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Phase I and Pharmacokinetic Study of Brequinar (DUP 785; NSC 368390) in Cancer Patients

Marcel de Forni, Guy G. Chabot, Jean-Pierre Armand, Xavier Fontana, Gonzalo Recondo, Christian Domenge, Patrice Carde, Mirela Barbu and Alain Gouyette

Brequinar (DUP 785, NSC 368390) is a 4-quinoline carboxylic acid derivative with broad spectrum antitumour activity in experimental models that acts as an antimetabolite by specific inhibition of *de novo* pyrimidine synthesis. We performed a phase I study of brequinar administered as a 10 min intravenous (i.v.) infusion for 5 consecutive days, every 4 weeks. 67 evaluable patients were entered in this study and a total of 130 courses were administered at doses ranging from 2 to 350 mg/m^2. The dose-limiting toxicity was myelosuppression with predominant thromobocytopenia. Myelosuppression was dose-related and non-cumulative, with considerable interpatient variability depending on haematological risk factors. The maximum tolerated dose of brequinar was 210 mg/m^2/day in poor risk patients whereas patients with good risk haematological profile tolerated higher doses (up to 350 mg/m^2/day). Other non-limiting toxicities included nausea and vomiting, mucositis and skin reactions. Brequinar plasma pharmacokinetic profiles were biphasic with alpha half-life ranging from 0.1 to 0.7 h, and beta half-life ranging from 1.5 to 8.2 h. Increase in brequinar area under the plasma concentration versus time curves (AUC) was nonlinear. Day 5 brequinar pharmacokinetics obtained in 21 patients indicated a significant increase in AUC (47%) and half-life beta (133%) compared to day 1 pharmacokinetics in the same patient. Brequinar plasma AUC and the per cent change in platelet count at nadir were correlated ($P < 0.001$). Although no objective response was observed in this study, one minor response was noted in cervical lymph nodes of a Hodgkin's disease patient.
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INTRODUCTION

ORIGINATING FROM the Medicinal Chemistry Section of Dupont Pharma and screened by the NCI Developmental Therapeutics Program, brequinar [Fig. 1; DUP 785; NSC 368390; 6-fluoro-2-(2'-fluoro-1,1'-biphenyl-4-yl)-3-methyl-4-quinoline carboxylic acid sodium salt] is a substituted 4-quinoline carboxylic acid which was selected for further investigation because of its preclinical antitumour activity and water solubility [1]. *In vitro*, brequinar was one of the most potent inhibitors of small cell lung and colon cancer cell lines [2, 3]. It also demonstrated high antitumour activity in murine models (L1210 leukaemia, B16 melanoma and colon adenocarcinoma 38) [1, 4] and human tumour xenografts (MX-I breast, LX-1 lung, BL/STX-1 stomach and CX-1 colon carcinomas) [5]. Antimetastatic properties of brequinar were also observed [6].

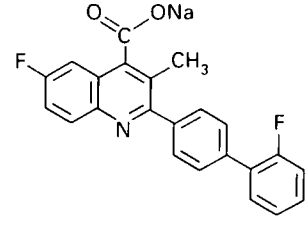

Fig. 1. Chemical structure of brequinar sodium (DUP 785; NSC 368390).

Correspondence to M. de Forni.
M. de Forni is at the Institut Gustave-Roussy, Unité La Grange 77176, Savigny Le Temple, France; J.-P. Armand, X. Fontana, G. Recondo, C. Domenge and P. Carde are at the Department of Adult Medicine; G. G. Chabot and A. Gouyette are at the Clinical Pharmacology Laboratory (CNRS URA 147; INSERM U 140), Institut Gustave-Roussy, 94805 Villejuif Cedex, France; and M. Barbu is at the DuPont Pharma S.A., Geneva, Switzerland.
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Brequinar is an antimetabolite which exerts its tumoricidal effect by inhibiting dihydroorotate dehydrogenase, a mitochondrial enzyme in the *de novo* pyrimidine nucleotide biosynthetic pathway [7, 8]. This inhibition can be reversed in cultured human cells by uridine. Brequinar antitumoral effect was found to be dose- and schedule-dependent *in vitro* and *in vivo*, with long term exposure producing optimal cell growth inhibition [9]. Single dose pharmacokinetic studies in mice, rats and dogs showed that brequinar has a relatively low clearance and a very high protein binding (96–99.7% bound). The elimination half-life in mice (4–7 h) was shorter than in other species (15–27 h). The urinary elimination accounted for 5–10% of the total injected dose [10].

In all animal species tested, the main side effects included gastrointestinal and bone marrow toxicities. These toxicities were schedule-dependent, i.e. repeated dosage was more toxic than single administration. In dogs, epithelial erosions and haemorrhages were observed. When brequinar was administered daily for 5 consecutive days in mice, the lethal dose for 10% of the animals (LD_{10}) was 192 mg/m^2/day, whereas the maximum tolerated dose in dogs was 6 mg/m^2/day.

Based on these preclinical data, we performed a clinical phase I and pharmacokinetic study of brequinar administered daily for 5 consecutive days. The objectives of this study were to define the clinical toxicity of brequinar, and determine its maximum tolerated dose (MTD) using the 5 consecutive days schedule. We also studied the relationships between pharmacokinetic parameters and brequinar pharmacodynamics.

PATIENTS AND METHODS

Patient selection

Selected patients with histologically confirmed advanced malignancy, but without standard alternative treatments, were entered into this phase I study. Eligibility criteria included: age between 18 and 75 years; a baseline WHO performance status ≤ 3; a life expectancy of at least 9 weeks; adequate bone marrow function (leucocytes > 4000/mm^3, platelets $> 100\,000$ mm^3), adequate renal and hepatic functions (serum creatinine ≤ 120 μmol/l, bilirubin < 25 μmol/l and other liver function tests < 2 times the normal upper range). No chemotherapy or extensive radiotherapy was administered 4 weeks before brequinar therapy (6 weeks for mitomycin-C and nitrosoureas). Patients with a history of gastric ulcers or neuropsychological problems were excluded. All patients gave their written informed consent.

Drug formulation and administration

Brequinar was supplied by DuPont Pharmaceuticals (Geneva, Switzerland) as a freeze-dried powder in vials containing 100 mg of active drug. The vial content was dissolved in distilled water for injection and further diluted in 100 ml 0.9% sodium chloride solution for injection before administration as a 10 min i.v. infusion through a peripheral or a central line.

Study design

All patients were treated on an inpatient basis at the Institut Gustave-Roussy, Villejuif. Patients had a complete history and physical examination before brequinar therapy, and were clinically evaluated at weekly intervals thereafter. Pretreatment laboratory tests included complete blood cell counts, complete biochemical profile, prothrombin time and urinalysis. Blood and serum biochemistry tests were repeated on days 3, 5, 8, 15, 21 and 28. A chest X-ray and electrocardiograms (ECG) were also performed every 4 weeks. When possible, baseline tumour measurements were taken before treatment and every 8 weeks. Toxicity assessment and tumour response were based on the WHO criteria.

Brequinar was administered as a daily single dose, for 5 consecutive days, every 4 weeks, according to patient tolerance or until disease progression. Based on preclinical toxicity data, the starting dose was 2 mg/m^2/day, which corresponds to 1/3 of the dog MTD. At least 3 patients were entered at each dose level after a 1 week interval. Subsequent groups of new patients were serially entered at higher dose levels following a modified Fiboncacci escalation scheme. Intrapatient dose modification was allowed as follows: dose reduction was possible if severe toxicity ($\geq$ grade 3–4) occurred, and dose escalation was permitted if no significant ($<$ grade 3) and reversible toxicity was observed in the first course, and if at least 3 patients had been safely treated at the higher dosage. Brequinar doses were escalated until the MTD was reached. The MTD was defined as the dose resulting in severe and reproducible toxicity(ies) of grade 3 or 4 in 50% or more of the patients. Above the 100 mg/m^2/day dose level, significant toxicity occurred, and patients were divided into good and poor risk groups, based on pretreatment, performance status and extent of disease. At the highest dose levels, good risk patients could be treated every 3 weeks, provided no severe toxicity occurred.

Pharmacokinetics

Blood sampling: Pre-infusion and post-infusion blood samples (8 ml) were collected on days 1 through 5 from an indwelling i.v. heparin lock in the arm contralateral to the infusion line. Complete pharmacokinetic sets were obtained in 28 courses on day 1, and in 21 courses on day 5. For these complete pharmacokinetic sets, blood samples were collected at the following time points following drug administration: 5, 10, 15, 30, and 60 min, and 2, 3, 4, 6, 8, 10, 14, 18 and 24 h postinfusion. The plasmas were separated by cold centrifugation and were immediately frozen at −20°C in polyethylene tubes until analysis.

High-performance liquid chromatography (HPLC) analysis. Plasma brequinar concentrations were assayed by HPLC at Laboratoire Riotton S.A. (Geneva, Switzerland). The extraction was accomplished as follows: 1 μg of the internal standard (DuPont compound DUP 416) was added to 1 ml of plasma and 200 μl tetrabutylammonium hydroxide (4 mmol/l); 10 ml of methylene chloride were added to the mixture, shaken at high speed for 30 min, and centrifuged for 10 min at 4°C; the aqueous layer was aspirated off, and the organic layer was transferred to a clean tube and evaporated to dryness under a nitrogen stream. The residue was reconstituted with 500 μl of mobile phase and 50 μl was injected onto the HPLC system. The mobile phase (flow rate, 1 ml/min) was composed of 63% acetronitrile and 37% phosphoric acid (46 mmol/l). Calibration curves were linear from 0.05 to 10 μg/ml, and were obtained from the peak height ratios of brequinar to the internal standard, plotted against the control sample. The peak height ratios of unknown plasma samples were used to determine concentrations according to the calibration curve. In these conditions, the lower limit of quantification was 25 ng/ml with 1 ml of plasma. The intra-day precision had a mean coefficient of variation of 4.3%, and the inter-day precision had a mean coefficient of variation of less than 10%.

Pharmacokinetic analysis. Plasma brequinar concentrations were fitted to a two-compartment model with a 10 min constant i.v. infusion input and first-order output, using the nonlinear regression program PCNONLIN (Statistical Consultants Inc., Lexington, Kentucky). Pharmacokinetic parameters were calculated for 28 complete pharmacokinetic data sets obtained on day 1, and 21 complete sets on day 5, using standard formulae [11]. Briefly, the half-lives were calculated as 0.693/alpha or beta; the areas under the plasma concentration vs. time curve (AUC_∞) were calculated by the trapezoidal rule from time zero to infinity; the apparent volume of distribution of central compartment (V_C) was calculated as the Dose/C_{max}, where C_{max} is the calculated maximum concentration at time zero. The apparent volume of distribution at steady state (V_{SS}) was calculated as

$$Vd_{SS} = \frac{\text{Dose} \times \text{AUMC}_\infty}{(\text{AUC}_\infty)^2} - \frac{\text{Dose} \times T}{2 \times \text{AUC}_\infty}$$

where $AUMC_\infty$ is the area under the first moment curve, and T is the infusion time. The total body clearance was calculated as Dose/AUC_∞. Data are presented as the mean ± the standard error of the mean (SEM).

RESULTS

Patient population

69 patients were entered in this phase I study and 67 patients were eligible. 2 patients did not complete their brequinar course due to complications (rupture of the carotid artery in 1 and neuro-psychiatric problems in another). Table 1 summarises the patients' characteristics. The median performance status (PS) was 2 (range 0–3), with 69% of patients presenting a PS ≤ 2. Predominant tumour types were head and neck and gynaecological cancers (71%). 37 patients (53%) had metastatic disease mainly localised to lungs (n = 20), liver (n = 14) and bone marrow (n = 11). The remaining patients were treated for loco-regional recurrence. Of the 65 patients who received prior chemotherapy, 33 received two or more chemotherapeutic regimens, and 58 patients received radiotherapy and chemotherapy.

Dose escalation

The dose escalation procedure is presented in Table 2. A total of 128 brequinar courses were administered during this phase I study. Patients received a median of two courses (range 1–7), and 15 patients were administered three courses or more. 22 patients were dose-escalated, and a dose reduction was required for 3 patients.

Table 1. Patients' characteristics

Characteristics	No. of patients
Total entered	69
Male/female	48/21
Total evaluable	67
Median age (range)	51 (21–71)
Performance status (range)	2 (0–3)
Tumour types	
Head and neck	30
Gynaecology	18
Lung	5
Colorectal	2
Other	13
Metastatic disease	37
Measurable disease	33
Prior therapy	
Chemotherapy	65
Radiotherapy	61
Chemotherapy and radiotherapy	58

Table 2. Brequinar dose escalation scheme

Dose (mg/m²/day)	No. of patients	No. of first course	Total no. of courses
2	4	4	5
4	3	3	3
8	4	3	6
16	4	3	5
24	3	2	4
36	6	3	7
48	6	3	9
65	7	5	10
90	3	2	4
100	6	5	9
140	9	8	12
170	16	9	20
210	15	13	18
250	6	3	8
300	5	3	7
350	1	0	1

There were 21 intrapatient dose escalations and three intrapatient dose reductions.

Haematological toxicity

The first 28 patients enrolled from the starting dose level (2 mg/m²/day for 5 days) to the 90 mg/m²/day dose level (9 dose levels; 53 courses) did not experience significant or reproducible toxicity. 2 patients however presented grade 2 thrombocytopenia at low dose levels (4 and 8 mg/m²/day), 1 of which had a documented bone marrow involvement.

At 100 mg/m²/day, a heavily pretreated 71 year-old breast cancer patient with widespread bone marrow and liver metastases developed grade 4 thrombocytopenia associated with mild (grade 1) neutropenia and anaemia. This toxicity was reversible and was an early indicator of the haematological toxicity profile observed at higher doses.

At doses ≥ 100 mg/m²/day, haematological toxicity—mainly thrombocytopenia—was confirmed as the prominent side effect, and more extensive evaluation at each dose level and more prudent dose escalation were required, as detailed in Table 2. 41 additional patients were included (26 of them belonging to a population of heavily pretreated patients) and received a total of 75 courses. The haematological toxicity in this group of patients is presented in Table 3. Overall, at dose levels ≥ 100 mg/m²/day, 38% of patients receiving brequinar as a first course (16/41 patients) developed thrombocytopenia as follows: 6 grade 1, 6 grade 2, 2 grade 3 and 2 grade 4. Platelet transfusions were required in 4 patients, including a patient who experienced lower gastro-intestinal bleeding. The median time of onset of brequinar-induced thrombocytopenia was day 10 (range day 7–15), with median nadir time at day 12 (range day 7–15) and median time of recovery at day 16 (range day 12–19). Thrombocytopenia was clearly found to be dose-dependent and non-cumulative in patients who received two courses of brequinar or more, at either the same dose level (n = 8), or after dose modification (depending on haematological tolerance) including dose escalation (n = 13) or dose-reduction (n = 3).

Table 3. Brequinar haematological toxicity for doses superior to 90 mg/m²/day (WHO scale)

Dose mg/m²/day	No. of courses	Platelets no. (grade)	WBC no. (grade)
100	9	1 (4)	1 (1)
140	12	2 (1)	1 (1)
		3 (2)	1 (3)
		1 (3)	
170	20	6 (1)	1 (1)
		5 (2)	2 (2)
		3 (2)	1 (3)
210	18*	3 (1)	1 (1)
		2 (2)	2 (2)
		2 (3)	1 (3)
		1 (4)	
Good risk patients			
250	8	1 (1)	2 (1)
300	7	2 (1)	1 (1)
350	1	—	—

*Including 5 poor risk patients.

Of the 5 poor risk patients treated at 210 mg/m²/day as first cycle, 2 had grade 3 and 1 had grade 4 thrombocytopenia. All of the good risk patients treated at dose level > 210 mg/m²/day presented low platelet toxicity, even in the 5 patients treated at 3 week interval. Dose-dependent leukopenia was observed in 15 patients (8 grade 1; 4 grade 2; 3 grade 3) with a kinetic profile similar to thrombocytopenia, i.e. with a median time of onset on day 8, nadir on day 12, and recovery time on day 17. No patient experienced leukopenia-induced sepsis. Grade 4 leukopenia and thrombocytopenia were associated in 1 poor risk patient at the 210 mg/m²/day dose level. 23% of patients presented significant anaemia during the first course, as defined by a decrease in baseline haemoglobin level > 2 g/100 ml. Bone marrow aspirates performed in 8 patients at the time of haematological nadir showed decreased cellularity associated with early signs of myeloid regeneration.

Other toxicities

Non-haematological toxicities were encountered above the 100 mg/m²/day dose level (Table 4). 4 patients developed toxic skin reactions described as annular and erythematous patches, mainly affecting rubbing and peri-orifice areas (elbow, periocular and intertriginous regions, perineum, mouth), and developing into hyperpigmented, thickening and non-ulcerative desquamation. Drug rechallenge resulted in skin reaction recurrence in only 2 patients. Brequinar-related dermatitis was not dose-limiting. Mucositis was mild, infrequent (2 grade 1, 6 grade 2) and was associated with skin reactions in 50% of patients. Other toxicities included nausea–vomiting ≤ grade 2 (8 patients), diarrhoea ≤ grade 2 (2 patients). 10 patients experienced local discomfort at the injection site at higher drug doses, but no case of veinitis or phlebitis was observed. One accidental drug extravasation did not produce local tissue necrosis. Brequinar tolerance to the local discomfort was improved by further diluting the drug (in 200 ml) and lengthening the infusion time from 10 to 30 min. No alteration in cardiac, hepatic or renal functions were observed.

Table 4. Brequinar non-haematological toxicities for doses superior to 100 mg/m²/day

Dose mg/m²/day	No. of courses	Skin no. (grade)	Mucosa no. (grade)	Nausea and vomiting no. (grade)	Local no. (grade)
140	12	1 (2)	2 (1)	2 (1)	2 (1)
		—	1 (2)	2 (1)	—
170	20	1 (2)	1 (2)	1 (1)	3 (1)
		—	—	2 (2)	—
210	18	2 (1)	2 (2)	—	2 (1)
		2 (2)	—	—	—
250	8	—	—	—	—
300	7	1 (2)	1 (2)	1 (2)	—
350	1	—	1 (2)	—	1 (2)

No drug related toxic death occurred during this phase I study. 5 patients died during the study due to cancer progression ($n = 4$) or to septic complications ($n = 1$, with no concomitant drug-related myelosuppression).

Responses

Although 24 patients received two courses of brequinar or more at dose level > 100 mg/m²/day, no objective response was observed in this study. One minor response was however noted in cervical lymph nodes of a Hodgkin's disease patient at 210 mg/m²/day.

Pharmacokinetics

Pharmacokinetic data were obtained in 28 patients on day 1 of the 10 min i.v. infusion of brequinar, and also on day 5 for 21 patients. Representative brequinar plasma concentrations versus time curves at doses ranging from the starting dose (2 mg/m²) to the highest dose administered (350 mg/m²) are presented in Fig. 2. Brequinar plasma disposition was biphasic with a distribution phase alpha half-life ranging from 0.1 to 0.7 h, and a terminal beta half-life ranging from 1.5 to 8.2 h (Table 5). The volume of distribution of the central compartment V_c ranged from 2.5 to 9.4 l/m² and the volume of distribution at steady state V_{SS} ranged from 5.1 to 13.2 l/m². The clearance ranged from 0.8 to 4.9 l/h/m².

The area under the plasma concentration versus time curves

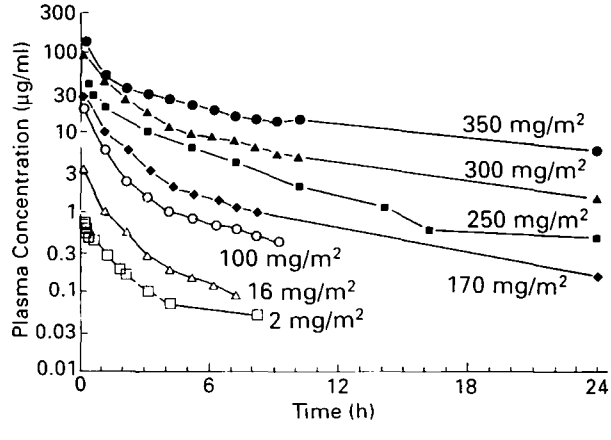

Fig. 2. Brequinar plasma concentrations in representative patients after a 10 min i.v. infusion on the first day of a 5 times a day drug administration schedule. Brequinar concentrations were determined using a high-performance liquid chromatographic assay described in the Patients and Methods section. Doses in mg/m² are indicated next to their corresponding curve.

*Table 5. Day 1 Brequinar pharmacokinetic parameters**

Dose mg/m² per day	No.	C_{max} µg/ml	Half-lives (h) alpha	beta	V_c l/m²	V_{SS} l/m²	AUC µg.h/ml	Clearance L/h/m²
2	2	0.6	0.2	1.6	2.7	5.1	0.9	2.4
4	2	1.0	0.1	1.5	3.2	6.3	1.3	3.8
8	5	1.8	0.4	2.9	4.1	8.2	3.2	3.2
16	3	2.7	0.4	3.1	4.7	11.6	3.8	4.9
24	1	5.1	0.3	2.0	4.0	8.7	5.6	4.3
65	1	16.5	0.4	2.4	3.6	5.5	36	1.8
90	1	19.1	0.5	4.0	4.2	10.8	26	3.5
100	2	15.6	0.3	3.6	9.4	13.2	53	3.0
140	1	40.2	0.2	2.5	2.9	6.7	66	2.1
170	2	30.6	0.2	2.1	4.7	8.3	54	3.3
210	2	79.1	0.5	3.6	3.7	8.4	111	2.4
230	1	43.0	0.5	5.5	4.0	10.2	147	1.6
250	2	54.5	0.7	4.6	4.3	8.6	157	1.7
300	2	86.5	0.7	8.2	3.3	9.2	284	1.1
350	1	127.0	0.4	6.2	2.5	6.4	428	0.8

*Brequinar was administered as a 10 min i.v. infusion.

(AUC) did not increase proportionally to the brequinar dose indicating that brequinar displays a dose-dependent (or non-linear) pharmacokinetics (Table 5 and Fig. 3).

In addition to day 1 pharmacokinetics, brequinar kinetic profiles were also obtained on day 5 in 21 patients. A comparison of day 5 versus day 1 pharmacokinetics revealed a significant mean increase of 47% in day 5 AUC ($P < 0.01$, Student's paired t-test) and a mean significant ($P < 0.005$) increase of 133% in half-life beta (Table 6). This increase in day 5 AUC could be due to an increase in half-life beta on day 5 (Table 6), since we observed a positive linear relationship between the per cent change in AUC and the per cent change in half-life beta ($r = 0.64, P < 0.01$).

Pharmacokinetic–pharmacodynamic relationships

Although a significant linear correlation was observed between the day 1 brequinar plasma AUC and the per cent change in platelet counts at nadir ($r = 0.506$, $P < 0.01$), the correlation was better between the $\log_{10}$ AUC and the per cent change in platelet count ($r = 0.709$, $P < 0.001$) (Fig. 4). A Spearman's rank correlation test between day 1 AUC and per cent change in platelet count at nadir was also significant ($r = 0.714$, $P < 0.001$, 25 patients).

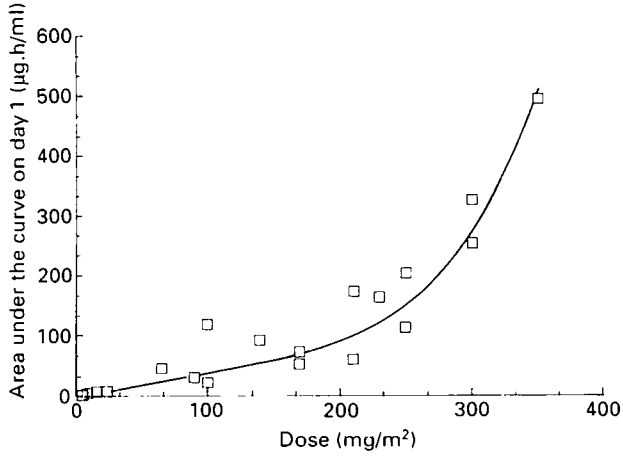

Fig. 3. Area under the brequinar plasma concentration curves (AUC; µg.h/ml) for the first day of treatment as a function of brequinar dose (mg/m²). The data were best fitted to a second order polynomial equation ($r = 0.997$; $P < 0.001$).

Table 6. Comparison of day 1 and day 5 brequinar pharmacokinetics in the 21 patients in which complete pharmacokinetics were available for day 1 and day 5

Patient no.	Dose mg/m²	AUC (µg/h/ml) Day 1	Day 5	% change	Half-life beta (h) Day 1	Day 5	% change
1	2	1.9	0.8	−59	1.8	2.4	34
2	2	1.3	1.1	−15	1.4	2.0	48
7	4	0.9	0.9	−1	1.1	1.8	59
4	8	2.5	2.5	0	2.9	1.3	−54
8	8	6.9	8.9	29	4.2	11.1	167
9	8	3.8	2.6	−33	3.4	1.6	−55
10	8	1.3	3.9	207	1.0	2.2	111
10	16	5.3	4.7	−11	5.9	5.6	−6
11	16	4.5	8.8	96	1.9	2.8	50
12	16	2.5	6.8	172	1.6	17.3	975
14	24	6.3	6.2	−2	2.0	1.9	−3
26	65	46	42	−8	2.4	2.6	9
30	100	22	32	49	3.0	2.8	−5
31	100	118	196	66	4.2	10.6	152
36	140	92	181	96	2.5	6.8	172
42	170	52	57	10	2.0	6.4	220
47	170	74	192	159	2.1	14.2	576
50	210	172	365	112	4.0	9.4	135
53	230	164	217	32	5.5	11.4	107
68	300	253	300	19	7.1	5.4	−24
69	350	497	833	68	6.2	13.3	115

DISCUSSION

Brequinar was brought into clinical evaluation based on preclinical data demonstrating a good spectrum of antitumour activity with acceptable toxicity, and also because of its unique chemical structure [1].

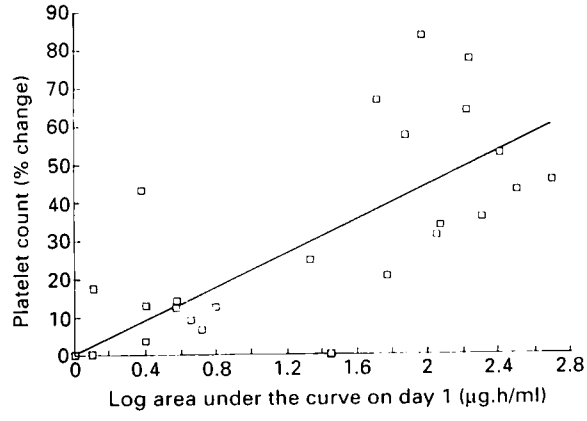

Fig. 4. Correlation between the $\log^{10}$ day 1 AUC (µg.h/ml) and the per cent change in platelet count at nadir. The line indicates the linear regression analysis ($r = 0.709$, $P < 0.001$). The formula used for the per cent change in platelet count at nadir is:

$$\frac{\text{count before treatment} - \text{count at nadir}}{\text{count before treatment}} \times 100.$$

Thrombocytopenia was the only dose-limiting toxicity in this phase I study, although less frequent and less pronounced leukopenia and anaemia were also observed. Myelosuppression was dose-related and non-cumulative. Heavily pretreated patients, or patients with poor performance status or widespread metastatic liver or bone marrow disease, were more sensitive to the brequinar-induced myelosuppression. For these poor risk patients, thrombocytopenia determined the brequinar MTD at 210 mg/m^2/day. However, good risk patients (i.e. without the above poor risk characteristics) showed acceptable haematological tolerance at doses up to 350 mg/m^2/day.

A similar pattern of brequinar haematotoxicity was reported in 3 of the 4 previously reported phase I studies of this drug administered as a 5 consecutive days every 4 weeks schedule [12], as a short term infusion every 3 weeks [13], and as a weekly or twice weekly administration [14]. In those trials, thrombocytopenia was also found dose-related and dose-limiting, although with considerable interpatient variability. Consequently, all clinical studies empirically divided patients into good and poor risk populations. Prior treatment seems to be the major factor which determined the haematological tolerance of high dose brequinar. Noe *et al.* [15] reported little myelosuppression at doses up to 300 mg/m^2/day using a 5 times a day schedule. Differences in patient selection (majority of colorectal cancer patients in the latter study) may explain this apparent discrepancy regarding the brequinar-induced haematotoxicity.

The main non-haematological side effects included skin reactions, mucositis, nausea–vomiting and diarrhoea, none of them being dose-limiting. The 4 cases of dermatitis were observed at high dose levels and in poor risk patients. The brequinar-induced dermatitis presented as maculo-papular desquamative lesions of the type, which were previously encountered in other phase I studies of brequinar [16] and, more generally, with anti-pyrimidine anticancer drugs [17]. Even though brequinar dermatitis was presumed to be dose-dependent, it did not define an additional dose-limiting toxicity, by contrast to the two previously reported phase I trials investigating a daily times 5 schedule [12, 15]. It is noteworthy that preclinical studies were predictive of both gastro-intestinal tract and bone marrow toxicities of brequinar.

Pharmacokinetic data indicated that brequinar displays non-linear pharmacokinetics in the dose range studied, in accordance with previous reports [13, 15]. This nonlinearity would be consistent with a saturation in metabolism and/or excretion of this drug, using the daily times 5 schedule. The observed correlation between brequinar AUC and thrombocytopenia confirms and extends the results obtained by Arteaga *et al.* [12], suggesting this pharmacokinetic parameter could potentially be predictive of platelet toxicity.

The poor antitumoral activity was also observed in other phase I reports which showed only two objective responses for a total of 243 patients included [12–15].

Based on this analysis, the recommended brequinar doses for phase II trials using a 5 consecutive days schedule, is 140–170 mg/m^2/day every 4 weeks in poor risk patients, as defined by poor performance status, heavy pretreatment or visceral metastases (liver, bone marrow). For good risk patients, with good performance status, no prior chemotherapy and no liver or bone marrow involvement, the MTD has not clearly been established in this study; however, a safe recommended phase II starting schedule could be 300 mg/m^2/day for 5 days, every 3 weeks.

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