P-450.

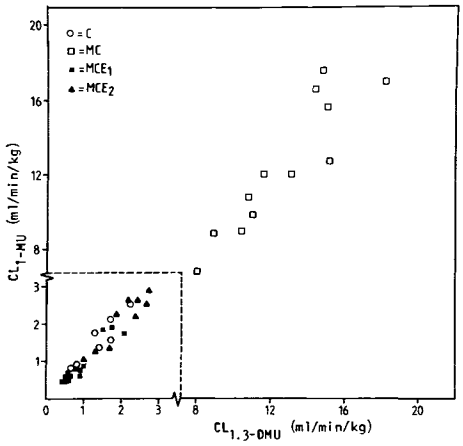

Fig. 2. Correlation diagram of the clearances for production of 1-methyluric acid and 1,3-dimethyluric acid after simultaneous administration of theophylline (1.3 mg) and antipyrine (5.0 mg) to rats with different pretreatments.

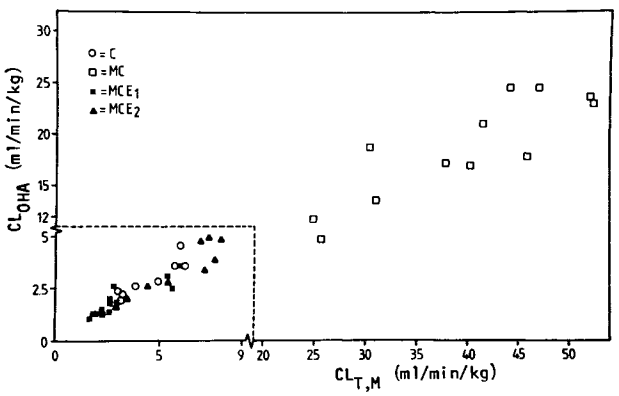

Fig. 3. Correlation diagram of the metabolic clearance of theophylline and the clearance for production of 4-hydroxyantipyrine after simultaneous administration of theophylline (1.3 mg) and antipyrine (5.0 mg) to rats with different pretreatments.

Table V. Slopes and Coefficients of Correlation Between Metabolic Clearance of Theophylline and Antipyrine Metabolic Clearance Data in Four Groups of Rats with Different Pretreatment and Combined Data[a]

	$CL_{A,M}$		CL_{OHA}		CL_{NORA}		CL_{HMA}		CL_{DOHA}		$CL_{OHA+DOHA}$	
	r	$s \pm SE$	r	$s \pm SE$	r	$s \pm SE$	r	$s \pm SE$	r	$s \pm SE$	r	$s \pm SE$
Untreated ($N = 8$)	0.73	2.11 ± 0.81	0.90	0.60 ± 0.12	0.57	0.40 ± 0.23	0.68	0.53 ± 0.23	0.82	0.36 ± 0.10	0.92	0.96 ± 0.17
MC ($N = 12$)	0.83	1.37 ± 0.29	0.87	0.46 ± 0.08	0.66	0.14 ± 0.05	−0.12	−0.01 ± 0.02	0.71	0.12 ± 0.04	0.88	0.58 ± 0.10
MCE1 ($N = 13$)	0.92	1.26 ± 0.16	0.89	0.47 ± 0.07	0.91	0.39 ± 0.05	0.58	0.20 ± 0.08	0.90	0.13 ± 0.02	0.90	0.59 ± 0.09
MCE2 ($N = 9$)	0.65	3.31 ± 1.48	0.92	0.62 ± 0.10	0.44	0.41 ± 0.32	0.23	0.16 ± 0.25	0.56	0.21 ± 0.12	0.84	0.83 ± 0.20
All rats ($N = 42$)	0.92	1.04 ± 0.07	0.99	0.45 ± 0.01	0.92	0.18 ± 0.01	−0.41	−0.03 ± 0.01	0.90	0.10 ± 0.01	0.98	0.55 ± 0.02

[a] r, correlation coefficient; s, slope ± SE.

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