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Phase I and pharmacokinetic studies with the pentacyclic pyrroloquinone mitoquidone*

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Summary. Mitoquidone (MTQ) is the first member of a new group of pentacyclic pyrroloquinones developed for clinical evaluation as a potential anticancer agent. MTQ demonstrated good activity in a range of experimental solid tumour models, but was weakly active against standard prescreens such as the P388 murine leukaemia. Bone marrow suppression or other significant toxicity was not observed in preclinical studies. Twenty-seven patients were treated with MTQ given as a 4-h infusion either once every 21 days (150–600 mg/m^2), once a week (200 mg/m^2 per week), or as 5 daily doses repeated every 28 days (60–180 mg/m^2 per day). The major adverse events encountered included nausea and vomiting (in virtually all patients), dyspnoea, tumour-related pain, and thrombocytopenia in several patients with pretreatment bone-marrow impairment. Phase I studies were suspended without a maximum tolerated dose being reached because of formulation difficulties. There were no major responses, although stable disease was observed in a number of patients with gastrointestinal malignancies. Temporary remission of B-symptoms occurred in two patients with lymphoma. The plasma pharmacokinetics of MTQ were investigated using an HPLC assay with fluorescence detection. Linear pharmacokinetics were observed with a terminal plasma half-life of 2.9 ± 2.1 h (n = 18 doses). The volume of distribution was 3.4 ± 2.6 l/kg and plasma clearance was 629 ± 469 ml/min per m^2. Several soluble analogues with similar antitumour activity are currently under investigation.

Introduction

Mitoquidone (MTQ, Fig. 1) is the lead compound of a recently developed group of pentacyclic pyrroloquinones which are being synthesised for clinical evaluation. MTQ is active in several murine solid tumour models (Sarcoma 180, D23 hepatoma), and in colon and human mammary tumour xenografts, whereas it is only weakly active in the murine leukaemias L1210 and P388 [2]. This antitumour spectrum from a novel chemical structure, of an agent without evidence of bone marrow toxicity in animals [2], led to clinical studies to identify the maximum tolerated dose, the optimal schedule and pharmacokinetic parameters in patients. Formulation problems necessitated suspension of the clinical protocols before the maximum tolerated dose was reached. This communication describes the preliminary clinical observations, assay methodology and plasma pharmacokinetics of MTQ.

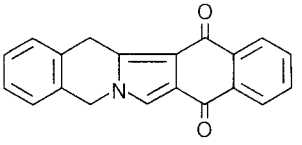

Fig. 1. Structure of mitoquidone

Patients, materials and methods

Patients. Twenty-seven adults with histologically confirmed malignancy, both pretreated and non-pretreated, an expected survival of over 2 months and a performance status of 0–2 on the ECOG scale entered treatment protocols. Patient diagnoses are shown on Table 1. Other inclusion criteria included: WBC $\geq 3.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l and normal or only slightly abnormal renal and liver function. Physical examination and monitoring

Table 1. Diagnoses of patients receiving MTQ

Carcinoma	19
Non-small cell lung	5
Small cell lung	1
Breast	2
Colorectal	2
Pancreas	1
Cervix	1
Ovary	1
Renal	1
Unknown primary	5
Lymphoma	6
Non-Hodgkin's	3
Hodgkin's	3
Melanoma	1
Osteosarcoma	1
Total	27

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of haematopoiesis, liver and renal function were repeated at frequent intervals. Toxicity was recorded and graded according to the WHO scale [5]. Tumour response was measured according to standard WHO criteria [5].

Formulation. Mitoquidone was developed and distributed by Glaxo Group Research Ltd (Greenford, UK). The compound is insoluble in water, and was administered as a microcrystalline suspension of 1 g/l MTQ in a vehicle containing 0.5 g/l soyabean lecithin, 25 g/l propylene glycol (PG) and 75 g/l polyethylene glycol 300 (PEG 300).

Clinical protocols. MTQ was administered as an (arbitrarily chosen) 4-h constant-rate infusion according to one of the following schedules: once every 21 days (Royal Marsden and Clatterbridge); daily for 5 days every 28 days (Amsterdam and Nijmegen); or once weekly (Essen). The initial dose was 150 mg/m^2 for the single administration schedule, 60 mg/m^2 per day for the daily for 5 days schedule, and 200 mg/m^2 for the once weekly schedule. The dose escalation schedule, numbers of courses given and patients treated at each dose level are shown in Table 2. One increment in dose was permitted for each patient and three patients received a total of at least four courses at each dose, in each protocol, before escalation.

The protocols were approved by the EORTC Protocol Review Committee (for the studies at Amsterdam, Nijmegen and Essen) and the ethical committees of each hospital. All patients gave informed consent.

Pharmacokinetic studies. Blood samples were obtained in heparinized tubes prior to, during and at various time points up to 24 h after the administration of MTQ. After centrifugation, plasma was separated and stored at −20° C until analysis. For high-performance liquid chromatography (HPLC) analysis 200 µl plasma was mixed with 200 µl acetonitrile (ACN) added with vortexing. After a short centrifugation step (10000 rpm, 1 min, Beckman Microfuge B), 100 µl supernatant was injected onto the HPLC column. The chromatographic system consisted of a Constametric III pump (LDC/Milton Roy, Stone, UK) and a 100-µl sampling loop. An ODS-Hypersil 10 µ column (100 × 4.6 mm) was eluted with ACN : water (50 : 50 v/v) at a flow rate of 1 ml/min. An LS-3 fluorescence detector (Perkin-Elmer, Beaconsfield, UK) was used with excitation at 388 nm and emission at 490 nm (lower level of quantitation 0.01 mg/l). MTQ concentrations were calculated from a calibration curve of peak height versus concentration over a concentration range of 0.01–50 mg/l (percentage SD of the slope was < 5%). For the calibration line, pure MTQ was dissolved in *N,N*-dimethyl acetamide (DMA, Analar grade BDH, Poole, UK), and aliquots were immediately added to plasma (the solution is photosensitive).

When plasma samples spiked with MTQ at concentrations in the range of 0.01–50 mg/l were assayed (n = 6 at each concentration), the coefficient of variation (CV) was < 4.1% at concentrations of > 0.1 mg/l, and up to 12.4% at concentrations of < 0.1 mg/l. Spiked plasma samples (n = 9) in the clinically important range of 0.01–9.0 mg/l were assayed in triplicate at different institutions (Glaxo Group Research, Institute of Cancer Research and St. Radboud University Hospital). Slight modifications to the method described above were permitted. Reproducibility within each laboratory was better than 6% CV, and between the laboratories was better than 15% CV. Storage of samples of 0.01–10 mg/l at −20° C led to a maximum loss of < 16% over a period of 28 days.

Plasma MTQ concentration data could be fitted to a two-compartment open model, using the NONLIN computer program [4]. The area under the plasma concentration-vs-time curve (AUC) was calculated by the trapezoidal rule corrected to infinity. The clearance (Cl) was calculated as dose/AUC and the volume of distribution (Vβ) as Cl/β, where β is the first-order rate constant for the terminal phase of disposition.

Table 2. Dose schedules used in the phase I studies of mitoquidone

Dose schedule	Total dose (mg/m^2 per course)			
	150	300	600	900
Daily × 5 q 28 days	–	4/7	8/12	5/5
Single dose q 21 days	3/4	9/21	5/7	–
Single dose q 7 days	–	–	1/1	–

Figures shown give no. of patients/no. of courses of treatment at each dose level

Results

Clinical

Patients' diagnoses are shown in Table 1, and the numbers of patients and of courses at each dose level are shown in Table 2.

There were no major differences in severity or incidence of toxicity between the three dose schedules. The analysis of toxicity by WHO criteria is shown in Table 3. The most frequently encountered adverse events were nausea/vomiting and dyspnoea. Nausea and/or vomiting oc-

Table 3. Toxicity of mitoquidone (WHO criteria)

Dose (mg/m^2)	Grade of toxicity (WHO-scale)					Total toxic observations per dose (%)
	0	1	2	3	4	
Nausea/vomiting						
150	1*	0	2	1	0	3 (75)
300	1	5	15	7	0	27 (96)
600	0	0	8	11	1	20 (100)
900	0	0	3	2	0	5 (100)
Thombocytopenia						
150	3	1	0	0	0	1 (25)
300	23	1	2	0	2[a]	5 (18)
600	15	2	0	0	3[a]	5 (25)
900	5	0	0	0	0	0
Dyspnoea						
150	3	1	0	0	0	1 (25)
300	22	1	2	2	1	6 (21)
600	15	1	2	2	0	5 (25)
900	3	0	0	0	2	2 (40)

[a] Entered despite protocol selection criteria
* No. of courses at which toxicity was observed

curred during 55/57 courses of MTQ, with no clear dose relationship for frequency or severity. Ten patients experienced dyspnoea during 14 courses, and in eight of these patients the dyspnoea was associated with chest pain. Two out of these ten patients had proven pulmonary emboli, and in seven of the remaining eight patients with dyspnoea, treatment was terminated because of adverse events.

Severe thrombocytopenia (WHO grade 4) without leucopenia or anaemia occurred after five courses in three patients. Two patients had received extensive prior chemotherapy, and the third had bone marrow infiltration by tumour. All three patients had pretreatment thrombocyte counts below 100×10^9/l (normal range $130-450 \times 10^9$/l) and therefore violated one of the criteria for entry into the study.

Mild and ill-defined low-grade fatigue and/or headache occurred in 15 (26%) of 57 courses. Pain at the tumour site was reported in eight patients during or immediately after treatment and was severe enough in one case to require re-admission to hospital.

Three patients died during the study. Although they all had very advanced disease, their deaths were unexpectedly sudden. Two developed severe abdominal pain related to the site of metastases within 48 h of the administration of MTQ and died 4 and 13 days later. One patient developed multiple pulmonary emboli and died 2 days later. Autopsy yielded no evidence, either macroscopic or microscopic, that the deaths were treatment-related.

Only 14 of the 27 patients were evaluable for response, the remainder having been withdrawn from treatment because of adverse events or clinical deterioration; 8 of the 14 evaluable patients had stable disease. On the "daily $\times 5$" schedule, 2 patients with Hodgkin's lymphoma and 2 with adenocarcinoma had stable disease for 3–6 months, with marked temporary improvement in B-symptoms in the lymphoma patients. Four patients treated according to the "once every 21 days" schedule had stable disease for 1.5–6 months. One of these was a patient with carcinoma of the pancreas, who showed significant clinical improvement; this, however, may have been partly attributable to the insertion of a Denver shunt. Six patients developed progressive disease during the treatment.

Pharmacokinetics

A typical MTQ plasma level/time profile is illustrated in Fig. 2. After termination of the infusion, a mean initial rapid half-life of 0.4 ± 0.3 h was observed, followed by a mean terminal half-life of 2.9 ± 2.1 h ($n = 18$). Pharmacokinetics were linear over the dose range studied (60–600 mg/m^2), as indicated by a plot of AUC as a function of dose (Fig. 3). Pharmacokinetic data have been summarized in Table 4. The mean volume of distribution was 3.4 ± 2.6 l/kg. Mean plasma clearance was high, at 629 ± 469 ml/min per m^2.

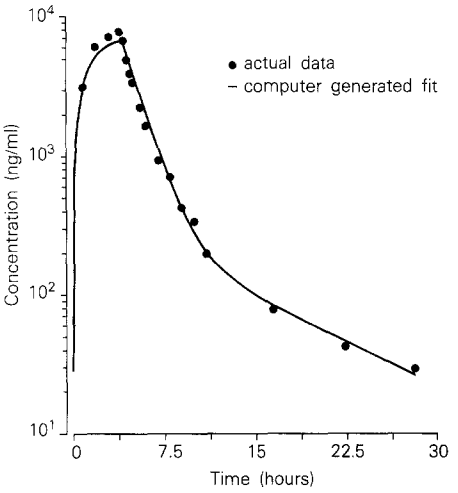

Fig. 2. Plasma levels of mitoquidone in a patient treated with 600 mg/m^2 over 4 h

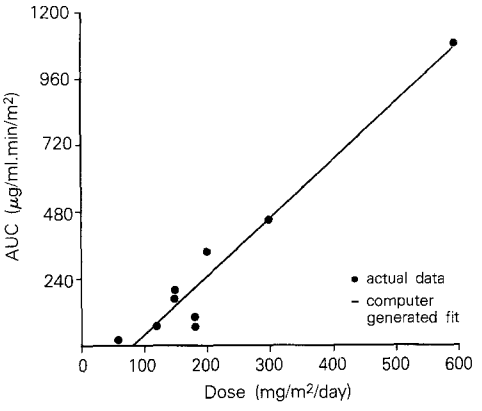

Fig. 3. Mitoquidone plasma AUC as a function of dose

Table 4. Pharmacokinetic parameters of mitoquidone
All values relate to day 1 of administration at each dose level

Dose mg/m^2 per day	n	Peak plasma concentration	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	V_β (l/kg)	Cl (ml/min per m^2)	AUC (µg/ml × min/m^2)
60	1	0.29	<0.01	0.5	1.1	1034	25
120	3	0.72±0.33	0.05, 0,03[a]	2.7±2.9	4.7±3.4	1101±737	72±37
150	2	2.09, 3.28	0.5, 0,2	6.4, 1.4	5.1, 1.6	349, 503	201, 166
180	2	0.75, 0.63	0.2[a]	2.5, 2.3	4.8, 8.9	1042, 1504	105, 69
200	1	2.98	0.07	1.5	1.1	326	341
300	5	3.80±0.53	0.5±0.1	2.1±0.6	1.7±0.5	362±86	472±132
600	4	6.85±1.33	0.6±0.3	4.6±2.5	3.9±2.6	363±90	1080±197

Values are means ± SD. When $n = <3$ individual values are quoted
n, number of courses for which pharmacokinetics are available; $t_{1/2\alpha}$, $t_{1/2\beta}$, initial and terminal half-lives; V_β, volume of distribution; Cl, plasma clearance; AUC, area under the curve
[a] $t_{1/2}$ not quoted for one data set; poor model fit

No accumulation of MTQ was observed in the daily $\times 5$ schedule as determined by peak plasma levels. On the contrary, there was some evidence of an increase in the clearance of MTQ at days 2–5, compared with day 1, in four of eight courses.

At least five uncharacterized metabolites were observed in plasma. Only trace amounts of MTQ itself were seen in urine, whereas large amounts of as yet unidentified, mostly polar, metabolites were present. In addition, some patients reported passing dark coloured urine, which may be attributed to the presence of MTQ degradation products.

Discussion

Mitoquidone met many of the criteria for development as a potential anticancer agent: novel structure, activity in a range of murine and xenograft solid tumours and lack of major toxicity (notably no bone marrow toxicity) in animals, though the mechanism of action is unknown [2].

A microcrystalline suspension formulation was developed and subjected to standard animal toxicology. MTQ formulated in this manner caused vomiting in dogs, but no evidence of bone marrow suppression or other toxicity was seen in extensive animal studies.

In humans, adverse events observed included nausea and/or vomiting in almost all patients, ill-defined respiratory problems in about one-third and reversible thrombocytopenia (without anaemia or leucopenia) in 3 patients with pretreatment thrombocyte values below 100×10^9/l. Whether thrombocytopenia was due to bone marrow suppression, increased consumption, or some other mechanism is unknown. Of the adverse events encountered, the unexpectedly high incidence of respiratory symptoms was of the greatest concern. Of the total of 57 courses of MTQ, 14 (25%) were associated with dyspnoea, and these resulted in discontinuation of treatment in 9 of the 10 patients affected. Two patients had proven pulmonary emboli. Pain at the tumour site was observed in several patients, though it is unclear whether or not this effect was drug-related.

Whilst there was some concern that the high frequency of pulmonary adverse events may have been due to the administration of a microcrystalline suspension, no such problems occurred when larger doses of MTQ were administered to animals repeatedly for a month. Moreover, similar presentations of small particles are used uneventfully in radiographic imaging. The occurrence of these events does, however, indicate that caution should be exercised in the use of microcrystalline suspensions.

In addition, escalation of MTQ doses above those reported here would have necessitated the administration of extremely large volumes of propylene glycol and polyethylene glycol, which have been associated with such toxicities as lactic acidosis, haemolysis and hyperosmolality [3, 6].

The plasma pharmacokinetics of MTQ indicated that over the dose range 60–600 mg/m^2 the plasma AUC was linearly related to dose (Fig. 3). Following the end of the infusion, plasma levels decayed biphasically and a two-compartment open model could be fitted to the data. The high total plasma clearance values observed (Table 4) suggest rapid elimination, probably by metabolism, a number of drug-derived (fluorescent) metabolites being observed in plasma. Studies in mice, dogs and monkeys using ^{14}C-MTQ have shown that there is rapid dissociation of total drug-derived material and intact MTQ (Glaxo Group Research, data on file).

In agreement with the human data, MTQ also undergoes rapid plasma clearance in animals (200–1600 ml/min per m^2), with plasma clearance increasing on repeat administration. Taken together, the clinical and preclinical data suggest the MTQ undergoes rapid degradation in vivo. However, the metabolites remain uncharacterized.

This pharmacokinetic study demonstrated that following the administration of the pentacyclic pyrroloquinone MTQ, substantial plasma levels of the parent compound were achieved, which exceeded the peak MTQ concentration associated with response in the mouse model. No correlation could be found between the preclinical and the human pharmacokinetic data, particularly the ratio of the AUC values at the mouse LD_{10} and the projected human MTD [1], since (a) mouse LD_{10} values were never obtained and (b) it is not known whether MTQ itself is the active compound.

No major responses were seen, as is to be expected in such early studies, although the temporary abolition of B-symptoms in two lymphoma patients was encouraging. The novel structure remains of interest. Several soluble analogues with similar antitumour activity have been identified and are currently under laboratory investigation. One has entered clinical evaluation.

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