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Role of Oxidation Polymorphism on Blood and Urine Concentrations of Amitriptyline and Its Metabolites in Man

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Summary. We have measured the metabolites (demethylated and hydroxylated) of amitriptyline in a group of seven normal volunteers. They were phenotyped as extensive or poor metabolizers using debrisoquine and bufuralol. The results demonstrate that the oxidative metabolism (aliphatic hydroxylation) of amitriptyline is under the same genetic control as that of debrisoquine and bufuralol.

However, phenotypic polymorphism cannot be used to predict amitriptyline blood concentration after a single oral dose, since the principal metabolic pathway of amitriptyline is demethylation and not aliphatic hydroxylation.

Key words: Amitriptyline – Nortriptyline – Hydroxylated metabolites – Oxidation polymorphism – Hydroxylation polymorphism – Pharmacogenetics – Interindividual differences – Pharmacokinetics

Introduction

It is well known that blood concentrations of drugs extensively metabolized in the liver, such as amitriptyline (AT), vary markedly between individuals (Baldessarini 1980). This is usually explained by "inter-individual differences" in the capacity of the liver to metabolize these drugs on their first-pass, as well as after their systemic distribution. Until recently, the role of genetic factors on the oxidative metabolism of drugs concerned only rare compounds. In recent years it has, however, been reported that hydroxylation may also be under genetic control. It is now believed that polymorphic hydroxylation of debrisoquine (Mahgoub 1977) is representative of the same pharmacogenetic entity as the one which controls the hydroxylation of sparteine (Eichelbaum et al. 1979), guanoxan (Sloan et al.

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1978), phenacetin (Sloan et al. 1978), and numerous other compounds (Eichelbaum 1982) including β-adrenoreceptor blocking agents (Alvan et al. 1982, Dayer et al. 1982a, b) and tricyclic antidepressant drugs such as nortriptyline (Mellström et al. 1981) and imipramine (Potter et al. 1982).

Since AT is also metabolized by aliphatic hydroxylation to 10-hydroxy-amitriptyline (HO-AT) (Fig. 1), and since it is possible that the same oxidative enzymes may play a role in the demethylation of AT to nortriptyline (NT), we have investigated the possible role of genetic polymorphism on the disposition of AT in man.

Methods

Subjects

Seven healthy (1 F and 6 M) volunteers, ranging in age from 22 to 29 years took part in the study. Their weight is given in Table 1, serum and urine biochemistry, hematology and clinical status were all normal. They were phenotyped with bufuralol according to the method proposed by Dayer et al. (1982b). There were three poor metabolizers (Th, K, G) and four extensive metabolizers (A, W, C, Ch), and all gave written informed consent to participate in the study.

Experimental Design

Each subject received 75 mg AT orally as a single dose of the parenteral solution (Laroxyl) with 100 ml of water in the fasting state. A total of 12 blood samples were drawn into E.D.T.A. tubes (Venoject) during the 48 h following the drug administration and stored at −20°C until analysed. Control blood samples were drawn before each administration to detect any substances interfering in the drug assay.

Analytical Procedure

Amitriptyline, a tertiary amine, is metabolized by N-demethylation and benzylic 10-hydroxylation, whereas the demethyl metabolite, NT, is primarily metabolized by 10-hydroxylation (Fig. 1). A sensitive and specific HPLC method was developed which was able to simultaneously measure these four substances in blood. The assay is a three-step extraction procedure: extraction into heptane at a basic pH, back extraction into an acidic aqueous phase and re-extraction into heptane at a basic pH. The internal standard is 4-methyl-propranolol.

The chromatography was performed on a Varian 5000 instrument equipped with a variable-wavelength UV detector at 240 nm. The stainless steel column (30 cm × 4 mm) was filled either with Micropack-MCH-10 (10μm) or Microbondapak-C-18. The mobile phase was a gradient of 25% CH_3CN, 75% KH_2PO_4 (pH = 3) at the beginning, increasing gradually to 55% CH_3CN, 45% KH_2PO_4 after 7 min with a flow rate of 2.3 ml/min. The lower limit of sensitivity was approximately 2 ng/ml. Based on the assay of four identical samples containing 10, 20, 40, 80 and 100 ng/ml of AT and its metabolites the coefficient of variation ranged from 15% to 18% for 10 ng/ml and from 4% to 7% for 100 ng/ml.

Pharamacokinetic Calculations

The apparent half-life of elimination ($t_{1/2}$) of AT was calculated by log-linear regression after visual inspection of the individual plots in order to determine the elimination phase. The $t_{1/2}$ of NT, HO-AT and HO-NT were not calculated since in this situation it was not possible to clearly distinguish the formation phase from the true elimination phase (Balant and McAinsh

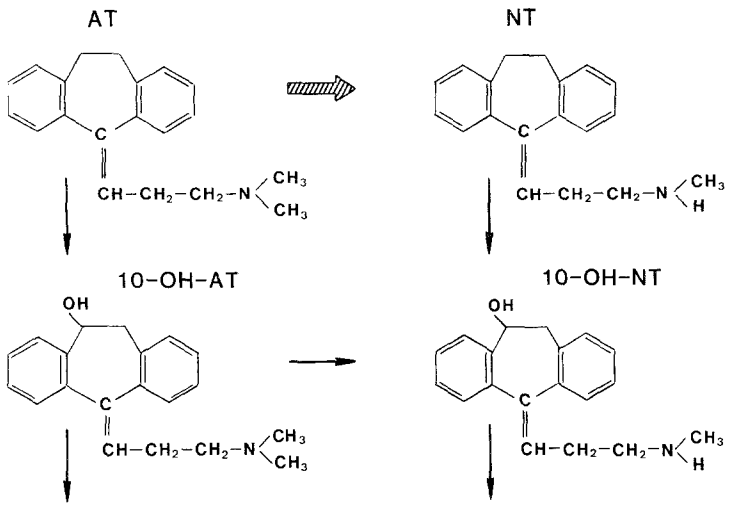

Fig. 1. Main metabolic pathways for amitriptyline

1980) The areas under the blood concentration-time curves (AUC) were calculated to the last experimental point by the linear-trapezoidal method; the AUC (AT) was extrapolated to infinity using the value of the elimination rate constant. The renal clearance was calculated by dividing the amount of drug or metabolite excreted in urine by the corresponding area under the curve.

Other pharmacokinetic parameters such as systemic availability *(F)*, volume of distribution (V_d), average steady state concentrations ($C_{ss,av}$) were calculated according the clearance concept of Rowland and Tozer (1980) with the assumptions classical for this pharmacokinetic approach (Rowland et al. 1973; Pang and Rowland 1978). The metabolic ratio calculated in urine is defined as the ratio obtained when the total (i.e. conjugated and unconjugated) amount of "parent compound" (i.e. AT or NT) excreted in the urine is divided by the amount of metabolite (i.e. HO-AT or HO-NT) excreted during the same sampling period. The metabolic ratio does not imply any assumption about reaction mechanisms.

No statistical methods such as *t*-tests were used in view of the small number of subjects involved and the a priori non-Gaussian distribution of the pharmacokinetic parameters due to the choice of our study population. Regression analysis was not performed since the choice of our population was deliberately made in order to have two "clusters" of subjects, i.e. extensive and poor metabolizers which artificially favors the production of high correlation coefficients.

Results

The results of our study are summarized in Tables 1 and 2 and in Figs. 2 and 3. It is apparent that the poor metabolizers can be distinguished from the extensive metabolizers by the absence in blood of detectable amounts of HO-AT (Table 1 and Fig. 3) and by a marked reduction of the urinary excretion of HO-AT and HO-NT whereas there are no important differences in the 48 h urinary excretion of AT and NT (Table 2). Basically our results are in agreement with those of Vandel et al. (1982) who measured steady state urinary excretion of AT and its metabolites.

If the behaviour of the parent compound (AT) in blood is considered, the situation is more complex since two out of four extensive metabolizers (C and

Table 1. Basic pharmacokinetic parameters derived from blood concentrations

Volunteer		Weight [kg]	Debrisoquine[a]	Bufuralol[b]	AT		AUC_0^{48} [ng · ml^{-1} · h]			
					CL_0^c [l · h^{-1}]	$t_{1/2}$ [h]	AT	HO-AT[d]	NT	HO-NT
A	●	72	0.15	0.30	272	18.1	218	96	499	585
W	■	67	0.20	0.73	260	9.6	278	58	547	785
C	▲	65	0.22	0.61	83	16.5	789	50	280	–
Ch	✦	65	0.89	2.64	88	20.1	667	99	716	728
T[e]	○	88	0.56	4.30	86	21.9	672	–	400	–
K♀	□	58	>40	11.7	61	24.3	950	–	956	–
G	▽	78	15.5	11.8	90	14.0	759	–	821	–

[a] Debrisoquine urinary metabolic ratio, antimode at 12.6
[b] Bufuralol plasma metabolic ratio, antimode at 3.55
[c] Calculated on the basis of AUC_0^∞
[d] Extrapolated from 11 to 48 h
[e] Subject presenting a "linkage desequilibrium" (see text)

Table 2. Urinary excretion of AT and its metabolites (in % of dose) 48 h after the oral administration

Volunteer	AT		HO-AT		NT		HO-NT		AT/HO-AT	NT/HO-NT	AT/NT
	Unconjugated	Total	Unconjugated	Total	Unconjugated	Total	Unconjugated	Total	M.R.[a]	M.R.[a]	M.R.[a]
A	0.21	2.4	0.93	19.5	0.49	0.72	18.7	57.2	0.12	0.013	3.34
W	0.41	2.3	0.22	8.2	0.59	1.4	19.3	46.3	0.29	0.029	1.71
C	0.48	3.0	0.78	9.9	0.53	1.1	16.5	47.8	0.30	0.023	2.73
Ch	0.48	1.9	0.99	8.1	0.89	1.8	16.9	50.8	0.23	0.036	1.04
T	0.46	5.4	0.16	5.3	0.63	0.62	2.7	12.1	1.01	0.051	8.63
K	0.56	2.6	0.31	2.6	0.62	1.5	4.6	14.2	1.03	0.11	1.77
G	0.32	2.7	–	2.1	0.81	0.98	2.3	6.6	1.31	0.15	2.73

[a] Metabolic ratio calculated on total amounts excreted (i.e. conjugated and non-conjugated)
Note: the differences found in urinary excretion of AT, HO-AT, NT and HO-NT are not resulting from differences of their renal clearance

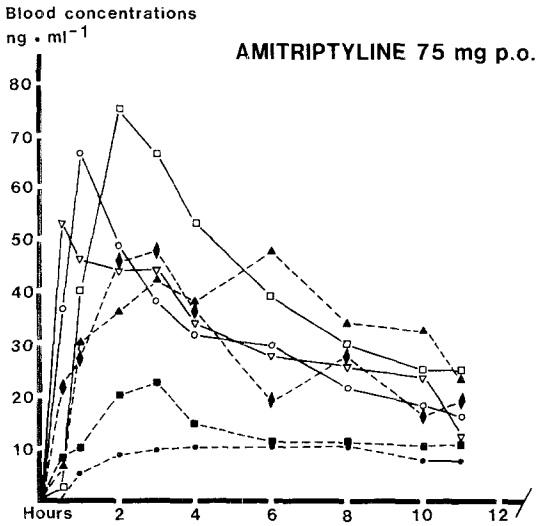

Fig. 2. Early blood concentrations of AT after its oral administration to seven healthy volunteers. The symbols are indicative of extensive (●■▲✦———) or poor (○□▽———) hydroxylators. Blood concentrations were also measured at 24, 36 and 48 h. During the later hours the two groups of subjects cannot be distinguished

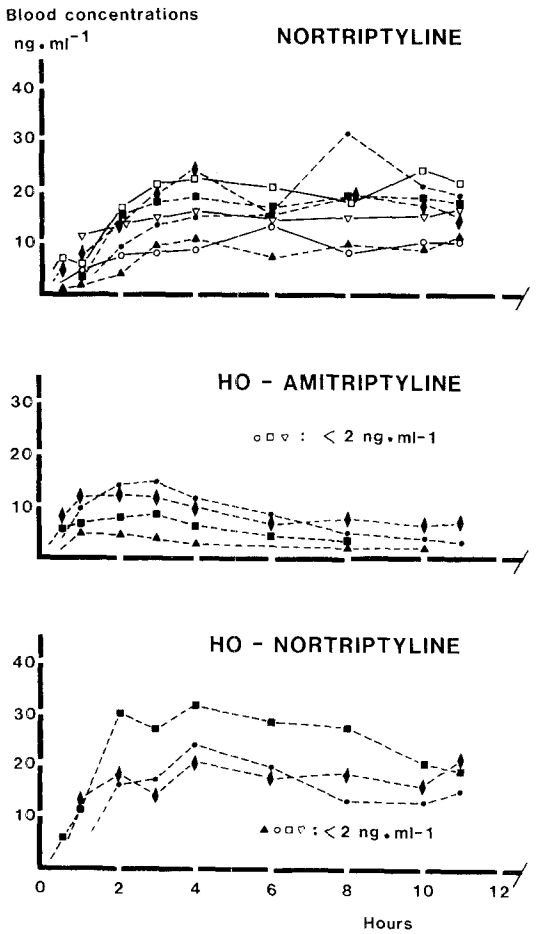

Fig. 3. Early blood concentrations of the main metabolites of AT. The symbols are the same as in Fig. 2. From 24 to 48 h it is still possible to measure NT and HO-NT in blood whereas the concentrations of HO-AT are below the detection limit of the assay

Ch) have a blood concentration profile close to the values observed in poor metabolizers, whereas the two other volunteers (A and W) have markedly lower blood concentrations (Fig. 2). The same difference may also be seen for the apparent oral clearance (Table 1) whereas there are no important differences between all the subjects as far as the $t_{1/2}$ of AT is concerned.

There are also no noticeable differences between our volunteers if the blood concentrations of NT are analysed (Fig. 3).

Discussion

Metabolic Ratios

Dayer et al. (1982) found that approximately 10% of the Swiss population were of the poor hydroxylator type for bufuralol. Similar results have been reported for debrisoquine by Dick et al. (1982). It has also been shown that phenotyping with these two substances usually correlates well (Dayer et al. 1982b). In the present study, the metabolic ratios for debrisoquine, bufuralol and AT are in agreement in six out of seven subjects (Tables 1 and 2). The exception is volunteer T: he is an extensive metabolizer of debrisoquine (Table 1), a poor hydroxylator of AT (Table 2) and bufuralol (Table 1). This subject is probably representative of the "linkage desequilibrium" as defined by Nebert (1981). This term is used to characterize individuals who are either extensive metabolizers for one substrate and poor metabolizers for another substance. This "linkage desequilibrium" occurred with a frequency of approximately 3% among a British population of 212 patients examined for their capacity to metabolize debrisoquine and sparteine.

The influence of the hydroxylation phenotype on the N-demethylation of AT to NT can probably not be analyzed on the basis of the present results, as was discussed by Rollins et al. (1980). It seems nevertheless that there is no direct relation between the two metabolic pathways in the case of AT (Table 2).

From our observations it can thus be concluded that the behaviour of AT in man is under the same genetic control as the enzymatic systems which govern the oxidation of other drugs for which this elimination pathways has been reported. The overall blood concentration pattern of AT is, however, not directly correlated to this phenotype since N-demethylation of AT to NT is the major pathway of elimination of this drug.

Pharmacokinetic Considerations

For a drug such as AT, which is only given over prolonged periods of time, the concentrations measured at steady state are of more clinical interest than the concentrations measured after a single dose. It is, however, well known that if one knows the pharmacokinetic parameters of a drug in a given subject, it is possible to estimate the drug concentrations at steady state. If we use this approach, we can predict that the steady state concentrations of patients who would show the same kinetic profile as our volunteers A and W would have steady state AT concentrations approximately three times lower than patients with the profile of the other subjects.

It is also possible to show that these differences are not the consequence of differences in the renal clearance of these compounds, but that both the values of the systemic availability (i.e. first-pass hepatic metabolism) and of the systemic clearance (i.e. liver metabolism after the distribution of the drug in the body) are of importance in this respect. Surprisingly, the two volunteers C and Ch are closer to the poor than to the extensive hydroxylators. This is probably also the consequence of N-demethylation being the most important pathway of AT elimination in man.

Clinical Consequences

The clinical relevance of our findings remain an open question if the behaviour of AT and NT after the oral administration of AT is considered. Our results show that in some extensive metabolizers, relatively low blood concentrations of AT may be observed, whereas in others, comparable AT blood profiles are seen as in poor oxidizers. In addition, the specific phenotype seems to have little importance on the blood concentrations of NT.

It would thus seem that when AT is to be administered orally, the determination of the phenotype of the patient might be of limited clinical value for predicting the steady state concentrations of AT and NT. This statement may, however, be reevaluated if it were found that the 10-hydroxylated metabolites of AT or NT have specific clinical effects not exhibited by the parent molecules and that the 10-hydroxylated compounds participate in the clinical effect of AT.

Conclusions

From the present results, it appears that for the time being, drug level monitoring is probably a better method of reaching safe steady state concentrations during AT therapy than phenotyping. Further population studies are, however, necessary to reach definitive conclusions.

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