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DRUG METABOLISM IN EPILEPTICS: *IN VIVO* AND *IN VITRO* CORRELATIONS

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1 The effect of inducing drug therapy on the relationship between *in vitro* (cytochrome P-450 content) and *in vivo* (antipyrine kinetics) was investigated by comparing eleven consecutively treated epileptics with two groups of controls, eleven subjects with normal liver histology and eleven disease matched non-epileptics, all underwent diagnostic liver biopsy.

2 The epileptics had significantly higher cytochrome P-450 level in biopsies and they also metabolized antipyrine faster than the controls.

3 Decrease in antipyrine half-life in epileptics was related with alterations in liver histology, whereas the level of cytochrome P-450 was not.

4 There was a linear relationship between these two indices of enzyme induction when regressed on logarithmic data.

Introduction

The use of anticonvulsants is commonly associated with increased ability of the liver to metabolize foreign compounds (Conney, 1967). Increased rate of drug clearance in patients on antiepileptic drugs have been reported (Petruch, Schuppel & Steinhilber, 1974; Welch, DeAngelis, Wingfield & Farmer, 1975; Neuvonen, Penttilä, Lehtovaara & Aho, 1975; Stevenson, O'Malley & Shepherd, 1976). Billing & Black (1971) demonstrated a significant rise in the microsomal cytochrome P-450 content in biopsies of subjects treated with phenobarbitone for a week before abdominal surgery, and Remmer, Schoene, Fleischmann & Held (1973) showed increased activities of drug-metabolizing enzymes in liver biopsy of a female epileptic treated with phenytoin and phenobarbitone. This suggests a relationship between liver enzyme activity and drug clearance in patients on antiepileptic therapy.

We compared the *in vivo* and *in vitro* parameters of drug metabolism in a group of epileptics submitted for diagnostic liver biopsy with the findings of two groups of controls, one with normal liver architecture and the other with liver changes like those found in the group of epileptics. Antipyrine was chosen as a model drug since it is completely absorbed when given orally, is extensively metabolized in the liver, is little bound to plasma proteins (Brodie & Axelrod, 1959) and is relatively safe (Sotaniemi, Kontturi & Larmi, 1973). Hence, the rate of antipyrine elimination reflects closely changes in the hepatic capacity to metabolize the drug. Cytochrome P-450 content, determined in liver biopsies, was used as an index of microsomal drug hydroxylation (Conney, 1967).

Methods

Subjects

Eleven consecutively treated epileptics (two females and nine males) undergoing diagnostic liver biopsy were investigated. Relevant clinical and biochemical data are given in Table 1. Some cases (Numbers 6, 8 and 11) were treated with digoxin and thiazides. During the investigation none of the patients had any sign of cardiac failure and their kidney function, judged by creatinine clearance was normal.

Two groups of control subjects were investigated. 'Normal controls' consisted of eleven non-epileptics (five females and six males), with the mean age of 41.2 years (range 27–54 years). They were referred to our hospital for diagnostic purposes; on admission some of them had slightly disturbed liver function tests, like a small elevation in the serum transferase or alkaline phosphatase levels but none of them was icteric. All the cases had normal findings in liver histology. 'Patient controls' consisted of eleven age and sex matched non-epileptics, whose histological findings in biopsies were closely resembling those of the group of epileptics. They were selected from our biopsy

Table 1 Clinical data and plasma antipyrine half-life and cytochrome P-450 in eleven epileptics

Patients	Age (years)	Sex	Body weight (kg)	Liver* histology	Abnormal** liver function tests	Anti-convulsants***	Antipyrine $T_{\frac{1}{2}}$ (h)	Cytochrome P-450 (nmol/g)
1	53,	M	56	N	–	DPH	2.3	16.48
2	54,	M	62	N	AP	DPH	2.8	20.33
3	43,	M	51	FL	ALAT, ASAT	DPH+CARB	3.7	17.14
4	43,	M	58	H	AP, ASAT	DPH+PB	3.9	11.54
5	40,	M	78,5	H	BIL, ASAT, ALAT	DPH	4.1	32.71
6	50,	M	55	C	AP, ALAT	DPH	5.0	18.68
7	24,	F	64	H	AP, ALAT	DPH	5.9	12.64
8	56,	M	77	H	AP, ALAT	DPH	6.0	11.00
9	39,	M	100	H+FL	–	DPH	6.2	6.00
10	58,	F	69	H	AP, ALAT	DPH+CARB	7.8	8.37
11	62,	M	94	C	BIL, ASAT, ALAT	DPH	9.2	12.75

* N = normal liver, FL = fatty liver, H = hepatitis, C = cirrhosis
** AP = alkaline phosphatase, BIL = bilirubin (total), ASAT = asparate aminotransferase, ALAT = alanine aminotransferase.
*** DPH = phenytoin, CARB = carbamazepine, PB = phenobarbitone.

material of about 170 consecutive patients with diagnostic liver biopsy merely on the basis of the histological findings. Also, their smoking and drinking habits were closely equal to that obtained among the group of epileptics.

Protocol

All subjects were investigated at the Clinical Research Unit. Blood samples for liver function tests were drawn after an overnight fasting. Three to four hours later the percutaneous liver biopsy was performed with a Thrucut needle. The biopsy material was divided into two parts: one fixed in formalin for histological examination and the rest was used for determination of cytochrome P-450. On the following day, again after an overnight fast, antipyrine (20 mg/kg) in loganberry juice was administered to each patient. Blood samples were obtained by venepuncture at zero time and 1, 3, 6, 9, 12, 24, 48 and in some cases 72 h after the administration of the drug. Informed consent was obtained before the antipyrine test was performed.

Liver histology

The preparation of the biopsy material for histological examinations was performed according to the method described by Ahlqvist (1970). On the basis of the histological findings the patients were divided into four subgroups, namely, those with normal liver structure, fatty liver, hepatitis and cirrhosis.

Determination of cytochrome P-450

The cytochrome P-450 content in the whole homogenate of the biopsy material was determined according to the method of Greim, Schenkman, Klotzbucher & Remmer (1970).

Antipyrine assay

The concentration of antipyrine in plasma was performed by a gas liquid chromatographic method (Prescott, Adjepon-Yamoak & Roberts, 1973).

Calculations

The plasma half-life of antipyrine ($T_{\frac{1}{2}}$) was read from the linear part of the time concentration curve on a semilog graph. The area under the plasma drug concentration curve (AUC) was calculated by the trapezoidal rule, and the area to infinite time was added by integration (C_t/k), where C_t is the last antipyrine concentration, and k, the elimination rate constant is calculated from the equation

$$k = \frac{0.693}{T_{\frac{1}{2}}}$$

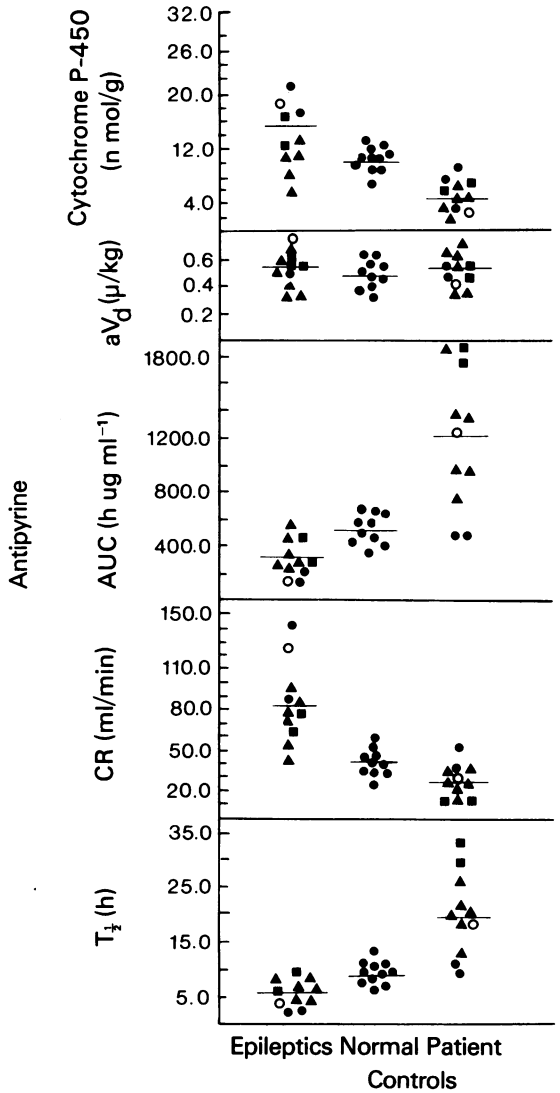

Figure 1 Individual values of plasma antipyrine half-life ($T_{\frac{1}{2}}$), clearance (CR), area under the plasma concentration curve (AUC), apparent volume of distribution (aV_d) and cytochrome P-450 (P-450) in epileptics and controls. ● normal liver; ○ fatty liver; hepatitis; ■ cirrhosis.

The apparent clearance (CR) was calculated from the equation

$$CR = \frac{D}{AUC}$$

where D is the oral dose of antipyrine. The apparent volume of distribution (aV_d) was calculated from the equation

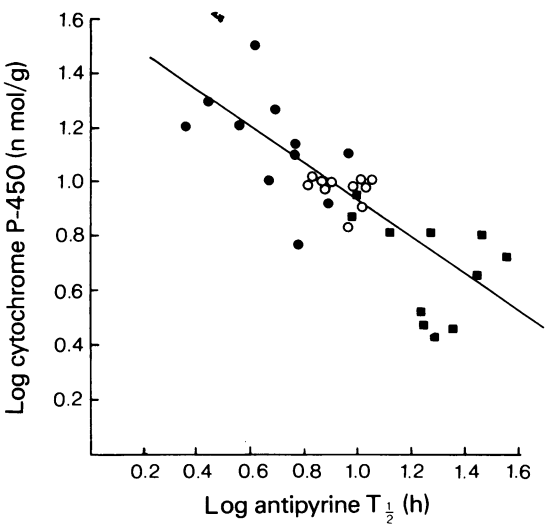

Figure 2 Relationship between cytochrome P-450 and plasma antipyrine half-life in epileptics (●) and controls (normal ●; patient ■). $YEST = EXP(-0.67 \times + 1.60, r = -0.79, P < 0.001)$.

$$aV_d = \frac{D}{AUC \times k}$$

Statistical analysis of the data was performed by Student's *t*-test and regression analysis.

Results

Table 1 lists the clinical and biochemical data of the epileptics. Figure 1 shows the individual values obtained from drug metabolism studies and the summarized results are given in Table 2. From these results it is clear that the mean cytochrome P-450 content in biopsies of epileptics was significantly higher, and the elimination rate of antipyrine significantly faster than in the control groups.

Table 1 demonstrates that the prolongation of antipyrine half-life in the epileptics was related to the changes in liver histology; patients with normal or fatty liver (cases 1–3) had a half-life 2.8 h (S.D. 0.7) as compared with 6.0 h (1.8) in those with hepatitis and cirrhosis ($P < 0.02$). The corresponding values for cytochrome P-450 levels, 17.9 nmol/g (2.0) and 14.2 nmol/g (8.3), however, indicate no significant difference between the patients ($P > 0.05$).

Figure 2 shows the relationship between the plasma antipyrine half-life and cytochrome P-450 content in biopsies and Table 3 gives the values of correlation coefficient (r) between the variables in the epileptics and the controls. As demonstrated, the fit between the logarithms of the variables was better than that between the original data. Also, the relationship

Table 2 Antipyrine kinetics and cytochrome P-450 contents in epileptics and controls

	Antipyrine				
Subjects	$T_{\frac{1}{2}}$ *(h)*	*CR (ml/min)*	*AUC (h μg ml^{-1})*	*aV$_d$/kg (l/kg)*	*Cytochrome P-450 (nmol/g)*
Epileptics					
Mean	5.8	82.2	311.3	0.54	15.2
s.d.	2.2	28.3	143.9	0.14	7.3
Normal controls					
Mean	8.8*	42.6	514.1*	0.48	10.7**
s.d.	1.9	11.5	119.1	0.09	1.7
Patient controls					
Mean	19.6*	27.7*	1204.4*	0.52	5.4*
s.d.	7.7	10.1	480.7	0.10	2.2

P-values for difference between means: * = 0.001, ** = 0.05
CR = apparent clearance rate, AUC = area under the plasma level curve, aV_d = apparent volume of distribution.

between the variables was better when epileptics were compared with patient controls than with normal controls, or only among themselves.

Discussion

Substrate interaction with cytochrome P-450 is assumed to be an essential preliminary step for drug oxidation in the liver (Imai & Sato, 1966; Remmer, Schenkman, Estabrook, Sasame, Gillette, Narasimhulu, Cooper & Rosenthal, 1966). Hence some correlations might be expected between drug kinetics and microsomal enzyme activities. Studies in animals, have in fact demonstrated relationship between antipyrine half-life and activities of drug-metabolizing enzymes in the liver, like antipyrine hydroxylase (Statland, Astrup, Black & Oxholm, 1973) and ethylmorphine N-demethylase (Vesell, Lee, Passananti & Shively, 1973). In man, the present status is confusing since either no or poor correlation (May, Helmsteaedt, Bustegens & McLean, 1974; Thorgeirsson, 1971; Davies, Thorgeirsson, Breckenridge & Orme, 1973) or a significant relationship (Remmer *et al.*, 1973) between *in vivo* and *in vitro* drug metabolism have been reported. The present results as well as our previous studies (Sotaniemi, Ahlqvist, Pelkonen, Pirttiaho & Luoma, 1977) clearly demonstrate the necessity to consider two independent factors, namely the alterations in liver parenchyma and the role of exposure to inducing agents when investigating drug metabolism in man. The compounds with enzyme inducing properties enhance the microsomal enzyme activity in the liver, but parenchymal changes, like fatty accumulation, amount of fibrous tissue or inflammatory process might interfere with the amount and activity of the enzyme system (Schoene, Fleishman, Remmer & von Olderhausen, 1972; Sotaniemi *et al.*, 1977) and the microsomal drug hydroxylation changes correspondingly. Furthermore, the liver parenchymal alterations also influence the drug uptake and metabolism in the liver by changing hepatic blood flow, availability of NADPH, drug penetration through the cell membranes or liver size (Wilkinson & Schenker, 1976; Remmer *et al.*, 1973; Pirttiaho, Sotaniemi, Pelkonen, Ahlqvist & Pitkänen, unpublished observations). Both, the changes in enzyme activities and in drug kinetics occur independently and the relationship between the variables is linear only in subjects with similar liver histology and with same

Table 3 Comparison of the correlation coefficients (*r*) of antipyrine half-life (h) and cytochrome P-450 (nmol/g) in epileptics and controls

Subjects	*Antipyrine* $T_{\frac{1}{2}}$ *Cytochrome P-450*	*Log antipyrine* $T_{\frac{1}{2}}$ *Log cytochrome P-450*
Epileptics	0.498	0.549
Epileptics and normal controls	0.558	0.624
Epileptics and patient controls	0.645	0.783

habits, like chronic alcoholics with fatty liver (Sotaniemi *et al.*, 1977) but non-linear in random sample of patients with different liver changes even though the drug therapy was identical, as in this study.

In man, the activity of drug-metabolizing enzymes in the liver can be assayed directly from samples taken during surgery or by percutaneous needle biopsy (Alvares, Schilling, Levine, Kuntzman, Brand & Mark, 1969; Billing & Black, 1971; Thorgeirsson, 1971; Schoene *et al.*, 1972; May *et al.*, 1974). We utilized a Thrucut needle for percutaneous biopsy obtaining enough material for both histological studies and determination of cytochrome P-450. The contents of cytochrome P-450 were closely equal to those reported by others (Remmer *et al.*, 1973; May *et al.*, 1974) for the normal human liver. Our findings also demonstrate that in some epileptics with cirrhosis the cytochrome P-450 level, and the antipyrine elimination rate, might be about the level obtained in our normal controls and markedly higher than in other cirrhotic patients without inducive drug therapy. This suggests that phenobarbitone or phenytoin may be a suitable drug to enhance drug oxidation in liver of subjects with impaired liver metabolism.

A major problem when investigating drug metabolism in drug treated patients is to find a suitable control material. To overcome the problem we compared epileptics with the two groups of controls and also classified the patients on the basis of their liver changes. The findings demonstrate that the picture we might receive is largely dependent on the selection of the control material. Comparison with healthy normals is always important, but it need not necessarily inform us the effect of disease process on the drug metabolism in man. The findings suggest that in evaluation of drug metabolism in certain patient groups the subjects should be classified on the basis of the disease process. Such a study might also give useful data also for practising doctors when prescribing drug for their patients. Our study also indicates that when evaluating relationship between *in vivo* and *in vitro* drug metabolism in man the studies should be carried out on the same subjects, otherwise difficulties may arise when interpreting the results.

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