THE PLASMA HALF-LIFE OF ANTIPYRINE IN CHRONIC URAEMIC AND NORMAL SUBJECTS

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1 Antipyrine was given intravenously in a dose of 18 mg/kg body weight to twelve patients with chronic renal failure (plasma creatinine > 4.9 mg/100 ml) who were not taking drugs and twenty normal subjects.

2 Plasma antipyrine levels were measured by a specific method, the plasma half-life of the drug was determined and used as an index of drug oxidation.

3 The mean ($\pm$ s.d.) plasma antipyrine half-life in patients with chronic renal failure (7.3 ± 2.0 h) was significantly shorter than in normal subjects (13.2 ± 4.3 h; $P < 0.002$). There was no difference in the apparent volume of distribution of antipyrine between the two groups ($P > 0.6$).

4 Pretreatment of five patients with chronic renal failure and seven normal subjects with antipyrine or phenobarbitone for two weeks significantly shortened the mean plasma antipyrine half-life from 7.4 ± 2.5 h to 5.0 ± 1.5 h in uraemics ($P < 0.005$) and from 13.2 ± 4.5 h to 6.9 ± 1.5 h in normal subjects ($P < 0.0025$).

5 These results suggest that oxidation of antipyrine by hepatic microsomal enzymes is increased in patients with chronic renal failure, but a state of maximal induction of these enzymes was not observed. The clinical implication of this finding with regard to the association between liver microsomal enzyme induction and vitamin D resistant osteomalacia is discussed.

Introduction

There are few published studies of drug metabolism in patients with chronic renal failure. Letteri, Mellk, Louis, Kutt, Durante & Glazko (1971) showed that the plasma half-life of phenytoin is considerably shortened in chronic renal failure and Rasmussen, Jervell, Storstein & Gjerdrum (1972) found that the plasma half-life of digitoxin was reduced in some patients with chronic uraemia. The explanation for these observations remains obscure. Since phenytoin and digitoxin are bound to plasma proteins it is possible that reduced protein binding that occurs with both these drugs in chronic uraemia makes them more rapidly available for oxidation by hepatic microsomal enzymes (Reidenberg, Odar-Cederlöf, Von Baher, Borga & Sjoqvist, 1971; Shoeman & Azarnoff, 1972). Alternatively, it is possible that the activity of the hepatic microsomal enzymes is increased in chronic uraemia. The present study was undertaken to determine whether there is increased hepatic microsomal enzyme activity in patients with chronic uraemia. This was done by measuring the plasma antipyrine half-life of patients with chronic renal failure and of normal control subjects. Antipyrine is exten-

sively oxidized by the liver (Brodie & Axelrod, 1950; Yoshimura, Shimeno & Tsukamoto, 1968) and is only 10% bound to plasma proteins (Soberman, Brodie, Levy, Axelrod, Hollander & Steele, 1949); it is distributed throughout body water and less than 5% of the dose is excreted in the urine in 24 h (Brodie & Axelrod, 1950). For these reasons, the plasma half-life of antipyrine is widely used as an index of drug oxidation (Vessel & Page, 1969; Kolmodin, Azarnoff & Sjoqvist, 1969; Breckenridge, Orme, Thorgeirsson, Davies & Brooks, 1971; O'Malley, Stevenson & Crooks, 1972).

Methods

Twelve patients (seven male and five female) with chronic renal failure in the age range 14 to 49 (mean 32.3) years were studied. Clinical details are shown in Table 1. Plasma creatinine in these patients ranged from 4.9 to 26.0 (mean 14.3) mg/100 ml. Eight patients were being treated with chronic haemodialysis and the other four patients had never received any form of dialysis. No drugs

had been taken by six patients (Nos. 5, 8, 9, 10, 11 and 12) for several months. The other six patients had stopped taking drugs two weeks before the study began; their drug treatment is shown in Table 1. None of the drugs taken by these patients are known enzyme inducers (Conney, 1967; Kock-Weser & Sellers, 1971). Dietary protein was restricted to 40 g daily in all patients except No. 6 who was on a normal diet.

The haemoglobin of these patients ranged from 5.1 to 9.3 (mean 7.3) g/100 ml. Plasma bilirubin and transaminases were normal in all patients although the plasma alkaline phosphatase was raised in six patients (Nos. 3, 4, 6, 7, 11 and 12) presumably due to renal osteodystrophy.

Twenty normal subjects (thirteen male and seven female) aged between 16-60 (mean 28.2) years were studied. Plasma creatinine, haemoglobin and liver function tests were normal in all control subjects.

Informed consent for this investigation was obtained from patients and controls.

Because of the possibility of poor gut absorption in uraemia, antipyrine was given by intravenous injection. A standard dose of 18 mg/kg body weight was given intravenously at 09.00 h over a period of 10 min since more rapid injection was associated with pain in the arm, faintness and nausea. Patients did not receive any form of dialysis during the 12 h after the intravenous dose of antipyrine. Four heparinized venous blood specimens were taken at 3-hourly intervals after antipyrine administration. The plasma was separated and samples stored at -20°C until assayed.

The effect of oral administration of phenobarbitone or antipyrine for 2 weeks on the plasma antipyrine half-life was studied in five of the uraemic patients and in seven normal subjects. Both these drugs are known to induce hepatic microsomal enzymes (Vessel & Page, 1969; Breckenridge *et al.*, 1971). Two uraemic patients in hospital (Nos. 8 and 9) were given sodium phenobarbitone 2 mg/kg body weight each day for 2 weeks. Because of the possible danger of sedating uraemic patients on home dialysis, antipyrine was used instead of phenobarbitone in the other three patients (Nos. 10, 11 and 12) in a dosage of 1 g daily for 2 weeks; the seven normal subjects (Nos. 13 to 19) each received 1 g of antipyrine daily for 2 weeks. Three days after the course of 'enzyme inducing' drug, the plasma antipyrine half-life determination was repeated in uraemic and normal subjects.

After the first dose of antipyrine a 24 h urine collection was obtained from patient No. 8 to determine the amount of drug excreted in the urine in chronic renal failure.

Plasma antipyrine levels were assayed by the method of Brodie, Axelrod, Soberman & Levy (1949). In preliminary studies the plasma was deproteinized and antipyrine measured in the supernatant spectrophotometrically. Unfortunately, with this procedure the plasma blank readings were so high with uraemic plasma as to make the method non-specific. Also, in uraemia there was the possibility of retention of antipyrine metabolites that are normally excreted in the urine, which might interfere with the assay of the parent drug. We therefore resorted to extracting

Table 1 Details of patients investigated

Case No.	Age (years)	Sex	Dialysis	Plasma creatinine (mg/100 ml)	Haemoglobin (g/100 ml)	Previous drug therapy
1	49	M	No	15.0	7.5	Clonidine, Nitrazepam
2	21	M	Yes	24.0	7.1	Perphenazine, Propranolol
3	38	F	Yes	7.0	5.2	Metaclopramide, Ampicillin
4	27	F	No	26.0	7.6	Metaclopramide, Methyldopa, Diazepam
5	26	M	Yes	16.0	7.7	None
6	14	F	No	4.9	9.3	Diazepam*, Pethidine*
7	48	F	No	11.1	8.7	Nitrazepam
8	24	M	Yes	12.0/14.0**	7.9	None
9	47	M	Yes	17.5/11.5	6.6	None
10	22	M	Yes	10.7/17.6	5.1	None
11	39	M	Yes	14.5/15.0	7.9	None
12	33	F	Yes	13.0/17.0	7.1	None

* Single dose.

** Values after antipyrine or phenobarbitone administration for 2 weeks.

antipyrine from alkalized plasma using chloroform (Brodie *et al.*, 1949); the uraemic plasma blank reading then became negligible. The efficiency of extraction was 90%. This method was shown to be specific for assay of antipyrine in uraemic plasma by the distribution technique of Brodie & Udenfriend (1945).

Result

The plasma antipyrine half-lives of patients with chronic renal failure and normal subjects are shown in Figure 1. In the 12 uraemic patients the plasma antipyrine half-life was 7.3 ± 2.0 h (mean $\pm$ s.d.) and in the 20 normal subjects 13.2 ± 4.3 hours. The difference between the two groups was statistically significant ($P < 0.002$) using Wilcoxon's sum of ranks test.

After phenobarbitone or antipyrine administration there was shortening of the plasma antipyrine half-life in both uraemic patients and normal subjects (Table 2), and the difference between the means of the two groups became non-significant ($P > 0.05$). In five uraemic patients the mean $\pm$ s.d. plasma antipyrine half-life was reduced from 7.4 ± 2.5 h to 5.0 ± 1.5 hours. The difference between the means was significant ($P < 0.005$). Seven normal subjects had a mean $\pm$ s.d. plasma antipyrine half-life of 13.2 ± 4.5 h before and

Figure 1 Plasma half-life and volume of distribution of antipyrine in normal subjects and in patients with chronic renal failure. Horizontal lines indicate mean values.

6.9 ± 2.5 h after the drug course. The difference between the means was statistically significant ($P < 0.0025$). There was no significant difference in the apparent volume of distribution of antipyrine between the 12 uraemic patients and the 20 normal subjects ($P > 0.6$). Phenobarbitone

Table 2 Plasma half-life and apparent volume of distribution (V_D) of antipyrine in chronic uraemic and normal subjects before and after administration of phenobarbitone or antipyrine as inducing agents.

Subject No.	Plasma antipyrine half-life (h)		V_D (litres)	
	Pre-induction	Post-induction	Pre-induction	Post-induction
Uraemics 8	10.0	7.0	31.4	32.0
9	8.8	5.8	38.4	41.0
10	5.8	3.8	28.0	27.0
11	8.5	5.3	38.4	38.4
12	4.0	3.3	50.5	51.4
Mean	7.4	5.0	37.3	37.9
$\pm$ s.d.	± 2.5	± 1.5	± 8.6	± 9.2
Significance of difference*	$P > 0.005$		$P = NS$	
Controls 13	8.0	5.3	42.8	40.1
14	10.5	4.8	36.7	40.4
15	18.0	9.0	41.4	41.4
16	15.0	6.5	40.5	38.2
17	9.8	7.0	36.5	36.5
18	20.0	8.5	40.4	43.3
19	11.0	7.0	40.6	38.3
Mean	13.2	6.9	39.8	39.7
$\pm$ s.d.	± 4.5	± 2.5	± 2.4	± 2.3
Significance of difference*	$P > 0.003$		$P = NS$	

V_D (litres) = Dose (mg)/Plasma level at zero time (mg/l)

* Paired Student's *t*-test. NS = $P > 0.15$.

or antipyrine administration had no significant effect on the apparent volume of distribution of antipyrine in uraemic patients or normal subjects ($P > 0.15$).

The amount of antipyrine excreted in the urine of a patient with chronic renal failure (No. 8) represented 1% of the dose administered.

Discussion

Antipyrine is extensively oxidized in man to 4-hydroxyantipyrine (Brodie & Axelrod, 1950) and 3-hydroxymethyl-2-methyl-1-phenyl-3-pyrazolin-5-one (Yoshimura *et al.*, 1968) by hepatic microsomal enzymes. Elimination of antipyrine from the blood is mainly due to metabolism, only 5% of a dose is normally excreted in the urine (Brodie & Axelrod, 1950). Similar results were obtained in chronic renal failure by Lichter, Black & Arias (1973) and in patient No. 8 in this study. The plasma half-life ($T_{1/2}$) of antipyrine is related to its hepatic clearance ($\bar{C}$) and its apparent volume of distribution (V_D) by the formula $T_{1/2} = K V_D / \bar{C}$; where K is the elimination constant (Dollery, 1972). Since there was no significant difference between the apparent volume of distribution of antipyrine in patients with chronic renal failure and normal subjects, the diminished plasma antipyrine half-life in uraemic patients suggests that the hepatic clearance of antipyrine is increased. With antipyrine the calculated hepatic clearance of the subjects taking part in this study was low (22-62 ml/min) compared with the normal hepatic blood flow (1500 ml/min; Sherlock, 1963) so that changes in hepatic blood flow are probably not an important factor affecting the hepatic clearance of antipyrine. It appears, therefore, that the diminished plasma antipyrine half-life in chronic renal failure is due to increased metabolism of the drug by hepatic microsomal enzymes.

One could argue that these results might reflect differences in body composition between chronic uraemic and normal subjects, particularly since the drug was given on a body weight basis. Patients with chronic renal failure have less fat and muscle and an increased body water compared with normal subjects of similar weight (Coles, 1972), but this would tend to increase the plasma half-life of antipyrine by increasing its volume of distribution in relation to the body weight.

Increased drug oxidation in chronic renal failure might be produced by induction of hepatic microsomal enzymes, so that the amounts of drug metabolizing enzymes in the liver are increased. Drugs must obviously be considered as possible inducing agents in these patients, although six of them had not taken drugs for several months. The

other six patients had stopped taking drugs 2 weeks before the study and none of these are known enzyme inducers. It thus seems unlikely that increased oxidation of antipyrine in these patients is related to drug therapy. Other possible inducing agents include endogenous substances that accumulate in uraemic plasma or substances found in the diet or water supply such as insecticides (Conney, 1967), particularly since patients on dialysis are exposed to very large volumes of water.

Another possible, but perhaps less likely, explanation for increased oxidation of antipyrine in chronic renal failure is that the amounts of hepatic metabolizing enzymes are normal but the activity of these enzymes is increased by substances that accumulate in uraemic plasma.

Although the plasma half-life of antipyrine is shortened in chronic renal failure it can be further diminished by administration of phenobarbitone or antipyrine for 2 weeks. This suggests that it is possible to induce drug metabolizing hepatic microsomal enzymes in these patients.

Recently, Lichter *et al.* (1973) have reported the plasma antipyrine half-lives of 13 patients with chronic renal failure after oral administration of the drug. They concluded from their study that the oxidative metabolism of antipyrine in these patients was normal or occasionally enhanced. All but two of the patients studied were taking drugs when the plasma antipyrine half-life determinations were made. These included allopurinol which is known to inhibit drug oxidation (Vessel, Passananti & Green, 1970) and might lead to prolongation of the plasma antipyrine half-life. Also, they gave the drug orally and there is the possibility of malabsorption in chronic renal failure. Their results, therefore, might not be a true reflection of antipyrine metabolism in chronic renal failure.

Finally, the increased hepatic drug oxidation in uraemic patients demonstrated in the present study may throw some light on the pathogenesis of vitamin D resistant rickets or osteomalacia in end-stage renal failure. Some patients on chronic haemodialysis with osteomalacia have low plasma levels of 25-hydroxy-cholecalciferol (Bayard, Bec, Ton That & Louvet, 1973) and rats with experimental chronic renal failure have lower 25-hydroxy-cholecalciferol levels than their paired controls (Russell & Avioli, 1972). In addition, 25-hydroxy-cholecalciferol in doses of 6000 to 12000 i.u. daily is effective therapy for uraemic bone disease in patients refractory to vitamin D in equivalent dosage (Witmer & Balsan, 1971). These observations would be compatible with enhanced hepatic inactivation of vitamin D in chronic renal failure similar to that encountered in patients

receiving prolonged anticonvulsant (Dent, Richens, Rowe & Stamp, 1970; Hunter, Maxwell, Stewart, Parsons & Williams, 1971) or glutethimide (Greenwood, Prunty & Silver, 1973) treatment.

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