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Phase I Study of Intravenous 4-Hydroxyanisole

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4-Hydroxyanisole is a depigmenting agent which has been shown to have activity against malignant melanoma when given intra-arterially in man. An intravenous dose escalation study has been carried out with the aim of obtaining maximum plasma concentrations in a 5 day schedule. 8 patients entered this study which was stopped because of drug toxicity after 3 patients had been treated at the third dose escalation of 15 g/m². 2 patients had WHO grade 4 liver and one also grade 4 renal toxicity and another had grade 4 haemoglobin toxicity. Extrapolated plateau plasma levels between 112 and 860 μmol/l were obtained, which *in vitro* studies suggested would be cytotoxic. Hopefully, newer analogues will have a greater specificity for the melanin pathway with less toxicity.
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INTRODUCTION

INTEREST IN 4-hydroxyanisole (4HA) in the therapy of melanoma arose following the demonstration that the long-term depigmenting effect of this agent when applied topically, is the result of melanocyte-specific cytotoxicity [1–3]. This is thought to result from the formation of toxic metabolites by the oxidation of 4HA by tyrosinase which is present only in melanocytes [4]. The orthoquinone oxidation product of 4HA [5] readily undergoes reactions with nucleophiles and the toxic action may be the result of covalent binding to protein thiols or to glutathione depletion [6], although direct inhibitory actions of 4HA on mitochondrial electron transport or ribonucleotide reductase may also be implicated [7]. An intra-arterial study in man [8] has demonstrated a reduction of tumour volume in 9 out of 20 patients with malignant melanoma, and several of these responses were almost complete [9]. The rationale for intra-arterial use was to obtain a high local concentration and avoid liver metabolism. To be more widely applicable for use in treating malignant melanoma, this drug would need to be given intravenously.

In vitro studies have suggested that a cytotoxic effect is seen at concentrations above 100 μmol/l (10;11;12;13). An intravenous dose escalation study was therefore instituted to determine the maximum tolerated dose and whether plasma levels greater than 100 μmol/l could be achieved. To obtain the maximum plasma concentrations required, infusions were administered daily for 5 days to mimic the intra-arterial studies where responses had been seen.

PATIENTS AND METHODS

Patient selection

Eligibility criteria required that patients had measurable metastases of malignant melanoma, refractory to conventional treatment, performance status of 2 or less (WHO), life expectancy of greater than 2 months, age under 75 years, appropriate haematological (haemoglobin (Hb) >10 g/dl, white blood cells (WBC) $>4 \times 10^9$/l, platelets $>100 \times 10^9$/l) and biochemical (bilirubin <20 μmol/l, creatine <150 μmol/l) parameters, no anti-cancer therapy over the preceding 4 weeks and no serious intercurrent non-malignant disease.

Drug administration

4HA was formulated as 10 ml ampoules of a 0.6 g/ml solution in absolute ethanol B.P. It was diluted in 0.9% sodium chloride in Polyfusor bags. It was infused daily for 5 days as indicated in Table 1 with the infusion time and volume dictated by the concentration of ethanol and solubility.

The starting dose was based on the clinical data available when given intra-arterially. Over 15 patients had received 80 g over 3 days with minimal toxicity (ref. 9 and B. Morgan, Mount Vernon Hospital). The two initial doses were expected to be non-toxic. The time interval between patient entry was 1 week at the first dose level and 3 weeks before entry into a higher dose level. At non-toxic doses one incremental increase in dose was permitted within an individual patient.

Clinical pharmacological studies

A pharmacokinetic study was performed at entry into the trial at all dose levels. Blood was collected into heparinised tubes prior to, during and up to 24 h after administration of the drug on the first day and if possible fifth day of the infusion (end of infusion, 5, 15, 30, 45, 60, 90, 120, 240 min after the infusion). Each sample was centrifuged (2000 rpm for 5 min) and the plasma was frozen and stored at −20°C until assayed. Urine was collected over the first 24 h from start of the first day's infusion.

Table 1. Dose increments of 4HA

Dosage level volume (ml)	Daily drug dose (g/m²)	Infusion time (h)
500	5	3
1000	10	5
1500	15	7

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Table 2. Sites of disease

Dose (g/m²)	Skin	Nodes	Liver	Lung	Bone
5	3	3	1	1	0
10	2	2	1	0	0
15	3	1	1	2	2

Drug analysis

4HA was determined using essentially the method described by Holden *et al.* [13] except that samples were dried down under N_2 and the eluent contained 0.1 M acetate buffer (pH 5) [14].

Pharmacokinetic calculations

Plasma concentrations were plotted against time and the best fit to the data was calculated assuming first order (exponential) kinetics.

RESULTS

8 patients were entered into the study and they were all considered evaluable for assessment of toxicity. A total of nine courses of the drug were administered. 5 days of therapy was given in the six courses to all 5 patients at dose level 1 and 2. At dose level 3, 4HA was only given over 4, 4 and 2 days, respectively because of toxicity. The sites of disease are shown in Table 2.

Toxic effects of 4HA

Treatment associated toxicity is shown in Table 3. Apart from drowsiness probably due to the alcohol diluent there was no toxicity at the first dose level. At dose level 2, 1 patient experienced flushing of the skin and had a grade I fever. 1 patient had grade 1 elevation of alkaline phosphatase and bilirubin whilst the other 2 patients had grade 1 fall in haemoglobin. Treatment was stopped early in all patients at dose level 3 because of progressively abnormal liver function, but in one patient primarily because her haemoglobin fell to 3.4 g/l by day 4. The last patient in the stdy developed acute liver and renal failure after 2 days of therapy and died 4 days after starting therapy. He had no evidence of liver or kidney metastases but permission for a post-mortem was refused. On day 3 his methaemoglobin concentration was 10.6%.

Pharmacokinetics

Plasma concentrations were determined in the first patient receiving 5 g/m²/3 h covering both the initial infusion and the post-infusion period, and these data are shown in Fig. 1. This illustrates the rapid attainment of a plateau concentration of approximately 120 μmol/l, indicative of rapid clearance. There is no evidence for any significant change in the kinetics over the 5 days in this patient. A line has been fitted by non-linear least squares to the initial portion of the curve giving a half-life of 27 min, although clearance appeared to be biphasic. Figure 2 shows the plasma concentrations measured following the end of

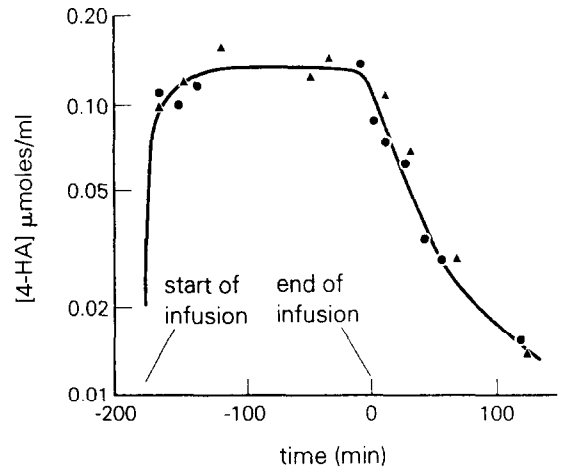

Fig. 1. Plasma concentrations of 4HA in a patient receiving 5 g/m² over 3 h on day 1, ▲ and day 5, ●.

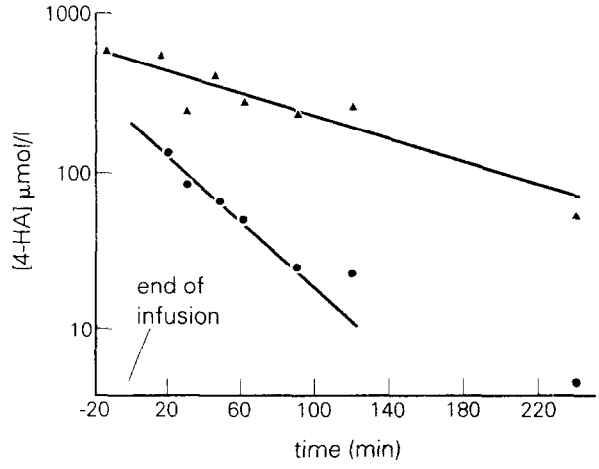

Fig. 2. Plasma concentrations of 4HA in a patient receiving 10 g/m² over 5 h, showing different clearance between day 1, ▲ and day 5, ●.

Table 3. Toxicity in individual patients after 4HA infusion

Dose level	Nausea Vomiting				Fever			Conscious				Flushing			Renal		Liver				Hb			
WHO Grade	0	2	3	4	0	1	2	0	1	2	3	0	1	2	0	4	0	1	2	3	4	0	1	2	4
1	3				3			2	1			3			3		3					3			
2	1	1		1	2	1		1		1	1	2		1	3		2	1				1	2		
3		1	1	1	2		1	2		2		2	1		2	1			1	2			1	1	1

0 = None, 1 = mild, 2 = moderate.

Table 4.

Dose (g/m^2)	Plateau (4HA) (μmol/l)	$t_{\frac{1}{2}}$ (min)
5	290	37
5	110	27
10	510	88
10	200	29
10	220	34
15	860	85
15	370	28
15	650	75

infusion on days 1 and 5 for a patient receiving 10 g/m^2/5 h, and shows very different kinetics. On day 1, the clearance appears linear with $t_{\frac{1}{2}}$ of 89 min, but by day 5 the kinetics had become non-linear, with a more rapid initial clearance ($t_{\frac{1}{2}}$ only 29 min), similar to that seen in Fig. 1.

Although we did not obtain full clearance curves for all the other patients on both days, there was sufficient data to suggest the existence of two populations, both able to clear 4HA rapidly or slowly. This is illustrated in Table 4 where those with the highest plateau level at a particular dose had the longest half life. This variation did not appear to be related to the particular dose of 4HA received. The data show that concentrations in excess of 100 μmol/l are easily achievable, which *in vitro* data suggests would be sufficient to obtain a significant cytotoxic effect. Less than 1% of the administered dose of 4HA was excreted unchanged in the urine over 24 h.

DISCUSSION

This study was stopped because of the unexpectedly high toxicity at the dose of 15 g/m^2. The absence of toxicity at the lowest dose of 5 g/m^2 suggests that toxicity is dose related. Although more patients should have been treated at doses around 10 g/m^2, to better define the maximum tolerated dose, the severity of the toxicity at 15 g/m^2 was such that peer pressure dissuaded us from continuing the study.

Analysis of the urinary excretion products of 4HA have shown that the drug is metabolised mainly to the 3,4-dihydroxy derivative which is excreted in a conjugated form [15]. This is, however, unlikely to be the cause of the hepatotoxicity as incubation of mouse hepatocytes with 3,4,diacetoxyanisole, the prodrug of 3,4-dihydroxyanisole was less cytotoxic than 4HA [16]. Their observation that glutathione depletion increased and that *N*-acetylcysteine abolished the cytotoxicity of 4HA is compatible with an as yet unidentified reactive metabolite of 4HA causing hepatotoxicity. The mechanism of methaemoglobin formation has recently been investigated by Stolze and Nohl [17] and involves the generation of reactive species which may deplete erythrocyte glutathione. This would explain the haemolysis observed in 1 patient.

Most patients treated by intra-arterial therapy received a dose equivalent to between 5 and 10 g/m^2 per infusion. There have been reports of patients feeling flushed, dizzy and nauseated following intra-arterial 4HA, as well as 2 cases of hepatocellular damage. It is probable that the tumour concentration obtained by intra-arterial infusion was considerably higher than obtained by the same dose when given intravenously, and we were unable, because of toxicity, to significantly increase the dose by using the intravenous route.

The higher tumour concentration after intra-arterial administration might be the reason for responses being seen after intra-arterial therapy. Although no responses were seen in this study, only 8 patients were treated. They all obtained plateau plasma concentrations over 100 μmol/l which was the concentration suggested from the *in vitro* studies to be cytotoxic. The suggestion of large variations in the clearance of 4HA both between patients and between doses would imply that pharmacokinetic monitoring would be desirable. These considerations and the fact that we had not determined a safe maximum tolerated dose have dissuaded us from proceeding with a phase II intravenous study. It will hopefully be possible to synthesise structural analogues of melanogenic precursors that have less tyrosinase-independent toxicity than 4HA and will therefore have an improved therapeutic ratio.

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