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URINARY PRIMAQUINE EXCRETION AND RED CELL METHAEMOGLOBIN LEVELS IN MAN FOLLOWING A PRIMAQUINE:CHLOROQUINE REGIMEN

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1 Red cell methaemoglobin levels were found to be significantly raised in healthy subjects given a 14-day course of primaquine with chloroquine on the first 3 days.

2 The methaemoglobin levels were not related to the quantity of primaquine excreted.

3 No primaquine could be detected in the plasma at 24 h following the last three daily doses.

Introduction

The 8-aminoquinolines have long been implicated in the formation of methaemoglobin during their use in the treatment of malaria in Caucasians (Clayman, Arnold, Hockwald, Yo 1952). The effect of a 14-day course of primaquine (15 mg base per day) on methaemoglobin levels was investigated by Cowan & Evans (1964). These workers were unable to show any significant variation of methaemoglobin levels from normal during or at the end of the 14-day period. It was decided to repeat the experiment of Cowan & Evans (1964) with one change, namely that for the first 3 days chloroquine (450 mg base) should be administered concurrently with the primaquine. This primaquine plus chloroquine regimen is the standard treatment for *P. vivax* malaria (WHO, 1967). As well as measuring methaemoglobin levels, it was decided to measure primaquine concentrations in plasma and urine. It was hoped that this would provide an indication as to whether primaquine or its metabolites were active in methaemoglobin production.

Methods

Instrumentation

The system used consisted of a Pye 104 gas chromatograph linked via a silicone membrane separator to an AEI MS 30 mass spectrometer operated at 4 kV with a trap current of 300 μA and an ionizing voltage of 70 eV. Selected ion monitoring was carried out using a six-channel multipeak monitoring unit (Kratos Ltd, Manchester) with signal display, via the UV galvanometer of the mass spectrometer. A glass column (1.5 m × 2 mm) packed with 3% OV-1 on 100/120 mesh Gas Chrom Q (Phase Separations Ltd, Queensferry, Clwyd) was used in the gas chromatograph. The GC oven was operated at 260°C with an injection temperature of 280°C. The flow rate of the helium carrier gas was 30 ml min^{-1}

Experimental design

Thirty Caucasian volunteers (healthy medical students and medical school staff—twenty-two males and eight females) received a standard course of chloroquine and primaquine as used for the treatment of *Plasmodium vivax* malaria. The oral regimen was a 15 mg (base equivalent) dose of primaquine on each of 14 days with chloroquine (450 mg base equivalent) on each of the first three days. Blood samples (20 ml) were taken by venepuncture before the beginning of the experiment and on day 7, 13, 14 and 15 of the experiment, the blood being taken before the drugs were given. A 100 μl sample of blood was immediately used for methaemoglobin estimation. The remainder of the blood was centrifuged and the plasma retained and stored at −20°C until analysis. A 24-h sample of urine was obtained after the final dose of primaquine. The volume of urine was recorded and 40 ml retained and stored at −20°C until analysis.

Analysis of samples

Methaemoglobin levels were determined by the method of Evelyn & Malloy (1938).

The method of analysis of samples for primaquine has been reported previously (Greaves, Evans, Gilles

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Table 1 Methaemoglobin concentrations (g 100 ml^{-1} blood) and primaquine excretion (mg 24 h^{-1})

		Range	*Mean*	*s.d.*
Methaemoglobin concentration	Day 0	0.03–0.28	0.16	0.06
	Day 7	0.03–0.77	0.37	0.20
	Day 13	0.20–1.56	0.57	0.29
	Day 14	0.13–1.93	0.59	0.32
	Day 15	0.09–1.48	0.61	0.29
Urinary primaquine concentration (ng ml^{-1})		5.1–47.6	190	100
Urinary volume (ml)		480–3770	1769	889
Urinary primaquine excretion: Day 14 to 15 (mg)		0.12–0.54	0.30	0.14

Methaemoglobin results all on 30 subjects.
Primaquine excretion result on 29 subjects.
(The sample from one subject was spoilt).

& Baty, 1979). Slight variations were employed for the results reported here. For all samples, the GC/MS was used to monitor M/Z 242 (primaquine) and M/Z 245 (internal standard). These ions were preferred because an increase in sensitivity was obtained when compared with the ions M/Z 403 and M/Z 406 previously used. The urine samples had 10 μg internal standard (viz. 6-trideuteromethoxylprimaquine) added and 2 ml were extracted. The urine concentrations obtained had 95% confidence limits of $\pm$ 22 ng.

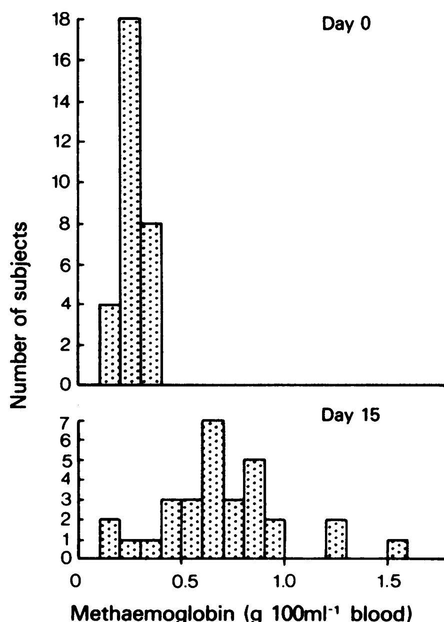

Figure 1 Frequency distributions of red cell methaemoglobin concentrations.

Results

The range, mean and s.d. of the methaemoglobin levels and the quantity of primaquine excreted are given in Table 1. No primaquine was detected in the plasma samples in any of the 29 subjects analysed. The effect of the drugs on methaemoglobin levels is shown in Figure 1. A one-way analysis of variance computed on methaemoglobin levels estimated on each of the 30 subjects on days 0, 7, 13, 14 and 15, yields F = 17.44 with degrees of freedom 4 and 145, $P < 0.001$. There was no significant correlation between methaemoglobin level and urinary primaquine excretion ($r = 0.12$). The individual with the highest methaemoglobin values was bled on days 17 and 27 and the methaemoglobin concentrations on those days were 1.36 and 0.35 g per 100 ml blood respectively.

Discussion

The cause of the methaemoglobin formation may be due to many factors but three appear likely. These are the presence of altered haemoglobin in the individual tested, variations in methaemoglobin reductase, and the direct effect of metabolites on haemoglobin. (1) It is unlikely that the individuals tested all possessed altered haemoglobin so this can be discounted. (2) Methaemoglobin reductase could exist in alternative molecular forms or differing numbers of molecules of the same form rendering some persons less able to reduce the increased amount of methaemoglobin present at a time of oxidant stress. (3) Varying amounts and types of primaquine metabolites produced by different individuals could also lead to the same end result.

The results showed an increase in the

methaemoglobin levels in the blood, but the lack of any detectable primaquine in the plasma meant that no correlation could be drawn between these parameters. There was no relationship between the amount of primaquine excreted and the level of methaemoglobin encountered.

Unfortunately it has not hitherto been possible to measure intra-erythrocytic primaquine concentrations. However, the slow build-up of methaemoglobin concentrations and the lack of build up of plasma primaquine concentrations, together with the slow decline of methaemoglobin concentrations after primaquine dosage was discontinued, strongly suggest the possibility that a primaquine metabolite is responsible for affecting the haemoglobin within the red cell.

The fact that the chloroquine : primaquine regimen caused an increase in methaemoglobin whereas primaquine on its own did not (Cowan & Evans, 1964) means that the chloroquine affects the process. This effect may be due to actions of chloroquine in the red blood cell or the liver cell. In the red cell, chloroquine might influence, for example, the effect of a primaquine metabolite on haemoglobin or methaemoglobin reductase. Alternatively, in the liver cell the chloroquine might cause variations in the type or amount of primaquine metabolites produced.

Methaemoglobinaemia may well be more pronounced after combined chloroquine and primaquine therapy when the red cells are parasitized (Jones, Hackson, di Lorenzo, Marx, Levy, Kenny, Gilbert, Johnston & Alving, 1953) than in the experimental situation where the red cells do not contain parasites.

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