PLASMA KINETICS AND URINARY EXCRETION OF PRIMAQUINE IN MAN

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- 1 The kinetics of primaquine have been studied in twenty volunteers after single and multiple dose regimes.
- 2 The kinetic parameters were similar in glucose-6-phosphate dehydrogenase (G6PD) normal Thais, G6PD deficient Thais and in Caucasians.
- 3 The Caucasian subjects showed about 1% of the dose was excreted in the urine.
- 4 The kinetic parameters obtained from multiple dose studies in Thais were very similar to those obtained from single dose studies in Thais.

Introduction

Since the development of an efficient method of synthesis (Elderfield, Mertel, Mitch, Wempen & Werble, 1955), primaquine has been the drug of choice against *Plasmodium vivax* malaria superseding other 8-aminoquinolines. It is also of increasing importance for use against other malarias because of the development of parasite resistance to the 4-aminoquinoline chloroquine (Strube, 1975). Primaquine is subject to toxic side-effects causing methaemoglobin formation (Data Sheet Compendium, 1979–80) and haemolytic anaemia in individuals who are erythrocyte glucose-6-phosphate dehydrogenase (G6PD) deficient (Beutler 1969).

The kinetics and metabolism of the 8-aminoquinolines in the body are largely unknown. Workers in the late 1940s and 1950s showed that pamaquine (Zubrod, Kennedy & Shannon, 1949) and pentaquine (Elderfield & Smith, 1953) were rapidly removed from the body and almost entirely metabolized. These studies, however, suffered from a lack of specific and sensitive techniques. This lack of understanding of the behaviour of the 8-aminoquinolines in the body stimulated work on primaquine in this laboratory. The work resulted in the identification of 6-methoxy-8-aminoquinoline as a metabolite of primaquine (Baty, Evans & Robinson, 1975) and to the setting up of an assay for the drug in body liquids using gas chromatography-mass

spectrometry (GC/MS) with mass fragmentography (Greaves, Evans, Gilles & Baty, 1979). We have used this assay to study the kinetics of primaquine in plasma after single dose and multiple dose administration. The effect of G6PD deficiency on the kinetics was investigated as was any variation between Caucasian and Thai subjects.

Methods

Experimental design

For the single dose studies, seventeen volunteers (eleven Thai, six Caucasian) received an oral dose of six tablets of primaquine phosphate-equivalent to 45 mg free base. The Thai subjects were subdivided into two groups. Six individuals were G6PD normal and five G6PD deficient. The drug was administered at 09.00 h after overnight fasting. Fasting was continued for 3 h when a light lunch was permitted. Blood samples were withdrawn into heparinized tubes at 0, 1, 2, 5, 4, 6, 9 and 24 h after administration. The regimen for the multiple dosing was an oral dose of two tablets primaquine phosphate (equivalent to 15 mg base) on each of 5 days with a dose of chloroquine equivalent to 1.5 g of free base administered 1 week previously. This regimen was one used therapeutically in Thailand and was conducted in three Thai volunteers. Blood samples were withdrawn into heparinized tubes before the experiment began and at 0, 0.5, 1, 1.5, 2.5, 4, 6 and 9 h on

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the final day. The samples were centrifuged and the plasma retained.

Urine samples were collected for 24 h from seven Caucasians after receiving a 45 mg oral dose. A pre-dose urine was obtained as a control sample. The volume of urine excreted was recorded and 40 ml retained for analysis. All samples were stored at -20°C whilst awaiting analysis.

Instrumentation

The primaquine concentration were measured on a GC/MS consisting of a Pye 104 gas chromatograph (GC) linked via a silicone membrane separator to an AEI MS12 mass spectrometer (MS). The MS was operated at 8kV with a trap current of 500 μA and an ionizing voltage of 70eV. Selected ion monitoring was carried out using a six-channel multiplex monitoring unit (Kratos Ltd, Manchester), linked to a Rikadenki multichannel chart recorder for signal display. A glass column (1.5 m $\times$ 2 mm) packed with 3% OV1 on 100/120 mesh Gas Chrom Q (Phase Separations Ltd, Queensferry, Clwyd) was used in the GC. The GC was operated at 250°C with an injection temperature of 280°C . The carrier gas (helium) flow was 30 ml min^{-1} .

A PDP 8/1 computer was used for calculation of the plasma and urine concentrations. The plasma kinetics were obtained by using the Nonlin Programme on a CDC 7600 computer at the University of Manchester Regional Computer Centre.

Analysis of samples

The development of method for the analysis of samples and preparation of calibration curves has been previously reported (Greaves *et al.*, 1979). The basic procedure is that 1 ml of plasma or urine has 1 μg 6-trideuteromethoxyprimaquine diphosphate (the internal standard) added and is then freeze-dried.

The sample is extracted with 2.5 ml trichloroethylene (freshly distilled) to which six drops of ammonia solution (specific gravity 0.880) had been added. The solvent is retained, evaporated and the residue reacted with 50 μl Trisil TBT (Pierce and Warriner U.K. Ltd, Chester) at 80°C for 20 min. The mass fragmentography was carried out on M/Z 403 and M/Z 406. These ions are the molecular ions of the bis TMS (bis trimethylsilyl) derivatives of primaquine and the internal standard.

Results

The plasma primaquine concentrations of the single dose studies are given in Table 1 and Figure 1. These values have 95% confidence limits of ± 18 ng. These values were then subject to computer analysis to obtain the kinetic parameters of primaquine in plasma. The computer program was a two-compartment iterative model to investigate absorption and removal of the drug from plasma. The results showed that insufficient data was available fully to investigate the kinetics of the absorption of the drug. The concentration-time curve of the primaquine showed a one-compartment exponential decay for the elimination of the drug. The data on the kinetic parameters are shown in Table 2. The estimates of urinary concentrations had a range of 39 to 173 ng l^{-1} , each estimate having a 95% confidence limit of ± 21 ng. In the seven Caucasian volunteers studied 0.24 to 0.74% of the oral dose of 45 mg was excreted in the 24 h following its ingestion.

The plasma primaquine concentrations from the multiple dose studies are shown in Figure 2, each result has a 95% confidence limit of ± 18 ng. The derived kinetic parameters are given in Table 3.

Table 1 Plasma primaquine concentrations (ng ml^{-1}) from single dose (45 mg) studies

Subjects		Time after dosing (h)						
		0	1	2.5	4	6	9	24
Six G6PD positive Thais	Range of concentrations	0-9	148-337	190-304	101-234	72-139	50-107	0-8
	Mean	4	240	233	166	113	78	4
	s.d.	4	67	47	49	29	21	3
Five G6PD negative Thais	Range of concentrations	0-8	79-385	102-384	91-315	66-206	56-145	6-22
	Mean	4	188	199	165	119	93	15
	s.d.	3	116	111	93	54	33	6
Six Caucasians	Range of concentrations	2-19	71-139	127-185	123-138	84-113	59-73	24-47
	Mean	12	111	162	131	101	65	36
	s.d.	7	30	20	7	13	6	10

Figure 1 Mean plasma primaquine concentrations ν time after single 45 mg oral doses in the three groups of subjects investigated. $\circ$ G6PD positive Thais $\triangle$ G6PD negative Thais $\bullet$ Caucasians

Discussion

The results obtained in this study confirm the conclusions of early work on other 8-aminoquinolines, that the parent compound is rapidly removed from the blood stream. This quick passage of the drug meant that in the 24 h following the dose, the compound was completely or almost completely removed from the plasma in all subjects. The rapid elimination of the drug from the plasma may be by excretion, or metabolism, or by its being subject to tissue localization. Of these possibilities, excretion of the drug, as the unchanged, unconjugated parent compound can be discounted as the quantity of primaquine in the urine showed that less than 1% of the dose was excreted over a 24 h period.

Figure 2 Plasma primaquine concentrations ν time after the final dose in the three Thai individuals who received multiple doses.

Table 2 Kinetic parameters obtained from single dose (45 mg) studies

Subject	Body weight (kg)	Elimination constant (h^{-1})	Elimination half-life (h)	Areas under curve ($ng\ ml^{-1}\ h$)	Volume of distribution (assumes bioavailability is 1) (l)	Clearance ($l\ h^{-1}$)
Six G6PD positive Thais	Range of parameter Mean s.d.	0.15–0.26 0.19 0.04	2.7–4.5 3.7 0.7	1009–2204 1682 455	11–172 149 28	20.4–44.6 28.7 8.9
Five G6PD negative Thais	Range of parameter Mean s.d.	0.081–0.17 0.12 0.04	4.2–8.6 6.2 1.8	1227–3219 1978 789	84–382 226 111	14.0–36.7 24.7 8.5
Six Caucasians	Range of parameter Mean s.d.	0.092–0.16 0.11 0.03	4.3–7.5 6.3 1.4	1306–2016 1661 270	214–267 241 23	22.3–34.4 27.7 4.6

Table 3 Kinetic parameters obtained from multiple dose studies

Subject	Body weight (kg)	Elimination constant (h^{-1})	Elimination half-life (h)	Area under curve ($ng\ ml^{-1}\ h$)	Volume of distribution (assumes bioavailability is 1) (l)	Clearance ($l\ h^{-1}$)
Thai						
1	45	0.17	4.0	808	320	55.7
2	68	0.12	6.0	593	660	75.9
3	40	0.17	4.0	933	280	48.3

Table 4 Amount of primaquine in total body plasma at time of maximum concentration

Subject	Maximum plasma concentration (ng/ml)	Body weight (kg)	Amount of body plasma based on body weight (ml)	Amount of primaquine in plasma (mg)	Maximum percentage of dose in plasma
G6PD					
1	216	47	1,927	0.416	0.92
2	190	50	2,050	0.390	0.87
3	304	64	2,624	0.798	1.77
Positive Thais					
4	223	49	2,009	0.448	1.00
5	337	48	1,968	0.663	1.47
6	235	48	1,968	0.462	1.03
1	385	44	1,804	0.695	1.54
2	216	49	2,009	0.434	0.96
3	178	47	1,927	0.343	0.76
4	102	56	2,296	0.234	0.52
5	150	62	2,542	0.381	0.85
1	159	67	2,747	0.437	0.97
2	185	67	2,742	0.508	1.13
3	170	68	2,788	0.474	1.05
Caucasians					
4	172	72	2,952	0.508	1.13
5	127	64	2,624	0.333	0.74
6	158	94	3,854	0.608	1.37

The pattern of the movement of the drug in the plasma as shown in Figure 1 indicated that there is little or no difference between the two groups of Thais. This is as would be expected, as it would not be anticipated that the G6PD deficiency would alter the metabolism of the primaquine. There are also no marked differences between the Thais and the Caucasians though there appears to be a small quantity of drug remaining in the plasma of the Caucasians 24 h after dosing.

The amount of drug present in the plasma over the 24 h of the test is reflected by the area under the plasma concentration-time curve between these points. This figure (see Table 2) for the drug in the plasma is within the same range for both ethnic groups as are the clearance values for the drug. The range of the results within each ethnic group, i.e. the inter-individual variation, may be accounted for by many factors, such as age, sex, body weight, diet, gut flora and protein binding (Curry, 1974). The extent of the inter-individual variation is shown by the standard deviation about the means of each group as shown in Table 1. It is not possible to determine the amount of drug in the plasma from the area under the curve values. It is, however, possible to make an estimate of the amount of drug in the plasma at any one time. The amount of drug in the plasma at time of maximum concentration has been calculated by the formula:

$$\frac{\text{concentration of primaquine } (\mu\text{g/ml}) \times \text{volume of body plasma}}{1000} \\ = \text{amount of primaquine in plasma (mg)}$$

These amounts are shown in Table 4 and as percentages of the dose administered. This table shows that only approximately 2% of the dose was present in the plasma at the time of maximum concentration. This suggests that the drug, if it is fully absorbed (for which there is no proof), is being sequestered immediately it enters the body. Experimentation is needed to determine at what point

this occurs, or whether the drug is failing to pass through the gut wall. Possible explanations are the degradation of the drug in the stomach contents, failure to absorb the drug in the gut, the metabolism of the drug in the gut mucosa or the sequestering of the drug by the liver in a 'first-pass' effect. The last of these effects seems more probable as the main action for which primaquine is employed is against the exoerythrocytic stages of the *Plasmodium vivax* life cycle, whilst the parasites are residing in the liver.

The multiple dose experiments showed that the primaquine did not build up in the plasma over the 5 days of dosing. This is demonstrated by the concentrations found by the pre-dose sample on the final day which contained no drug. The concentration-versus-time graph on the fifth (final) day for these three individuals is shown in figure 2. The pattern of primaquine movement through the plasma compartment is very similar to that in the other Thai subjects who received a single dose of the primaquine (Figure 1). This indicates that the chloroquine and multiple dosing with primaquine have very little, if any, effect on plasma primaquine kinetics, despite the fact that the chloroquine influences methaemoglobin production by primaquine (Greaves, Evans & Fletcher 1980), and also its antimalarial efficacy (WHO, 1967). It is to be noted that the plasma half-life of chloroquine is 6–7 days (McChesney, Fasco & Banks, 1967), and it will, therefore, still have been present in the plasma at the start of the primaquine administration.

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