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QSAR study on species differences in microsomal *N*-oxygenation of *N,N*-dimethylalkylamines

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Abstract—The metabolic *N*-oxygenation of nine long chain *N,N*-dimethylalkylamines and tri-n-butylamine has been studied using hepatic microsomal homogenates from mice, dogs and guinea-pigs. The relative oxidizability of amines (ROA) was correlated with structure, lipophilicity and nucleophilicity parameters of substrates and corresponding amine oxides formed in this biological reaction. The highest conversion of amines to *N*-oxides was found with male guinea-pig, followed by dog (male, female) and the lowest amount of amine oxides has been produced with male mice microsomal homogenates. The analyses were carried out by GLC and the results quantified using QSAR methodology. ROA is parabolically dependent upon structure and physicochemical properties of the substrates and products which was proved by the high statistical significance of the regression equations. The biological *N*-oxygenation of these amines is controlled by lipophilicity, stereochemistry and electronic effects.

It is generally accepted that the oxidation of xenobiotic amines by oxygen is catalysed by NADPH-dependent mono-oxygenases, mostly concentrated in liver microsomes (Bridges et al 1972; Gorrod 1978a; Gorrod & Damani 1985). Tertiary amines of the aliphatic and aniline type are oxygenated to the corresponding *N*-oxides by flavin-containing mono-oxygenases (Masters & Ziegler 1971; Gorrod et al 1975a,b; Gorrod 1978b; Ziegler 1980). In a report published by Sabourin et al (1984) the authors showed that hog and mouse liver enzymes, which were purified to homogeneity by Ziegler & Mitchell (1972) (hog), and by Sabourin et al (1984) (mouse, pig), are immunologically and mechanistically similar, although some quantitative differences in kinetic constants for amine substrates were observed. However, as pointed out by Ziegler (1985), there have been no studies that show significant quantitative differences in the purified liver mono-oxygenase from different species.

This is a continuation of our earlier work (Devínsky & Gorrod 1987) on the influence of substrate structural changes upon the microsomal *N*-oxidizability of *N,N*-dimethylalkylamines. The purpose of this communication is to report results from a study using QSAR methodology on the effect of structure and some physico-chemical properties (lipophilicity, nucleophilicity) of substrates and products upon the relative rates of *N*-oxide formation using a series of *N,N*-dimethylalkylamines ($C_mH_{2m+1}N(CH_3)_2$ m = 4 to 18) with hepatic microsomal homogenates prepared from various species.

Materials and methods

Chemicals. *N,N*-Dimethylalkylamines and the corresponding *N*-oxides were prepared in our laboratories according to reported methods (Devínsky et al 1978, 1982). Solvents used for extraction and dissolution of compounds in metabolism studies were purchased from BDH (UK) and Fison's (UK). Support and stationary phase materials used for packing columns for GLC were obtained from Phase Separation Ltd (UK). Glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADP were from Boehringer Mannheim (UK).

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Animals. Hepatic microsomal homogenate preparations from male LACA albino stock mice, male Dunkin-Hartley albino guinea-pigs and female (F) and male (M) beagle dogs and male (M) greyhound were used.

Instrumentation. GLC was carried out on a Perkin Elmer Model F 33 gas chromatograph equipped with a flame-ionization detector. The precoiled glass column (1 m), i.d. 3 mm, was packed with 8% Apiezon L on Chromosorb G AW DMDCS (80–100 mesh) and 10% KOH and conditioned for 48 h before use. Gas pressures were set as follows [kN m^{-2}]: air 140, hydrogen 125, nitrogen (carrier gas) 175. The injection port temperature (250–300°C) and the oven temperature (50–280°C) were varied until optimum conditions were obtained for each compound, i.e. all compounds had a retention time between 5 min 45 s, and 6 min.

Enzyme preparation, incubation, extraction, isolation, and identification of substrates and products were carried out as described previously (Devínsky & Gorrod 1987).

Results and discussion

In the previous study on microsomal *N*-oxygenation of long chain non-aromatic tertiary amines we have shown that the relative oxidizability (ROA) of these substrates using mouse liver microsomal homogenates is parabolically dependent upon the structural changes within the series of compounds as well as upon their lipophilicity (expressed in the free energy-related log P terms calculated according to Rekker (1977) and isoretention time values $\log(T)_i$) and the nucleophilicity (represented by stability constants $\log K_c^{AD}$) of the products formed in this biological reaction i.e. the corresponding amine oxides ($C_mH_{2m+1}N(O)(CH_3)_2$).

Such a parabolic dependence of *N*-oxygenation rate of some tertiary amines upon their lipophilicity expressed by the calculated values for log P using purified pork liver microsomal mixed function amine oxidase system have also been reported by Tong & Lien (1976).

The results of our study (Table 1, 2) using the same variable as before (Devínsky & Gorrod 1987) clearly shows the parabolic course of ROA upon the structural and physicochemical parameters characterizing the substrates and products. The resulting curves are smooth and the data well fit the equations for a parabola as proved by statistical analysis (all functional relationships are statistically significant at a high confidence level) (Craig et al 1971). Therefore we consider the described relationships real and significant and find this parabolic fit interesting in the light of the earlier statement of Hansch et al (1965) that "the movement of organic compounds into tissue is parabolically rather than linearly dependent on π or log P".

Following the generalization claimed by Ziegler (1985) on a very close similarity of flavin-containing mono-oxygenases in various mammals one should not expect any, or at most, only small differences in microsomal *N*-oxygenation of the same substrate using different species. Our results (Table 1) seem only to confirm this assumption clearly in the case of dogs. The differences between the male and female dogs are small. It should be noted that the experiments were run with only three dogs (2

males and 1 female) and therefore from a statistical point of view the intersexual differences are insignificant.

The differences between mouse microsomes (the lowest conversion of amines to amine oxides) and the guinea-pig (the highest amounts of *N*-oxides observed in this study) are significant and on average guinea-pig liver microsomes produce 7·5 times more *N*-oxide than mouse liver microsomes. (The average ROA differences between guinea-pig vs M, F beagle dogs and M greyhound are 2·3, 2·9 and 2·5, respectively, in favour of guinea-pig, M, F beagle and M greyhound dogs vs mouse 2·4, 3·3 and 4·3, respectively, in favour of dogs, F vs M beagle 1·3 in favour of F dog, M greyhound vs M beagle 1·7 in

Table 1. Physicochemical constants and microsomal *N*-oxygenation data used in deriving the equations in Table 2 for *N,N*-dimethylalkylamines ($C_mH_{2m+1}N(CH_3)_2$)

				log ROA[a]					
m	log P[b]	log $(T)_t$[b]	log K_c^{AD}[d]	Mouse	M dog[e]	M dog	F dog	G. pig	Pig[f]
1	0·02	2·466[g]	3·2707	—	—	—	—	—	−6·8386
4	1·58	2·5094[h]	3·3310	−8·5379	−7·6018	−7·9775	−7·9519	−7·1572	−6·4724
6	2·61	2·5418	3·4887	−8·0512	−7·2802	−7·7359	−7·5773	−6·9613	—
8	3·65	2·5946	3·6313	−7·4997	−6·7769	−7·1334	−7·0228	−6·7051	−6·1261
9	4·17	2·602[g]	3·6513[i]	—	—	—	—	—	−6·0799
10	4·69	2·6316	3·7086	−6·9678	−6·5992	−6·8727	−6·6231	−6·5244	—
12	5·73	2·6704	3·7509	−6·8519	−6·4987	−6·6978	−6·5185	−6·4569	−6·0883
14	6·77	2·7017	3·8314	−6·9263	−6·5619	−6·6249	−6·5015	−6·4773	—
16	7·80	2·7268	3·9002	−7·2596	−6·6452	−6·7288	−6·6109	−6·6180	−6·3107
18	8·84	2·7428	3·9563	−7·3485	−6·7813	−6·8622	−6·8482	−6·6992	—
2-ATD[j]	6·25	2·6841	3·3762	−6·7191	−6·4378	−6·5262	−6·3977	−6·4087	—
TBA[k]	4·69	2·6108	3·4803	−7·9963	−7·9474	−7·9606	−7·9362	−7·9019	—

[a] [mol.dm^{-3} (mg prot.)$^{-1}$ per 20 min] [b] Calculated according to Rekker (1977) [c] [K]
[d] For the corresponding *N*-oxides taken from Devínsky et al (1985), (dm^3 mol^{-1}) [e] Greyhound
[f] Purified pig liver microsomal mixed function amine oxidase, (mol dm^{-3} (mg prot.)$^{-1}$ min^{-1})
[g] Calculated from eqn 2 (Devínsky & Gorrod 1987) [h] At 105 kN m^{-2}
[i] Calculated from relationship log K_c^{AD} = f (m) [j] (l-Methyldodecyl)dimethylamine [k] Tri-n-butylamine

Table 2. Non-linear relationships between relative oxidizability (ROA) and structure (m), lipophilicity (log P, log $(T)_t$), nucleophilicity (log K_c^{AD}) for *N,N*-dimethylalkylamines with various mammalian species

				log ROA = $ax^2 + bx + c$						
x	Species	a	b	c	n[a]	s[b]	r_i^c	$F_{2,5;0\cdot001}$[d]	x[e]	Eq.
m	Pig	−0·008	0·178	−7·022	6	0·021	0·999	748·9[h]	11·1	(1)
	G. pig	−0·009	0·234	−7·982	8	0·042	0·989	114·8	13·0	(2)
	M dog[f]	−0·014	0·355	−8·830	8	0·067	0·990	117·8	12·7	(3)
	M dog	−0·014	0·388	−9·403	8	0·091	0·989	111·8	13·9	(4)
	F dog	−0·017	0·461	−9·601	8	0·077	0·993	173·0	13·6	(5)
	Mouse	−0·021	0·547	−10·459	8	0·120	0·984	76·4	13·0 13·2[g]	(6)
log P	Pig	−0·031	0·313	−6·860	6	0·021	0·998	490·6[h]	5·05	(7)
	G. pig	−0·034	0·418	−7·767	8	0·042	0·987	117·1	6·15	(8)
	M dog[f]	−0·050	0·636	−8·503	8	0·066	0·990	119·5	6·36	(9)
	M dog	−0·051	0·698	−9·044	8	0·090	0·989	109·8	6·84	(10)
	F dog	−0·064	0·827	−9·175	8	0·077	0·994	178·7	6·46	(11)
	Mouse	−0·078	0·977	−9·955	8	0·120	0·984	76·4	6·26 6·41[g]	(12)
log $(T)_t$	Pig	−27·736	146·053	−198·331	6	0·013	0·999	1298·9[h]	2·6329	(13)
	G. pig	−28·458	151·740	−208·770	8	0·050	0·987	96·8	2·6660	(14)
	M dog	−38·712	208·775	−288·189	8	0·080	0·991	139·0	2·6965	(15)
	M dog[f]	−42·748	228·314	−311·376	8	0·033	0·997	482·9	2·6705	(16)
	F dog	−50·846	272·524	−371·716	8	0·090	0·992	141·4	2·6799	(17)
	Mouse	−65·989	352·173	−476·821	8	0·086	0·986	87·4	2·6684 2·6763[g]	(18)
log K_c^{AD}	Pig	−4·401	32·271	−65·240	6	0·076	0·980	36·4[i]	3·6663	(19)
	G. pig	−3·230	24·478	−52·920	8	0·097	0·943	20·1[j]	3·7892	(20)
	M dog	−3·851	30·279	−66·243	8	0·173	0·957	27·5[j]	3·9313	(21)
	M dog[f]	−4·835	36·810	−76·667	8	0·117	0·967	36·4[j]	3·8066	(22)
	F dog	−5·478	42·162	−87·750	8	0·188	0·955	26·0[j]	3·8484	(23)
	Mouse	−7·379	56·071	−113·606	8	0·147	0·943	20·1[j]	3·7993 3·8349[g]	(24)

[a] Number of data points used in deriving the regression equations
[b] Standard deviation from the regression
[c] Correlation index
[d] Theoretical value for $F_{2,5;0\cdot001} = 37·12$
[e] Optimal values calculated by setting d(log ROA)/dx = 0
[f] Greyhound
[g] Average value, the values from pig not included into calculation
[h] $F_{2,3;0\cdot001}$; theoretical value for $F_{2,3;0\cdot001} = 148·5$
[i] $F_{2,3;0\cdot01}$; theoretical value for $F_{2,3;0\cdot01} = 30·82$
[j] $F_{2,5;0\cdot005}$; theoretical value for $F_{2,5;0\cdot005} = 18·31$

favour of greyhound and M greyhound vs F beagle 1·4 in favour of greyhound). These values can serve only as a rough guide as there is no linearity in the ROA differences between the compounds in the series. The highest interspecies differences of ROA have been observed with the short chain compounds (m = 4,6) whereas the substrates with the highest *N*-oxide formation relative rates (m = 10 to 14 and (l-methyldodecyl) dimethylamine (2-ATD)) the differences were considerably lower. These results indicate that the compounds containing decyl to tetradecyl radicals in the molecule are good substrates for microsomal *N*-oxygenation for all species. From this point of view 2-ATD seems to be the best substrate amongst the investigated compounds. By contrast, tri-n-butylamine does not follow any relationship (except the slight increase of *N*-oxide formation in the order: mouse < dog < guinea-pig) and is apparently one of the worst substrates for *N*-oxygenation by microsomal homogenates (Table 1).

For comparison, we included in Table 1 and into our calculations (Table 2) Ziegler et al (1969) results from a study of *N,N*-dimethylalkylamines *N*-oxygenation using purified oxidase from pork liver. All the relationships derived from this data are parabolic (eqns 1, 7, 13, 19) and it is apparent (see values of regression constants, Table 2) that the purified enzyme is, as expected, clearly better in the conversion of amines to amine oxides than microsomal homogenates. The only exception is the relationship log ROA = f(log K_c^{AD}) i.e. the dependence of the *N*-oxidizability of amines on the nucleophilicity of products formed (amine oxides). An explanation of this deviation requires further study.

The quantification of the results via the regression equations and the analysis of coefficients of these relationships (Table 2) confirm unambiguously the above findings. Although the course of all relationships is almost the same, they are slightly shifted in the y-axis direction. That means, the higher the positive shift of coefficients a and c the higher is the ROA.

The optimum values of m, log P, log $(T)_t$ and log K_c^{AD} (Table 2) for maximum ROA calculated by setting d(log ROA)/dx = 0 (for x see Table 2) are close to those observed for 2-ATD. In fact this compound was metabolized (*N*-oxygenated) at the highest rate among the substrates under investigation regardless of the origin of the liver microsomal homogenates (Table 1).

Because of the great similarity of all the relationships discussed we can assume that factors controlling the *N*-oxygenation of our substrates with hepatic microsomal homogenates derived from different species are almost the same.

We have shown in our previous study on this topic that there are at least three characteristics of *N,N*-dimethylalkylamines influencing their ROA with mouse microsomes: the lipophilicity, the stereochemistry and the nucleophilicity. This seems to also hold for the present case (Devínsky & Gorrod 1987).

In conclusion, it is clear that there exists definite quantitative differences between various species in microsomal *N*-oxide formation using the same substrates. These differences are not great, nevertheless they are significant. The highest conversions of *N,N*-dimethylalkylamines to the corresponding *N*-oxides were found to occur with guinea-pig, followed by dog; the lowest amount of *N*-oxides being formed by mouse. The QSAR analysis confirmed the experimental findings and showed that this approach can serve as a powerful and useful tool in the evaluation of even small differences in an observed set of results and enables some generalizations to be made which can lead to the better understanding of experimentally obtained data.

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