ANTIPYRINE METABOLISM IN RELATION TO POLYMORPHIC OXIDATIONS OF SPARTEINE AND DEBRISOQUINE

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1 Thirty-five healthy subjects who had been classified as extensive or poor metabolisers of both sparteine and debrisoquine were given a single oral dose of antipyrine. Saliva concentration of antipyrine and urinary excretion of its three major oxidation metabolites were measured.

2 All the parameters of antipyrine metabolism which were estimated had similar distributions in both the 28 EM and 7 PM genetic phenotypes defined by the metabolism of sparteine and debrisoquine.

3 The clearance of antipyrine by the formation of 4-hydroxy-antipyrine and 3-hydroxy-antipyrine respectively were closely correlated ($r = 0.83$, $P < 0.001$) and both were significantly higher in smokers than in non-smokers. Demethylation of antipyrine also seemed to be influenced by smoking, but not to a statistically significant extent. These findings confirm the influence of the environmental factor of smoking in antipyrine oxidative biotransformations.

Introduction

If a standard dose of a drug is administered to patients, large variations in drug response and therapeutic outcome are observed. One major factor contributing to these inter-individual differences in drug response is a marked variation in the capacity to metabolise drugs which results in pronounced inter-individual differences in steady-state plasma concentrations. Both genetic and environmental factors have been shown to be important determinants for the activity of drug metabolising enzymes (Vesell, 1977; Sjöqvist *et al.*, 1980). Whereas the impact of environmental factors has been the subject of numerous studies, until quite recently the influence of genetic factors on oxidative drug metabolism has been a relatively neglected area.

It is important to differentiate between genetic polymorphism controlling drug metabolism by oxidation due to 'genes of major effect' on the one hand, and polygenic control due to 'genes of minor effect' on the other.

During the last few years a genetic polymorphism of sparteine metabolism (Eichelbaum *et al.*, 1978, 1979) and a genetic polymorphism of the 4-hydroxylation of debrisoquine (Mahgoub *et al.*, 1977) have been reported. In population studies it was observed that the production of the main metabolites occurred with a bimodal frequency distribution with

about 5% of the population being 'poor metabolisers'. Furthermore, it has been demonstrated that the same polymorphism controls the oxidation not only of these 2 drugs but also of other drugs, e.g. phenacetin (Idle & Smith, 1979; Ritchie *et al.*, 1980), phenytoin (Sloan *et al.*, 1981), phenformin (Oates *et al.*, 1981; Wiholm *et al.*, 1981) and nortriptyline (Mellström *et al.*, 1981) (for review see Eichelbaum, 1982). Antipyrine has been used as a probe drug in studies of oxidative metabolism (Stevenson, 1977; Vesell, 1979). The total clearance has been considered to be due to polygenic control, and there is a conflict between two pieces of evidence. An estimate of 'heritability' (Falconer, 1964) computed using intra-class correlation coefficients derived from the basic twin data (Vesell & Page, 1968) is 0.76 indicating an important measure of control by genes of minor effect. However, a family study (Blain *et al.*, 1982) failed to reveal a significant heritability suggesting that environmental influences were of more importance than genetic factors. The study by Blain and co-workers (1982) used the clearance of the parent drug and did not use information about individual specific pathways of metabolism, whereas recently Penno *et al.* (1981) described that the formation of the major antipyrine metabolites was under genetic control.

Figure 1 Metabolism of antipyrine to 4-hydroxyantipyrine (4-OH-A), 3-hydroxymethylantipyrine (3-OH-MA) and norantipyrine (norA).

Antipyrine is metabolized by three major metabolic pathways indicated in Figure 1. As 60–80% of the antipyrine dose is recovered as these three metabolites in urine (Eichelbaum *et al.*, 1976; Danhof & Breimer, 1979; Danhof, 1980; Eichelbaum *et al.*, 1982b), measurement of the urinary excretion of these metabolites should give an insight into three different oxidative drug metabolic reactions. In a preliminary study (Bertilsson *et al.*, 1980b) involving only eight subjects, we found no relationship between total antipyrine clearance and the debrisoquine hydroxylation phenotype. Similarly Danhof *et al.* (1981) found no clear relationship between debrisoquine hydroxylation and the metabolism of antipyrine through these three pathways, but there was a tendency for poor metabolizers of debrisoquine to form less 3-hydroxymethylantipyrine. We now report data on the kinetics and metabolism of antipyrine in relation to polymorphic oxidation of debrisoquine and sparteine in 35 unrelated subjects. The impact of smoking habits on antipyrine metabolism was also investigated.

Methods

Thirty-five healthy volunteers (16 Germans and 19 Swedes; 25 males and 10 females; age between 20 and 48 years; body weight between 48 and 86 kg; 11 smokers and 24 nonsmokers) consented to participate in the study. The study was approved by the Ethical Committees of the Karolinska Institute and the University of Bonn. Prior to the administration of antipyrine the volunteers had been phenotyped according to their ability to metabolize sparteine and debrisoquine (Eichelbaum *et al.*, 1982a). Seven subjects had a metabolic ratio of both sparteine and

debrisoquine > 20. These were therefore designated as poor metabolizers (PM) of both drugs. The remaining 28 subjects had a metabolic ratio of both sparteine and debrisoquine < 10 and were thus phenotyped as extensive metabolizers (EM). The close relationship between the metabolism of sparteine and debrisoquine in these subjects has recently been reported (Bertilsson *et al.*, 1980; Inaba *et al.*, 1980; Eichelbaum *et al.*, 1982a).

The subjects fasted overnight, and at 08.00 h they received 10 mg of antipyrine/kg body weight as an aqueous solution. Two hours after drug administration they received a light breakfast. Saliva samples were collected at 0, 3, 6, 9, 12, 24, 36 and 48 h after drug administration. Urine was collected during the periods 0–12, 12–24, 24–36, and 36–48 h. The samples were kept at –20°C until analyzed. Antipyrine in saliva was analyzed according to Eichelbaum & Spannbrucker (1977). Antipyrine, 4-hydroxy- (4-OH-A), 3-hydroxymethyl- (3-OH-MA) and norantipyrine (norA) in urine were determined as described previously (Eichelbaum *et al.*, 1981). The pharmacokinetic parameters of antipyrine were calculated from the salivary antipyrine concentrations assuming a one compartment open model. The elimination rate constant (k) was determined by least square regression analysis of the terminal slope of the salivary antipyrine concentration time curve. Volume of distribution (V) was calculated from

$$\frac{\text{Dose}}{C(0)}$$

Clearance (CL_{sal}) was calculated as

$$CL_{\text{sal}} = \frac{\text{Dose}}{C(0)} \times k_{\text{el}}$$

where $C(0)$ is the 'zero-time' salivary concentration calculated as the intercept of the regression line. Bio-availability of antipyrine was assumed to be complete as recently demonstrated (Danhof *et al.*, 1982a; Eichelbaum *et al.*, 1982b). The antipyrine clearance to the three different metabolites (CL_{m}) was calculated from: $CL_{\text{sal}} \times f_{\text{mur}}$.

For the statistical analysis the unpaired Student's t -test and linear regression analysis was used.

Results

There were no significant differences between extensive and poor metabolizers of debrisoquine and sparteine in any of the kinetic parameters of antipyrine (Table I). So it would appear that the genes of major effect controlling the polymorphic oxidation of sparteine and debrisoquine do not exert any influence on any of the three oxidative biotransformations of antipyrine.

Table 1 Pharmacokinetic parameters of antipyrine derived from saliva concentrations following an oral dose of antipyrine to 35 volunteers. The urinary recovery of unchanged drug, 4-OH-A, 3-OH-MA and norA was measured during 48 h after the dose. The subjects were subgrouped according to metabolism of debrisoquine and sparteine (PM: poor metabolizer; EM: extensive metabolizer) and to smoking habits (NS: non-smoker; S: smoker). Mean $\pm$ s.d. is given.

Subjects	Antipyrine kinetic parameters			Urinary recovery (% of dose)
	CL_{sal} ($ml\ h^{-1}\ kg^{-1}$)	$t_{1/2}$ (h)	V ($l\ kg^{-1}$)	
All ($n = 35$)	42.4 ± 12.2	10.6 ± 2.5	0.60 ± 0.05	65.4 ± 7.5
EM ($n = 28$)	41.7 ± 10.1	10.7 ± 2.5	0.61 ± 0.05	64.7 ± 6.8
PM ($n = 7$)	45.2 ± 19.3	10.2 ± 3.0	0.59 ± 0.07	68.2 ± 9.8
NS ($n = 24$)	39.4 ± 9.2^a	11.2 ± 2.6^c	0.60 ± 0.05	63.8 ± 5.9^e
S ($n = 11$)	48.9 ± 15.7^a	9.2 ± 1.7^c	0.61 ± 0.06	68.8 ± 9.5^e
EM-NS ($n = 21$)	39.9 ± 9.1^b	11.2 ± 2.5^d	0.61 ± 0.05	63.7 ± 6.1
EM-S ($n = 7$)	47.2 ± 11.7^b	9.1 ± 1.6^d	0.60 ± 0.06	67.4 ± 8.4
PM-NS ($n = 3$)	36.2 ± 11.0	11.2 ± 4.3	0.54 ± 0.02	63.9 ± 5.3
PM-S ($n = 4$)	52.0 ± 22.9	9.5 ± 2.1	0.63 ± 0.07	71.4 ± 12.0

a,b,c,d,e, $P < 0.05$;

The influence of smoking could be examined within phenotypes. There was an increased elimination (increased clearance and shorter half-life) of antipyrine in smokers (S) compared to non-smokers (NS) within the EM group. A similar difference was seen between S and NS within the PM group. These results show an influence of smoking on the pharmacokinetics of antipyrine independent of the polymorphism—an inference corroborated by finding no significant difference between either EM-NS and PM-NS, or between EM-S and PM-S.

There was no significant difference in volume of distribution of antipyrine between any of the subgroups (Table I).

The recovery of antipyrine and its 3 metabolites 4-OH-A, 3-OH-MA and nor-A in urine collected during 48 h after the dose was slightly but significantly higher ($P < 0.05$) in smokers ($68.8 \pm 9.5\%$) than in non-smokers ($63.8 \pm 5.9\%$).

Correlations between the 3 separate metabolic clearances of antipyrine are shown in Figure 2. The clearance to 3-OH-MA correlated to the clearance to both 4-OH-A ($r = 0.83$) and nor-A ($r = 0.79$). The coefficient of correlation between the clearance to 4-OH-A and nor-A was lower ($r = 0.70$) but still highly significant ($P < 0.001$).

Clearances of antipyrine to its 3 metabolites were not significantly different between the 2 phenotypes

Figure 2 Relationships between the clearances to 4-OH-A, 3-OH-MA and norA ($ml\ h^{-1}\ kg^{-1}$) in EM (circles) and PM (squares). Smokers are represented by filled symbols and non-smokers by open symbols. Correlations were calculated using all subjects ($n = 35$). All three correlations are significant ($P < 0.001$).

Table 2 Clearance ($\text{ml h}^{-1} \text{kg}^{-1}$) to the three metabolites of antipyrine calculated from total antipyrine clearance $\times$ fraction of the dose excreted as metabolite in urine. Abbreviations as in Table 1.

Subjects	4-OH-A	3-OH-MA	norA
All ($n = 35$)	12.6 ± 4.6	6.6 ± 2.1	8.6 ± 3.4
EM ($n = 28$)	12.4 ± 3.9	5.3 ± 1.7	8.2 ± 3.0
PM ($n = 7$)	13.8 ± 7.2	6.5 ± 3.4	9.9 ± 4.6
NS ($n = 24$)	11.3 ± 3.3^a	4.8 ± 1.3^a	8.0 ± 3.3
S ($n = 11$)	15.6 ± 5.8^a	7.3 ± 2.5^c	9.8 ± 3.4
EM-NS ($n = 21$)	11.6 ± 3.4^b	4.8 ± 1.3^d	7.9 ± 3.2
EM-S ($n = 7$)	14.7 ± 4.4^b	6.9 ± 1.6^d	9.0 ± 2.2
PM-NS ($n = 3$)	9.5 ± 1.8	4.5 ± 1.5	8.3 ± 4.5
PM-S ($n = 4$)	17.1 ± 8.2	8.1 ± 3.8	11.1 ± 5.0

^b $P < 0.05$; ^{a,d} $P < 0.01$; ^c $P < 0.001$

(Table 2). Within the group of EM no correlation between the metabolic ratio of debrisoquine and sparteine and the formation of metabolites of antipyrine was observed.

As shown in Table 2, S had a significantly higher clearance than NS by formation of all three metabolites within phenotypes. This achieved statistical significance within EM for 4-OH-A and 3-OH-A.

Discussion

This study clearly shows that the genes of major effect which control the polymorphic metabolism of debrisoquine and sparteine do not control the three oxidative pathways of antipyrine shown in Figure 1. These results corroborate those of Danhof *et al.* (1981).

The study also confirms a fact previously known (Vestal *et al.*, 1975), *viz.* smoking induces the metabolism of antipyrine as indicated by the pharmacokinetics of the parent compound.

From an *in vivo* and *in vitro* comparison of the

three major metabolic pathways of antipyrine, Boobis *et al.* (1981) concluded that the *in vivo* metabolic clearances provide a useful measure of the activity of those enzymes involved in their production. Clearances to 3-OH-MA and 4-OH-A were significantly increased in smokers (Table 2), while the clearance by demethylation to nor-A was not significantly increased, although there was a trend. The strong relationship between the clearances to 3-OH-MA and 4-OH-A in the present material (Figure 2; $r = 0.83$) and that of Danhof (1980) ($r = 0.815$; $n = 63$) indicates that the hydroxylations in 4-position and on the 3-methyl group might be performed to a large extent by one isoenzyme of the cytochrome P-450 system. The *N*-demethylation to norantipyrine seems to be performed by a different entity, because (a) rifampicin (Toverud *et al.*, 1981), antipyrine and pentobarbitone (Danhof *et al.*, 1982b) induce the demethylation of antipyrine to a higher degree than the two hydroxylations of antipyrine; (b) the induction by smoking is less pronounced on this metabolic pathway than on the other two pathways; (c) the correlation between the clearance by demethylation and the clearance by the two hydroxylations are less pronounced than between the 4-OH-A and 3-OH-MA (Figure 2 and especially shown by Danhof, 1980).

It can thus be concluded that the regulation of the polymorphic oxidations of debrisoquine and sparteine differs from the regulation of the three oxidative reactions of antipyrine. Most probably different cytochrome P-450 isoenzymes are involved. It also seems possible that the formation of 4-OH-A and 3-OH-MA is performed to a large extent by one enzyme, which might be different from the antipyrine *N*-demethylase.

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