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ORIGINAL ARTICLE

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A phase I trial of amsalog (CI-921) administered by intravenous infusion using a 5-day schedule

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Abstract *Purpose*: Amsalog, a derivative of 9-aminoacridine, is an inhibitor of topoisomerase II. Early studies of intravenous amsalog administered either once weekly, or daily for 3 days repeated every 3 weeks, showed that myelosuppression is the dose-limiting toxicity (DLT). Phase II studies showed only limited activity in breast, head and neck, and non-small-cell lung cancer. The activity of other topoisomerase inhibitors is schedule-dependent. We therefore performed a phase I study to evaluate the use of amsalog on a more prolonged schedule. *Methods*: A group of 19 patients with refractory malignancies were treated in six cohorts using 2-h infusions of amsalog daily for 5 days, repeated every 3 weeks. *Results:* Myelosuppression was seen as DLT at 200 mg/m^2 per day. Other toxicities included nausea and vomiting, fatigue, and, when administered via a peripheral venous line, severe phlebitis necessitating administration via an indwelling central venous catheter for doses greater than 100 mg/m^2. Pharmacokinetic studies showed a linear relationship between C_{max} and AUC, and dose. The terminal half-life was 2 h, consistent with previous studies. *Conclusion*: We conclude that amsalog can be safely given on a 5-day schedule every 3 weeks at doses up to 200 mg/m^2. The dose recommended for further studies is 180 mg/m^2 per day for 5 days repeated every 3 weeks. However, in view of the phlebitis, which necessitated the use of central venous catheters for administration, other routes of administration, for example oral formulations, should be explored.

Key words Amsalog · Phase I trial · Topoisomerase inhibition · Pharmacokinetics

The authors have written on behalf of the Cancer Research Campaign Phase I/II Trials Committee.

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Introduction

Amsalog (*N*,5-dimethyl-9-[(2-methoxysulphonylamino) phenylamino]-4-acridinecarboxamide, CI-921) is a derivative of 9-aminoacridine synthesized in the Cancer Research Laboratory, University of Auckland School of Medicine, New Zealand [1]. Like amsacrine, the parent compound, it is a topoisomerase II inhibitor [20], and binds to DNA by intercalation [1], but amsalog is more lipophilic (0.5 log p units) and is also a considerably weaker base (pKa 6.40 compared with pKa 7.43) [1, 15]. It shows cytotoxic activity in vitro, with IC_{50} values ranging from 6.7 to 800 n*M*, indicating that it is two to four times more potent than amsacrine [1]. Antitumour activity has been seen in vitro in multidrug-resistant cell lines, for example p388/ADR [3], and in p388 leukaemia and Lewis lung cancer xenografts in nude mice in vivo [1, 2]. Of 16 tumour models in which amsalog has been found to be active, in 10 it is more active than amsacrine [13].

Preclinical pharmacokinetic studies have shown that the drug is highly protein-bound in plasma [5], with only $0.33 \pm 0.04\%$ free drug found in rabbits [15], and that in mice and rats most (86%) of the drug is eliminated in the feces, mainly as a glutathione conjugate [18, 19]. In addition, the uptake of amsalog into Lewis lung carcinoma in mice is more efficient than the uptake of amsacrine, with a longer tumour half-life and a greater tumour AUC [12]. There is 20% uptake of the drug into the brain, implying that it can cross the blood-brain barrier [4]. Toxicology studies in mice, rats, rabbits and dogs [7, 10, 17] have shown that the dose-limiting toxicity is

myelosuppression. Gastrointestinal toxicity, elevation of alkaline phosphatase and generalized lymphoid depletion are also seen.

In view of the promising preclinical data, a number of clinical studies have been carried out. In initial phase I studies, a 3-day schedule has been evaluated, with amsalog administered as a 15-min intravenous infusion at doses ranging from 13 to 270 mg/m^2 per day repeated every 3 weeks. The dose-limiting toxicity was myelosuppression, and mucositis and phlebitis were also seen. The recommended dose for phase II studies was 216 mg/m^2 per day for 3 days. Pharmacokinetic studies have shown high plasma binding and the terminal half-life is 2.6 h (range 1.08–4.98 h). No objective responses have been seen [8].

Subsequent phase II studies performed using a number of schedules were disappointing. In non-small-cell lung cancer, one partial response was seen out of 16 patients [9] using daily administration of 216 mg/m^2, again as a 15-min infusion for 3 days repeated every 3 weeks. In a multicentre study of weekly administration using 270 mg/m^2 per week as a 1-h infusion, four responses were seen in 132 evaluable patients including 2 of 19 breast cancer patients, 1 of 14 head-and-neck cancer patients and 1 of 36 non-small-cell lung cancer patients [21]. Amsalog was therefore not felt to have significant clinical activity using these schedules. However, other topoisomerase inhibitors such as etoposide are strongly schedule-dependent, with single doses being less active than prolonged administration [6]. Indeed in animal models, amsalog also shows greater activity when administration is prolonged [2]. It was therefore decided to perform a further phase I study to evaluate amsalog administered using a longer infusion time and more prolonged schedule in order to establish the dose-limiting toxicity, the maximum tolerated dose, and the dose to be recommended for further phase II studies.

Patients and methods

Patient population

A group of 19 patients with histologically confirmed cancer, no longer amenable to standard therapies, or known to be of a histological type refractory to standard therapies, were included in this single-centre phase I study. Patient characteristics are shown in Table 1. The criteria for entry into the trial included: age greater than 18 years, WHO performance status 0–2, and normal haematological indices at presentation (leucocytes $\geq 4\times10^9$/l, platelet count $\geq 150\times10^9$/l). Exclusion criteria were: a history of or concurrent symptoms of ischaemic heart disease; hepatic dysfunction, i.e. liver function tests greater than two times the upper limit of normal for the institution (ULN); severe renal dysfunction, defined as serum creatinine greater than two times the ULN; pregnancy or lactation; more than one previous chemotherapy regimen; any chemotherapy within the previous 4 weeks; previous extensive radiotherapy; evidence of brain metastasis; and significant electrolyte disturbance, i.e. hypokalaemia (<3.5 mmol/l) or hypercalcaemia (>3.0 mmol/l). Prior to entry, all patients gave written informed consent to participate in the trial. The trial was approved by the ethics committee of Nottingham City Hospital, and followed Good Clinical Practice guidelines.

Table 1 Patient characteristics (values are numbers of patients, except age in years)

Number of patients	
Total	19
Male	17
Female	2
Age (years)	
Mean	54
Range	34–69
Tumour type	
Lung	4
Unknown primary	3
Soft tissue sarcoma	3
Pancreas	2
Melanoma	2
Renal cell	2
Colon	1
Mesothelioma	1
Prostate	1
Previous therapy	
Chemotherapy	9
Radiotherapy	9
Hormonal	2
WHO performance status	
0	3
1	11
2	5

Initial clinical evaluation and on-study assessment

Initial assessment included a full clinical history and physical examination; full blood count with differential white cell count and platelets; serum biochemistry including urea and electrolytes, calcium, total protein, albumin, and liver function tests; and chest radiography. Since disease response was not a primary endpoint in this study measurable disease was not required as an entry criterion but if present was assessed with appropriate staging investigations.

Patients were seen every 3 weeks whilst on study, with assessment of toxicity, physical examination, and blood tests as above. In addition, full blood counts with differential white cell count and platelets were performed weekly.

For the first cycle of treatment plasma samples for pharmacokinetic analysis were taken from each patient at –2 h (before infusion), –1 h (during infusion) and 0 h (end of infusion), and at 5, 15, and 30 min and 1, 2, 4, 8 and 24 h after the infusion. Analysis of the samples was performed by high-performance liquid chromatography as previously described [11].

Treatment

Amsalog was supplied by Professor B.C. Baguley of the Cancer Research Laboratory, University of Auckland School of Medicine, New Zealand, and was formulated by the Cancer Research Campaign Phase I/II Clinical Trials Committee Formulation Unit at the Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow, UK. It was administered as an intravenous infusion in 500 ml 5% dextrose over 2 h, on five consecutive days, repeated every 3 weeks. Initially this was administered peripherally, but at higher doses central venous catheters were used. The starting dose, based on previous studies, was 100 mg/m^2 [8]. Doses were escalated by 20 mg/m^2 per cohort, in cohorts of three patients, in the absence of dose-limiting toxicity. This was defined as any toxicity of grade 3 or more, as assessed by the Common Toxicity Criteria [14]. One incremental intrapatient dose escalation was allowed. The study was to be terminated if two or more patients in a cohort developed toxicity of grade 3 or more. Patients could receive a maximum of six cycles of treatment.

Results

Dose levels and number of cycles of treatment

A total of 19 patients were treated in six cohorts. The dose levels were 100, 120, 140, 160, 180, and 200 mg/m^2. One patient in the fifth cohort did not complete the first cycle of treatment and therefore a further patient was entered in this cohort. In total, 34 cycles of chemotherapy were administered. A median of one cycle of chemotherapy was administered to each patient (range one to five). Two patients in the first cohort, and one in the second, were allowed a dose increment for the second and subsequent cycles. One patient in the sixth cohort had a dose reduction of 25% for the third cycle due to neutropenia in previous cycles. One patient in the second cohort had the second cycle of treatment delayed by 1 week due to phlebitis caused by the peripheral infusion of amsalog.

Toxicity

The main toxicities reported are summarized in Table 2. The dose-limiting toxicity was seen at the sixth dose level, 200 mg/m^2, when two out of three patients developed grade 3 or 4 neutropenia and fever, requiring hospitalization for intravenous antibiotics. One of these patients was allowed a further course at a 25% dose reduction, and developed grade three neutropenia during that cycle. Anaemia was commonly seen, with 13 out of 19 patients developing up to grade 2 anaemia which was felt to be due to the treatment. One patient developed grade 4 thrombocytopenia at a dose of 160 mg/m^2.

Grade 2 phlebitis at the site of the peripheral infusion of amsalog was seen in all three patients in the first cohort, and grade 3 in the first patient in the second. Because of this, all subsequent patients were given amsalog via an indwelling central venous catheter. Of the 15 patients with central venous catheters, 2 developed axillary vein thrombosis, and one a line infection, a complication rate of 20%.

Ten patients reported nausea due to the treatment. In three patients this was grade 3. Vomiting was also seen, again in ten patients, and was severe in three patients. After patient number 5, prophylactic antiemetics were routinely prescribed, using a variety of different antiemetic combinations. Five patients reported fatigue and tiredness caused by amsalog, two grade 2 and three grade 3. Surprisingly, this was not seen at the highest dose levels.

Responses

Of the 19 patients, 12 were evaluable for evidence of response. There were two partial responses:

1. A patient with undifferentiated non-small-cell lung cancer responded clinically and radiologically. Clinical measurements of nodal disease decreased from 90×60 mm to 10×10 mm, and a lung mass on chest radiograph from 16×12 mm to 10×7 mm.
2. A patient with metastatic carcinoma of the prostate, whose disease had progressed whilst taking cyproterone acetate and was therefore felt to be hormone-resistant, entered the study but continued to take cyproterone. Clinical measurements of a supraclavicular fossa nodal mass decreased from 90×65 mm to 5×5 mm.

One patient, with clear cell renal carcinoma, had stable disease after five cycles of treatment. Nine patients had progressive disease.

Pharmacokinetics

The results of the pharmacokinetic analysis are shown in Table 3, and Figs. 1 and 2. Differences between the mean values for each dose level were assessed by ANOVA using Statview (SAS Institute, Cary, N.C.). There were no significant differences between dose levels for the mean values for clearance, α half-life or β half-life. The AUC and C_{max} were significantly different between dose

Table 2 Toxicity: maximum experienced by each patient

Toxicity	Dose level (mg/m^2)																													
	100					120					140					160					180					200				
	CTC grade					CTC grade					CTC grade					CTC grade					CTC grade					CTC grade				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Nausea or vomiting	1	1	1			1	1	1			1			2		1	1	1			3			1		1	1	1		
Fatigue	1		1	1		3					1			2		2		1			4					3				
Neutropenia	3					2		1			2			1			1		1	1	1	2	1					1	1	1
Thrombocytopenia	3					3					2	1				1	1			1	3	1				2	1			
Phlebitis[a]			2	1			1		1																					

[a] In cohort 2, the third patient had a central venous catheter inserted. Only two patients therefore were assessable for peripheral phlebitis in this cohort. Central venous lines were used at higher doses

Table 3 Pharmacokinetic results

Dose (mg/m^2)	α $T_{1/2}$ (h)		β $T_{1/2}$ (h)		Vss (ml/kg)		AUC (μM·h)		C_{max} (nM)		Clearance (ml/kg/h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100	0.33	0.31	1.71	0.67	313	92.1	29.10	9.40	11.97	1.71	173	59.5
120	0.51	0.37	2.09	0.64	234	54.8	53.30	12.06	18.00	1.06	102	22.1
140	0.26	0.02	1.64	0.29	247	34.0	46.83	15.59	18.27	5.44	147	50.5
160	0.60	0.49	2.89	1.79	549	562	68.33	11.66	21.47	1.75	105	10.0
180	0.23	0.11	1.55	0.43	265	84.7	50.73	13.59	22.33	7.12	169	61.5
200	0.40	0.09	2.16	1.13	204	27.8	71.25	15.63	27.00	3.68	116	20.5

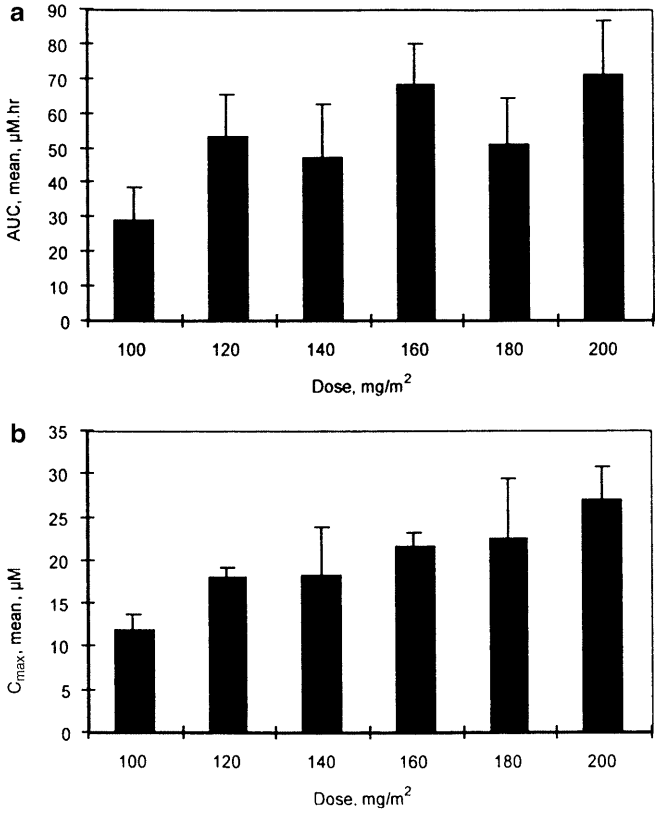

Fig. 1 **a** Mean AUC; **b** mean C_{max}. Error bars represent one standard deviation

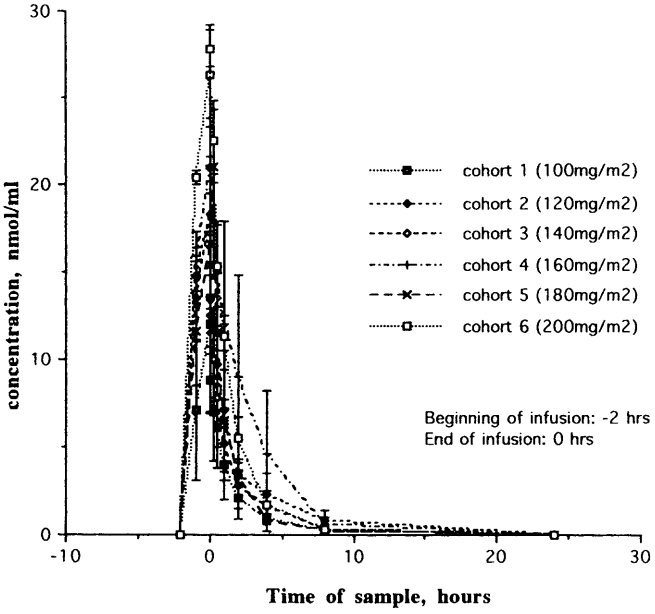

Fig. 2 Amsalog pharmacokinetics: plasma concentrations

levels ($P = 0.0077$ and $P = 0.026$, respectively), and there was a significant linear trend for these mean values: C_{max} slope 1.31, $r^2 = 0.63$, $P = 0.0003$; AUC slope 3.08, $r^2 = 0.36$, $P = 0.005$. This suggests linear kinetics over this dose range. The mean α half-life was 0.38 h (range 0.14–1.16 h) and the mean β half-life was 2.01 h (range 1.12–4.95 h). These results are very similar to those found in the previous phase I studies of amsalog (0.5 h, range 0.2–1.1 h, and 2.6 h, range 1.1–5.0 h) [16].

Discussion

Amsalog has been shown to have antitumour activity both in preclinical studies [1, 2] and in earlier clinical trials of schedules using short infusions, either weekly or daily for 3 days repeated every 3 weeks [9, 21]. However, in these clinical trials the activity was disappointing. Since other topoisomerase inhibitors appear to be more effective using prolonged administration schedules, we now report a phase I clinical trial using amsalog administered as a 2-h infusion daily for 5 days repeated every 3 weeks. The pharmacokinetic results were comparable to those of previous studies [8], and the dose-limiting toxicity was myelosuppression, as seen previously, and at a similar dose to that obtained in a study using a daily for 3 days regimen. There was clinical evidence of antitumour activity with two patients having a partial response. However, because of the severe phlebitis seen, necessitating the use of indwelling central venous catheters to administer the drug, the intravenous route of administration for prolonged scheduling is not convenient and cannot be recommended for further phase II studies. In animal studies, amsalog has been shown to be absorbed orally with reasonable bioavailability. Further studies are therefore planned to assess the practicality of using an oral formulation.

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