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A phase I and pharmacology study of GR63178A, a water-soluble analogue of mitoquidone

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Summary. GR63178A is a water-soluble analogue of mitoquidone, a pentacyclic pyrroloquinone. This group of drugs exhibit a novel structure and activity against several murine solid tumours and xenografts. In the present phase I study the toxicity and pharmacokinetics of GR63178A given on 5 consecutive days of a 21-day cycle were examined. A total of 24 patients presenting with a wide range of tumours were treated at 5 doses escalated to reach the maximal tolerated dose (MTD). Linear pharmacokinetics was documented over the dose range studied, and there was no difference in parent drug handling between day 1 and day 4 of dosing. A number of metabolites were detected. The toxicity profile was unusual in that pain occurred in 20/24 patients, most often at the site of known disease. This was the dose-limiting toxicity. Other side effects included nausea and vomiting (23/24), phlebitis at the infusion site (6/24) and headache (7/24). No treatment response was seen in this study. The MTD was demonstrated to be 160 mg/m^2 daily (total, 800 mg/m^2 per treatment cycle). The drug has now entered phase II trials at 120 mg/m^2 daily $\times 5$, repeated every 21 days.

Introduction

Mitoquidone was the first of a new group of pentacyclic pyrroloquinones developed as potential new anti-tumour agents, which presented problems in phase I studies due to its insolubility [5]. GR63178A is a water-soluble analogue of mitoquidone and has been shown to be active in several murine solid tumours (sarcoma 180, MAC 30T adenocarcinoma, colon 38, D23 hepatoma) and in human colon, lung and mammary xenografts. It is not active in vitro or in mouse leukaemias L1210 and P388 [2]. Preclinical animal toxicology data has shown a high therapeutic index in rodents [2]. In animal models the drug was most effective when given by chronic dosing rather than as a cyclical regimen. In preclinical studies the lethal dose for 10% of the mice tested (LD_{10}) was 191 mg/kg (about 600 mg/m^2) [2]. A dose of 200 mg/m^2 daily was given to beagle dogs for up to 35 days and produced no significant toxicity [2]. The starting dose for this phase I trial was 40 mg/m^2 daily $\times 5$ every 21 days [4] and dose escalations were made according to a modified Fibonacci scheme. Two other centres adopted different schedules of administration for parallel phase I trials of the same drug [1, 6].

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Patients and methods

Patients. To be eligible to enter this study, patients had to exhibit active malignant disease (for which no conventional therapy was likely to result in a disease response), a life expectancy of >12 weeks and a WHO performance status (PS) of 2 or better. Informed consent was obtained from all subjects. Renal and hepatic function as determined by routine laboratory tests were shown to be within defined limits before the start of treatment. A normal ECG was required. The age limits for inclusion were set at 18–75 years, and the toxic effects of previous chemotherapy were required to have fully resolved. A total of 24 patients were entered (Table 1); 8 had not previously been treated, and 1 had been treated with radiotherapy only. The mean age of the patients was 52.6 (range, 22–72) years.

Drug dosage and dose escalations. GR63178A reconstituted with water was given in 250 ml 5% dextrose (final concentration range, 0.2–1.16 mg/ml) as a 20-min (for actual times see Table 2) intravenous infusion. The starting dose of 40 mg/m^2 daily for 5 consecutive days was calculated to be approximately one-third of the LD_{10} in mice and was escalated through four steps to the maximal tolerated dose (MTD). Dosage levels were 40 (four patients, five completed courses), 80 (four subjects, seven courses), 120 (three patients, five courses), 140 (one subject, two courses) and 160 mg/m^2 daily (four patients, three courses). In addition, 8 patients were subsequently recruited at 120 mg/m^2 daily to increase the data base for use in phase II trials to a total of 20 completed courses at this dose.

Drug analysis and pharmacokinetics studies. Measurements of GR63178A, its 9-hydroxy metabolite GR 54374X (M1) and an internal standard (GR70440A) were carried out in plasma and urine by reversed-phase high-performance liquid chromatography (HPLC) using gradient elution and selective detection at 380 nm. This followed sample prepara-

Table 1. Patients' characteristics

Number treated	24
Mean age	52.6 (range, 22–72) years
Diagnosis:	
Colorectal	9
Lung	4
Melanoma	3
Breast	2
Cervix	1
Lymphoma	1
Ovary	1
Osteosarcoma	1
Oesophagus	1
Nasopharynx	1

tion by solid-phase extraction with 500 mg of Bond Elut C_2 40-μm silica gel in 2.4 ml reservoirs as described in detail elsewhere [3]. Standards of GR63178A (sodium salt), GR54374X and GR70440A (sodium salt) were kindly supplied by Glaxo Group Research Ltd (Greenford, England). Due to light instability at concentrations of <100 μg/ml, all sample preparation was performed in a darkroom under red light and standard solutions were always made up fresh.

Blood samples for pharmacokinetics studies were collected at 10, 20, 30 and 45 min and at 1, 2, 4, 6, 9, 12 and 24 h after the beginning of the GR63178A infusion on days 1 and 4 of each treatment cycle. A pre-dose blood specimen was also taken. Urine was collected over a 24-h period after the initiation of the GR63178A infusion as three aliquots (0–4, 4–12 and 12–24 h), and a pre-dose urine specimen was also obtained when possible. All samples were placed in opaque glass or plastic containers and stored at –40° C prior to drug analysis by HPLC.

Pharmacokinetic parameters were derived by graphical methods as follows: the half-life was obtained by non-linear regression analysis, the area under the curve ($AUC_{0-24\,h}$) was calculated by the trapezoidal rule and the peak concentration was taken directly from plasma profiles and usually coincided with the end of the infusion. Clearance was calculated using the formula dose/$AUC_{0-24\,h}$.

Results

Toxicity

The toxicity profile was unusual. The most striking adverse event was the occurrence of pain, usually at the site of known disease. Of the 20 patients affected, 5 experienced pain of WHO grade 1; 8, grade 2; and 7, grade 3. Two of four subjects (50%) treated at the MTD of 160 mg/m^2 daily experienced WHO grade 3 pain; one case involved a severe exacerbation of pre-existing chest-wall pain, and one patient (mammary carcinoma with left axillary node involvement) complained of severe right lower pleuritic and shoulder-tip pain that was possibly related to metastatic disease in the right lung (there was no ECG evidence of pulmonary embolism, although this had been considered on clinical grounds). Treatment was discontinued in this last case. Of the affected patients, 11/20 experienced exacerbation of pre-existing pain and 9/20 perceived pain arising de novo that was usually associated with known metastatic disease. In all but one case, pain arose within 24 h of drug administration, but not always after the first dose. The pain lasted for an average of 16.5 (range, 1–96) h.

Two patients experienced severe, persistent right-upper-quadrant pain that was thought to be of hepatic origin. Exacerbation of previously existing pain in 11 subjects did not differ in nature from the original pain but required an increase in analgesia during treatment. Two patients developed neuraesthenic-type pain (probably disease-related) that became worse on treatment and did not respond well to opiates as compared with the other types of pain experienced, which responded to an appropriate level of analgesia. Three subjects experienced fever associated

Table 2. Pharmacokinetics of GR63178A

Dose (mg/m^2)	Day	Infusion (min)	Peak concentration (μg/ml)	Half-life (h)	$AUC_{0-24\,h}$ (μg ml^{-1} h)	Clearance (l h^{-1} m^{-2})
40	1	20	8.6	4.03	4.78	8.4
	4	30	10.8	0.87	10.3	3.9
80	1	20	14.2	1.48	10.8	7.4
	4	22	18.7	9.63	11.6	6.9
80	1	30	11.6	4.47	17.6	4.5
	4	30	9.9	1.8	10.8	7.4
80	1	20	16.6	7.37	17.9	4.5
	4	20	17.4	1.92	15	5.3
120	1	35	15	1.08	18.3	6.6
	4	45	11.2	6.19	28.4	4.2
120	1	35	24.2	6.66	40.7	2.9
	4	45	10.5	2.05	19.4	6.2
120	1	20	12.9	1.31	7.5	16
	4	30	7	1	7	17.1
120	1	20	24.2	1.4	23.2	5.2
160	1	30	23.3	5.9	32.1	4.9
160	1	20	24.2	1	39.1	4.1
160	1	68	17.7	2.7	28.2	5.7
160	1	35	25.6	7.7	35.8	4.5

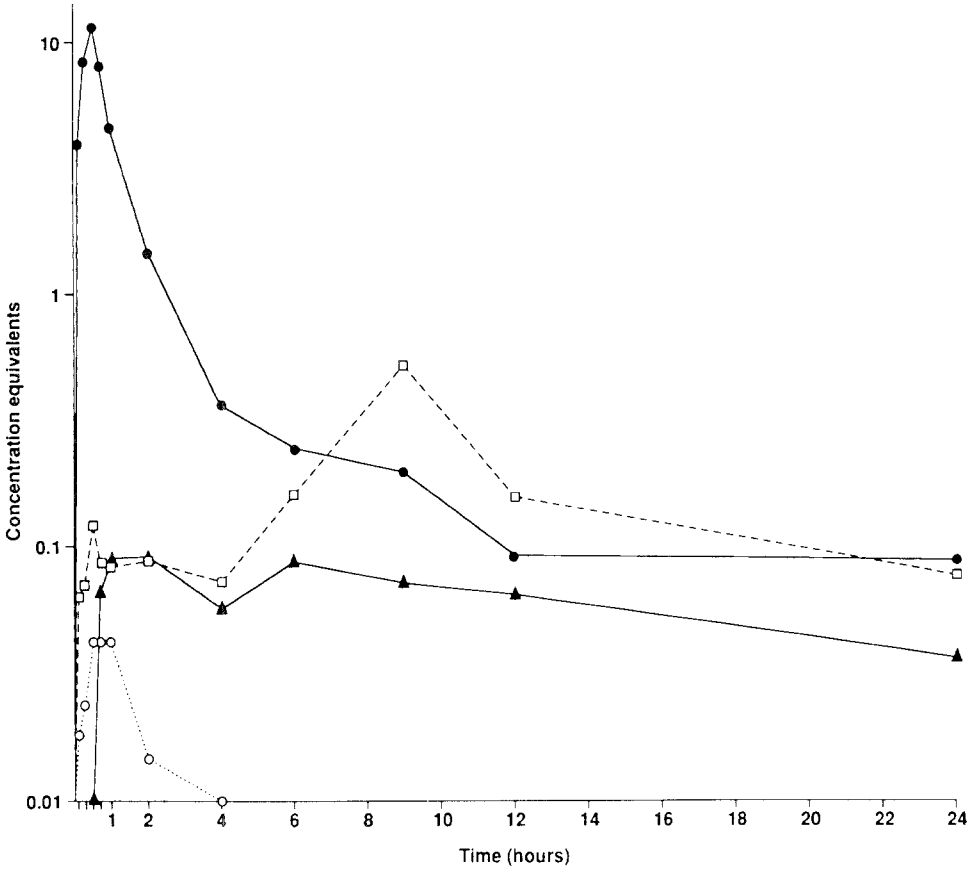

Fig. 1. Plasma profiles of GR63178A and its metabolites after administration of 80 mg/m^2. GR63178A, ●——●; M1, ○ · · · · ○; M2, ▲——▲; M5, □– – – –□. Concentration equivalents represent μg/ml for GR63178A and M1 and integrated HPLC peak areas for M2 and M5, which have yet to be identified chemically

Table 3. Values obtained for 24-h cumulative urinary excretion of GR63178A

Dose (mg/m^2)	Day 1		Day 4	
	Amount (mg)	% Dose	Amount (mg)	% Dose
40	ND	ND	1.56	3.12
80	1.62	1.2	4.58	3.27
80	ND	ND	4.34	2.89
120	5.93	2.76	4.6	2.14
120	5.16	2.35	17.97	8.17
120	6.87	4.58	ND	ND
120	4.08	1.7	ND	ND
120	ND	ND	4.63	2.1
160	8.09	2.79	8.31	2.86
160	4.76	1.9	ND	ND

ND, Not determined

with headache (1), pleuritic (1), and abdominal (1) pain. One patient (120 mg/m^2) complained of severe loin pain that was not apparently related to disease. Patients receiving a second cycle of treatment did not invariably experience recurrent pain; however, when pain recurred, it did so in the same site.

Nausea and vomiting (23/24 patients) that was readily controlled by standard anti-emetics was apparent at all dose levels (WHO grade 1 in 6 cases, grade 2 in 12, and grade 3 in 5). This effect did not appear to be dose-related. Other toxicities were minor and included headache (7/24 patients), phlebitis at the site of injection (6/24), shivering (1/24), fever (3/24), paraesthesia (1/24) and loss of taste (2/24). One man developed anti-D auto-immune haemolytic anaemia on a background of heavily pretreated stage IV Hodgkin's disease. His haemoglobin value dropped by 2 g/dl to 7.6 g/dl during the second cycle of treatment, and a short course of steroid therapy was eventually required to maintain a satisfactory red cell count. Two patients treated at 120 mg/m^2 developed WHO grade 2 anaemia, but no other significant haematological or biochemical disturbance occurred.

Pharmacokinetics and urinary excretion

The pharmacokinetics of GR63178A are shown in Table 2, their main features being a short terminal half-life (3.6±2.8 h) and a high rate of clearance (6.6±3.8 l h^{-1} m^{-2}). At doses of ⩾80 mg/m^2, AUCs were >7 μg ml^{-1} h, the value in rats after a dose of 75 mg/m^2 that proved to be therapeutic against the D23 hepatoma [2]. Over the dose range studied (40–160 mg/m^2), there was no evidence of saturation of clearance mechanisms and there was a good relationship between dose and AUC (P <0.05), thus illustrating the linear pharmacokinetics previously demonstrated for mitoquidone [5]. No significant difference was observed in half-life, $AUC_{0-24\,h}$ or clearance between day –1 and day –4 administration within patients (Student's paired t-test).

Only a small fraction of the delivered dose was excreted in the urine as the parent drug (day 1, 2.47%±1.1%; day 4,

3.51% ± 2.1%; Table 3). However, up to seven metabolites, including the parent, were also detected in urine. There were marked quantitative and qualitative inter-patient variations in GR63178A urinary metabolites, which normally consisted of glucuronide conjugates of the parent drug and its 9-hydroxy metabolite GR54374X [3].

Responses

Assessments of the response status of patients on the basis of International Union Against Cancer (UICC) criteria revealed no change (NC) in seven patients and progressive disease (PD) in eight cases; nine subjects were not considered to be evaluable for response.

Discussion

GR63178A has exhibited a novel structure, an interesting spectrum of activity and an unusual toxicity profile in animal studies. In the present phase I study we showed that there was no haematological toxicity at the MTD or at levels at which the AUC was greater than that achieved by therapeutic doses in the rat. The most striking adverse effect was the occurrence of pain at all dose levels, which was the side effect that defined the MTD. Nausea and vomiting was a common feature but was readily controlled. Other side effects were mild.

The nature of the pain experienced was diverse. The WHO grade was assigned according to the level of analgesia required. In this study, headaches were moderately severe at worst. In some cases the pain was described as being similar to that associated with a "flu-like illness". Considering this observation and that activity is seen only in in vivo models, not in vitro, it has been suggested that GR63178A or a metabolite may mediate these effects via stimulation of biological response modifiers. In other cases the pain was more like ischaemic pain, which may indicate some disturbance of tumour vasculature. Although there was no significant increase in lactic dehydrogenase (LDH), other possible markers of ischaemia such as creatine kinase were not specifically measured.

In an initial characterisation, six major metabolites (M1–6) of GR63178A were detected [3]. In the present work a further metabolite (M7) was detected. GR54374X was designated M1 on the basis that it was the first metabolite to be detected in plasma; it normally disappeared below the limit of detection of our assay in <1 h. A typical plasma profile is shown in Fig. 1 along with those for M2, M5 and GR63178A. It should be noted that the profiles illustrated for M2 and M5 in Fig. 1 reflect only the relative variation in those two species with time rather than their absolute concentrations, since they have yet to be identified chemically. M2, a non-urinary species, was a dominant plasma metabolite and always appeared after M1 (with which it shares chemical properties) [3]; M2 exhibited a longer half-life than did the parent drug. Two glucuronide conjugates of GR54374X have also been detected, and these have been designated M3 and M4. Although both are normally present in urine, only M3 was detected in plasma.

Chemically, the most unusual material is M5 [3]. In the present study it showed an unusual plasma profile (Fig. 1), achieving peak levels as late as at 20 h after drug administration and exhibiting marked inter-patient variation, occurring at significant levels in plasma and urine in only 6 of 19 subjects. By careful use of HPLC with diode-array detection, we could show that M5 is not after all a drug metabolite of GR63178A but an endogenous component occurring in small amounts in pre-dose plasma (and urine) in most of the 19 subjects tested. The reason why M5 concentrations in plasma increased in only six patients and the clinical consequences of this phenomenon are presently under investigation.

The direct glucuronide conjugate of GR63178A, M6, was never detected in plasma. The newly identified metabolite, M7, was found in only eight cases and showed a plasma profile similar to that of M1. M7 exhibited an HPLC retention time and a UV-visible absorption spectrum corresponding to the major product of base-catalysed hydrolysis of GR63178A, which appears preferentially to break the ester linkage between the benzyl ring and the phosphate group rather than that between the pentacyclic pyrroloquinone ring system and the phosphate group, which would produce GR54374X. If this is the case, then benzyl alcohol should be released as a side product in these patients. However, this compound is not known to produce any severe toxicity in humans.

Consequent to the establishment of the MTD for humans at 160 mg/m^2 daily in this phase I trial, GR63178A has now entered European Organisation for Research and Treatment of Cancer (EORTC) and Cancer Research Campaign (CRC) phase II clinical trials at a dose of 120 mg/m^2 daily for 5 consecutive days in a 21-day cycle.

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