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A Phase I Clinical and Pharmacokinetic Study of Brequinar Sodium, DUP 785 (NSC 368390), Using a Weekly and a Biweekly Schedule

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Abstract—*Brequinar, DUP* 785, *is a substituted* 4*-quinoline carboxylic acid derivative which in preclinical studies has shown broad antitumor activity. It is a novel antimetabolite blocking pyrimidine nucleotide synthesis. In a clinical phase I study,* 83 *patients were treated on a weekly schedule and* 18 *patients on a biweekly schedule. The drug was given intravenously as a short infusion. Three patients were entered on each dose level from a starting dose of* 6 mg/m^2 *up to* 2600 mg/m^2 *weekly. The dose ranges on a biweekly schedule were* 500–850 mg/m^2. *There was no dose escalation in individual patients. Pharmacokinetic studies were performed in* 19 *patients on a weekly schedule and in two patients on a biweekly schedule. A biphasic decay in plasma was observed with a median half life of* 10 h (5.1–23.4). *The main dose-limiting toxicity was thrombocytopenia. Of non-hematologic side-effects, stomatitis/mucositis occurred frequently. Skin eruptions occurred rarely, but were a major problem when found. All side-effects were fully reversible; there were no signs of cumulative toxicity. Antitumor activity was observed in one patient with a lung metastasis from a bladder cancer and in a patient with an unknown primary tumor. The recommended doses for phase II trials with DUP* 785 *are:* 1500–2000 mg/m^2 *on a weekly schedule and* 500–750 mg/m^2 *on a biweekly schedule dependent on status before treatment.*

INTRODUCTION

SUBSTITUTED 4-quinoline carboxylic acid was found in the National Cancer Institute, U.S.A. screening system to have antitumor activity. An analog, DUP 785 (NSC 368390), Brequinar (Fig. 1), was selected for clinical evaluation because of its water solubility and its broad antitumor efficacy. In preclinical studies, the compound was active against L 1210 leukemia and colon tumor 38 as well as against human xenografts from breast, lung, stomach and colon carcinoma [1]. DUP 785 acts as an antimetabolite by blocking *de novo* pyrimidine biosynthesis [2]. The mechanism of action has been demonstrated to be an inhibition of the mitochondrial enzyme dihydroorotic acid dehydrogenase (DHO-DH) [2, 3]. The inhibition can be reversed by uridine in cell cultures [3, 4]. The antitumor activity of the drug is dose- and schedule-dependent [1] with greater activity following longer time of exposure up to a certain concentration observed in *in vitro* studies [4]. Preclinical pharmacokinetic studies elicited an elimination half life in plasma of 4.5 h in mice, 17 h in rats, and 24 h in dogs. About 5–10% of the drug was excreted in the urine.

In mice, rats and dogs the main dose-limiting toxicity was gastrointestinal, but bone marrow hypoplasia was also a primary toxic effect in rats and dogs, with the dog being much more sensitive than the mouse, rat and monkey. The toxicity was schedule dependent, with LD_{10} in mice being 456 mg/m^2 following i.v. single dose vs. 192 mg/m^2 in a five daily dosage schedule. Repeated dosages were investigated in oral toxicity studies up to 90 days at different schedules. The results were quantitatively

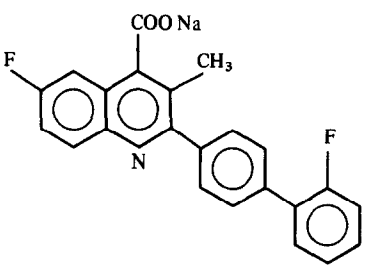

Fig. 1. Chemical structure of Brequinar, DUP 785: 6*-fluoro-*2*,* 2*'-fluoro-*1*,*1*'-biphenyl-*4*-yl)-*3 *methyl-*4*-quinoline carboxylic acid, sodium salt.*

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similar comparing the oral and i.v. routes. Evidence of venous destruction and phlebitis was observed following i.v. administration.

Based on the preclinical data of DUP 785 it was decided within the EORTC New Drug Development Plan to conduct phase I studies at different dose schedules varying from a single dose every 3 weeks [5] to a daily × 5 dosage every 3 weeks [6]. This report describes the results of clinical phase I studies of DUP 785 using a weekly and a biweekly schedule. The results of a pharmacokinetic study, which was carried out simultaneously, are also described.

PATIENTS AND METHODS

Patients

All patients entering the study had microscopically confirmed malignant solid tumor, and had either been unsuccessfully treated with standard therapy or were untreated because no recognized effective therapy was available. Informed consent was required to enter the study in addition to a life expectancy of at least 9 weeks and a performance status of ≤3 (WHO). Eligibility criteria included also normal hematologic values: leucocytes $>4.0 \times 10^9$/l and platelets $>100 \times 10^9$/l, normal renal and liver function tests, serum creatinine ≤120 μmol/l, bilirubin <25 μmol/l and other liver function tests within 2 times the normal upper limits. None of the patients had received systemic antineoplastic treatment or radiotherapy within the preceding 4 weeks (6 weeks for nitrosoureas and extensive radiotherapy) or had unresolved toxicity from prior treatment before entering the study, with the exception of alopecia in some patients. Excluded from the study were patients with gastrointestinal disorders, such as gastric or duodenal ulcers, and patients with clinical signs of brain metastases. Clinical assessment during the study included physical examination and blood cell counts before each treatment and more frequent if indicated clinically. The following measurements of serum or plasma values were performed before start of treatment and repeated every 3 weeks: alanine aminotransferase, (ALAT), lactate dehydrogenase (LDH), alkaline phosphatase, bilirubin, albumin, coagulation factors II + VII + X (PP), creatinine, sodium, potassium, and calcium as well as urine analyses for blood, glucose and protein. Chest X-ray and ECG were repeated every 4 weeks. In patients with evaluable disease, the various parameters, X-rays, ultrasonic or CT scans were repeated according to clinical status or routinely every 4–8 weeks. The WHO criteria [8] were applied for assessment of toxicity and tumor response.

Treatment plan

Brequinar, DUP 785, was supplied by Du Pont Pharmaceuticals, Geneva, Switzerland as a freeze-dried powder in vials containing 100 mg DUP 785, 40 mg sodium cholate and 40 mg of glycine or 500 mg DUP 785, 80 mg sodium cholate and 80 mg of glycine. The drug was dissolved in sterile water and diluted in isotonic saline varying from 50 to 2000 ml and administered as an intravenous infusion in 10 min up to 2 h depending on the dose. According to EORTC guidelines [9, 10], a starting dose of 6 mg/m^2 was based on 1/3 of the maximum tolerated dose (MTD) in dogs, which was the most sensitive species in preclinical studies. At least three patients were scheduled to enter each dose level with 1 week passing before the entry of the next two patients. At least 4 weeks should pass before dose escalation took place. When it became clear that the drug was well tolerated in humans, a modified Fibonacci escalation scheme with an increase of 1/3 per dose level was followed. Dose escalation was not permitted in individual patients.

Retreatment at the same dose level was given provided platelets were $\geq 100 \times 10^9$/l and/or leucocytes were $\geq 3 \times 10^9$/l. If hemoglobin was ≤6.0 mmol/l blood transfusion was given before treatment. Dose modification was applied by a delay in treatment until recovery from hematologic as well as non-hematologic toxicity. Reduction in dose was carried out in some patients with grade 3–4 toxicity. A minimum of 4 weeks of treatment was scheduled. Patients were regarded as evaluable for acute toxicity after receiving one course followed by at least 1 week's observation time. Treatment was discontinued in case of severe toxicity or when progressive disease was evident. In the case of stable disease or tumor regression, treatment was continued until progression, but with a limitation of 1 year unless recurrent complications made discontinuation necessary.

Pharmacokinetics

Plasma samples were collected from patients at various dose levels during the first course before drug administration and at 15, 30, 60, 120 min after infusion and thereafter with regular intervals for up to 4 days. The concentration of DUP 785 was analyzed by high-performance liquid chromatography (HPLC), as previously described [11]. Plasma AUC (area under the concentration vs. time curve) was calculated using the trapezoidal rule. The half-life was calculated from the semi-log graph of the serum concentration vs. time, $T_{1/2} = \ln 2/\text{slope}$. The total body clearance was calculated by dividing the total dose administered by the AUC, $CL_{tot} = D/AUC$.

RESULTS

Patients

From December 1985 to 1987 a total of 101 patients were admitted to the study, 83 on a weekly and 18 on a biweekly schedule. Of these patients, 61 had lung cancer, 22 gynecologic tumors with 12 ovarian, eight cervical and two endometroid carcinomas. Six patients had bladder cancer and 12 miscellaneous types of malignant solid tumors. More than 90% of the patients had a performance status (PS) of 2 or better according to WHO criteria, with 60 patients having a PS of 0–1 and 31 of 2. Of the remaining 10 patients with a PS = 3, four were excluded from further analyses, in three cases because of early death, unrelated to DUP 785, i.e. within 1 week after start of treatment, and one because of non-compliance. Forty-six of the patients had received prior chemotherapy and 27 were previously treated with radiotherapy, whereas 34 were previously untreated. Patient characteristics are summarized in Table 1.

Dose escalation

Weekly schedule. From a starting dose of 6 mg/m^2 28 patients were entered up to 54 mg/m^2 in nine steps. As no toxicity occurred, a further 55 patients were entered covering the dose ranges: 70, 90, 125, 160, 210, 280, 370, 490, 650, 860, 1140, 1500, 2000, 2300 and 2600 mg/m^2. At least three patients were included at each dose level until dose-limiting toxicity occurred at 2600 mg/m^2. Further evaluation of the dose levels 1500 and 2000 was made in four and eight patients, respectively. Seventy-nine evaluable patients received a total of 782 doses/courses, with 70 patients having ≥4 courses, 40 patients ≥8 courses and 19 patients ≥12 courses. Eight patients were treated for more than 20 weeks at different dose levels, and three patients were treated for as long as 43, 50 and 53 weeks, respectively (Table 2). Dose escalation did not take place within individual patients, whereas the dose was reduced in four patients at the highest dose levels; scheduled treatment was interrupted according to hematologic requirements in 15 patients, and because of non-hematologic toxicity in six patients. Discontinuation of treatment because of toxicity was required in four patients.

Twice weekly schedule. Based on preliminary results from the weekly schedule as well as on available pharmacokinetic data and also *in vitro* studies indicating greater activity of the drug following longer time of exposure [4], a biweekly schedule was

Table 1

	Schedule	
	Weekly	Biweekly
Patient characteristics		
No. of patients included	83	18
No. of patients excluded	4	0
Male/female ratio	44/39	9/9
Age: median (range)	54 (18–73)	50 (44–67)
Performance status (WHO)	0: 22	5
	1: 25	8
	2: 27	4
	3: 9	1
Prior therapy	none: 26	8
	chemotherapy: 39	7
	radiotherapy: 24	3
Diagnoses		
Lung cancer	50	11
adenocarcinoma (WHO III)	18	3
squamous cell (WHO I)	15	5
large cell (WHO IV)	7	2
small cell (WHO II)	4	1
mesothelioma	6	0
Gynecological tumors	20	2
ovary	10	2
cervix uteri	8	0
corpus uteri	2	0
Urinary bladder	4	2
Kidney	3	1
Other	6	2

Table 2. Dose escalation

No. of patients	Dose (mg/m^2)	No. of courses in the individual patient
3	6	4/7/8
3	12	4/15/21
2	18	11/5
3	24	3/8/16
4	30	3/6/6/50
3	36	43/14/
2	42	8/16
3	48	5/5/18
3	54	2/6/5
3	70	9/4/2
3	90	5/9/12
3	125	22/3/22
3	160	53/8/9
3	210	7/8/9
3	280	13/15/15
2	370	14/6
4	490	4/5/12/3
3	650	4/12/4
5	860	9/2/32/4/4
4	1140	5/7/9/9
4	1500	1/25/4/4
8	2000	8/4/12/16*/8/4*/10/20
3	2300	1/3/4
1	2600	2–5*

*Dose reduction.

applied in 18 patients covering the range 500, 600, 750 and 850 mg/m^2 twice weekly. The number of patients and courses per dose level are shown in Table 3.

Toxicity

Hematologic. Bone-marrow suppression was the main dose-limiting side-effect consisting primarily of thrombocytopenia. A decrease in platelet counts below 100 × 10^9/l was observed after two to four courses at dose levels above 1000 mg/m^2 (Table 4). Concomitant leucopenia and anemia occurred as well but were less pronounced. A complete recovery within 1–2 weeks following a delay in treatment was seen in all patients. A great inter-patient variation within the same dose level was observed. Up to a dosage of 2000 mg/m^2 weekly, no consistent significant hematologic toxicity was seen (Table 6). Consequently, one patient was exposed to 2600 mg/m^2. After two courses this patient developed grade 4 thrombocytopenia and grade 2 leucopenia as well as anemia. Pronounced non-hematologic toxicity occurred simultaneously. Three patients were therefore evaluated at 2300 mg/m^2. Of these, two previously treated patients developed hematologic as well as non-hematologic toxicity of grade 3. MTD was then settled to be 2300 mg/m^2 on a weekly schedule (Table 4).

At a biweekly schedule the MTD was found to be 600 mg/m^2 in previously treated patients vs. 850 mg/m^2 in previously untreated patients (Table 5).

Non-hematologic toxicity. Stomatitis/mucositis (SM) was observed in 50% of the patients at dose levels above 1000 mg/m^2. Symptoms of stomatitis often started 1–2 days after treatment and consisted of pain and soreness of the mouth developing into objective signs of erythema of the mucosa, eventually with a tendency to ulcerations especially of the lips, including the commissures, and the regions around the eyes. Up to and including the level of 2000 mg/m^2 the cases were mild to moderate (grade 1–2) and transient with relief within 1–2 weeks in spite of continued treatment. At dose levels above 2000 mg/m^2, two of four patients developed severe mucositis, including esophagitis (grade 3). Nausea and vomiting (grade 1–2) during infusion were reported in 40% of the patients at dose levels above 1000 mg/m^2. Antiemetics were rarely indicated. Grade 1–2 diarrhea or loose stools 1–2 days after treatment was observed too. Pain along the injection vein occurred frequently, but could be avoided by extra saline and prolongation of infusion time up to 2–3 h. Grade 2 alopecia was seen in one patient on the biweekly schedule (Tables 4 and 5).

Table 3. *Weekly* × 2

No. of patients	Dose (mg/m^2)	No. of courses in the individual patient
2	500	13/2
3	600	4/25/35
4*	600	4/25/35
3	750	3/40/21
2*	850	10/20†
4	850	4/10/50/4

*Prior treatment.
†Dose reduction.

Toxic skin reaction constituted a major problem, occurring in five of 97 patients (5%) at the following dose levels: 12, 160, 2000, 2300 mg/m^2 (weekly) and 750 mg/m^2 (biweekly). At the lowest dose levels, skin reactions occurred after several courses and consisted of diffuse erythemia, thickening and dry desquamation of the skin of the face and hands. Accordingly, the reaction was assumed to be photoallergic. However, at the dose level of 2300 mg/m^2, one patient had a pronounced skin eruption of the hands, wrists and along the inner side of the arms as well as of the neck and face, but also the intertrigineous areas and the perineum were affected. The reddening and patchy maculo-papular nodular reaction with ulcerations around the mouth and eyes was observed. Severe mucositis occurred as well. The patient recovered completely after 2 weeks of local steroid therapy. Retreatment was given with a 50% reduced dose. This time exactly the same type of reaction occurred, but much less severe. Retreatment was also carried out in a patient at 160 mg/m^2 without recurrence and in a patient at 750 mg/m^2 biweekly because both patients responded to treatment. The latter patient suffered especially from recurrent fungus infections appearing as exematous eruptions of the intertrigineous regions. Angioneurotic edema of the arm at the infusion side was seen in one patient at the first drug administration of 750 mg/m^2. He was retreated together with antihistamine for several courses without recurrence. Phlebitis of superficial veins was not observed. There was one accidental paravascular injection which did not cause necrosis. Three patients at 1500, 2000 and 2300 mg/m^2, respectively, developed hypotension during infusion of DUP 785. Among the latter patients, one (1500 mg/m^2) had cardiac failure treated with digitalis and diuretics while the patient treated with 2000 mg/m^2 had respiratory insufficiency because of a bronchogenic carcinoma. Treatment was immediately discontinued in two of these patients, while further treatment was tolerated following prolongation of infusion time in the patient with lung tumor.

No systemic allergic reactions were observed. All types of toxicity were fully reversible within 1–2 weeks. No cumulative hematologic toxicity was

Table 4. Toxicity of DUP 785 on a weekly schedule. No. of patients with toxicity grade 0–4 (WHO)

Dose (mg/m²)	Total No. of patients	Thrombocytopenia 1	2	3	4	Leucopenia 1	2	3	4	Anemia 1	2	3	4	Stomatitis 1	2	3	4	Nausea vomit/diarrhea 1	2	3	4	Skin reaction 1	2	3	4
6	3	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
12	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
30	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
70	3	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
125	3	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
160	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
370	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
490	3	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
650	3	1	—	—	1	1	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—
1140	4	2	1	—	—	—	1	—	—	—	1	—	—	1	—	—	—	1	1	—	—	—	—	—	—
1500	4	—	—	1	—	1	—	—	—	2	—	1	—	1	1	—	—	2	—	—	—	—	—	—	—
2000	8	2	1	1	—	—	2	—	—	3	1	—	—	2	1	—	—	3	1	—	—	1	—	—	—
2300	3	1	—	2	—	—	2	—	—	1	1	—	—	—	1	1	—	—	1	—	—	—	—	1	—
2600	1	—	—	—	1	—	1	—	—	1	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—

Table 5. Toxicity of DUP 785 on a biweekly schedule. No. of patients with toxicity grade 0–4 (WHO)

Dose (mg/m²)	Total No. of patients	Thrombocytopenia 1	2	3	4	Leucopenia 1	2	3	4	Anemia 1	2	3	4	Stomatitis 1	2	3	4	Nausea vomit/diarrhea 1	2	3	4	Skin reaction 1	2	3	4
500+	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—
600	3	—	—	—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
600+	4	—	1	2	2	—	1	—	—	1	1	1	—	3	1	1	—	1	—	—	—	—	—	—	—
850	4	2	—	1	—	—	—	—	—	—	1	—	—	—	1	1	—	—	1	—	—	1	—	—	—
850+	2	—	1	—	1	—	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
750	3	—	1	—	—	—	—	—	—	—	—	—	—	—	1	1	—	1	—	—	—	—	1	—	—

Table 6. *Median (range) nadir values in eight patients treated with* 2000 mg/m² *of DUP* 785

Week No.	Hemoglobin (mmol/l)	Leucocytes (× 10⁹/l)	Thrombocytes (× 10⁹/l)
1	8.0 (6.5–9.2)	7.9 (5.7–9.3)	332 (248–506)
2	7.9 (7.0–8.9)	4.9 (3.7–6.6)	264 (171–367)
3*	7.3 (6.2–9.0)	4.5 (3.0–5.6)	133 (50–244)
4	6.9 (5.6–8.8)	4.9 (2.7–6.7)	180 (86–396)
5*	7.2 (5.1–8.8)	5.5 (3.3–10.3)	254 (176–966)
6*	6.5 (5.8–8.8)	4.8 (4.3–8.7)	361 (64–545)
7*	6.9 (5.9–8.7)	6.1 (4.5–10.7)	340 (52–500)
8	7.1 (6.3–9.3)	8.6 (3.3–11.0)	256 (136–384)

*Dose modification in patients with platelets <100 × 10⁹/l or leucocytes <3 × 10⁹/l.

recorded even after long-lasting treatment up to 53 courses. There were no biochemical or physical indications of toxicity related to liver or renal function nor was damage to the central or peripheral nervous system observed. No toxic deaths occurred.

Recommended doses

In order to establish an optimal dose level for further investigation of the drug, more patients were included at dose levels below the MTD. A total of eight patients were treated with 2000 mg/m² weekly—all were previously untreated. Median nadir values of hemoglobin, leucocytes and thrombocytes are shown in Table 6 and Fig. 2. In conclusion, a mild to moderate degree of hematologic as well as non-hematologic toxicity occurred in six of eight patients at 2000 mg/m² (Table 4). A delay in treatment of 1–2 weeks was required in eight of the total of 73 courses, whereas dose reduction was done in two patients after two and eight courses, respectively. One of the four patients at 1500 mg developed grade 3 thrombocytopenia after two courses. This patient had been heavily pretreated for ovarian carcinoma. Based on these data (Tables 4, 5 and 6), the recommended doses for phase II studies are 1500–2000 mg/m² on a weekly schedule depending on the prior treatment and performance status of the patient. On a biweekly schedule, the recommended doses are 500 mg/m² to previously treated and 750 mg/m² to previously untreated patients, respectively.

Pharmacokinetic results

Pharmacokinetic studies were performed in 19 patients on a weekly and in two patients on a biweekly schedule at dose levels ranging from 24 to 2000 mg/m². A biphasic decay in plasma was observed (Fig. 3). The elimination half life, $T_{1/2}$, had a median of 10 h, range (5.1–23.4). Total body clearance, CL_{tot}, varied from 9.6 to 96.9 ml/min, decreasing with increasing dose (Table 7). The AUCs did not increase linearly with the dose (Fig. 4). The plasma concentrations measured during the biweekly schedule are shown in Fig. 5.

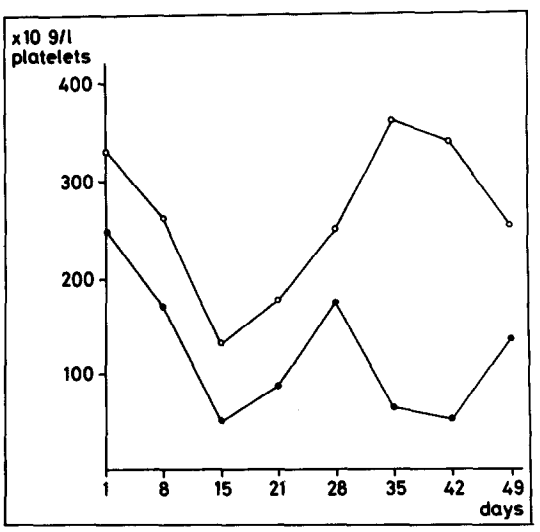

Fig. 2. *Median (top) and nadir values of platelets during treatment of eight patients with DUP* 785, 2000 mg/m² *weekly.*

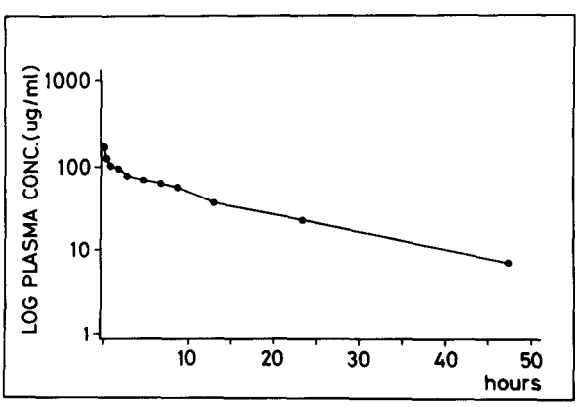

Fig. 3. *Decrease in plasma concentration of DUP* 785 *in patient No.* 67 *treated with* 1500 mg/m².

Table 7. Pharmacokinetic parameters of DUP 785

Patient No.	Dose (mg/m²)	AUC (μg*h/ml)	$T_{1/2}$ (h)	Cl_{tot} (ml/min)	V_d (1/kg)
Weekly schedule					
15	24	16.7	10.0	45.9	0.53
16	30	29.3	6.9	31.4	0.29
31	54	17.2	10.0	96.9	1.09
33	70	40.8	11.0	57.2	0.67
34	70	46.2	6.9	36.0	0.49
37	90	64.9	6.9	43.7	0.34
39	125	143.4	8.5	23.2	0.29
43	160	195.5	10.4	22.2	0.33
44	210	80.2	5.1	70.7	0.50
50	280	219.1	7.8	38.0	0.38
54	370	275.1	12.3	31.5	0.74
57	490	458.6	13.6	32.0	0.55
58	650	504.0	9.1	38.5	0.55
61	860	1330.0	16.5	19.4	0.46
68	1140	3537.0	14.5	9.6	0.25
67	1500	1720.0	14.4	30.3	0.53
82	1500	3235.0	16.4	14.9	0.24
72	2000	3212.0	14.7	17.6	0.35
81	2000	4987.0	23.4	12.9	0.37
Biweekly schedule					
3	600	2089	35.8	8.9	0.46
13	750	653	17.4	43.4	0.74

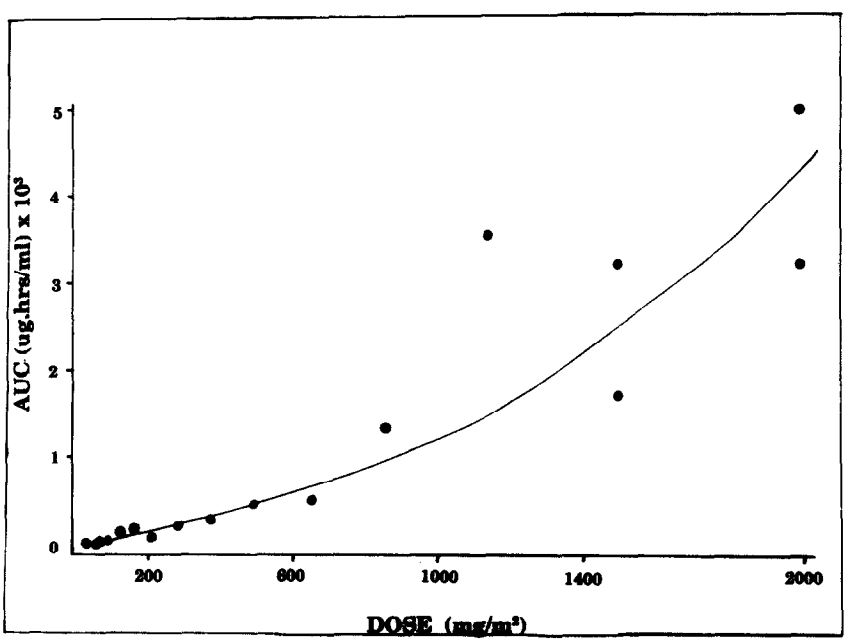

Fig. 4. AUC (area under the concentration vs. time curve) at various dose levels of DUP 785, range 24–2000 mg/m².

Antitumor activity

A partial remission of a biopsy verified lung metastasis from a bladder cancer was obtained in a 71-year-old woman after 14 weeks of therapy with DUP 785 at a dose level of 160 mg/m² weekly. The remission was maintained during treatment at the same dose level for 1 year. Surgical resection of the tumor was then performed disclosing a residual tumor of a clear cell type different from the biopsy taken before the start of treatment, which had shown transitional cell carcinoma. Interestingly, the primary resected bladder cancer represented a mixed tumor of clear cell as well as transitional cell carcinoma. Unfortunately, the patient died 3 months later of brain metastases. An autopsy was performed and brain metastases, histologically of

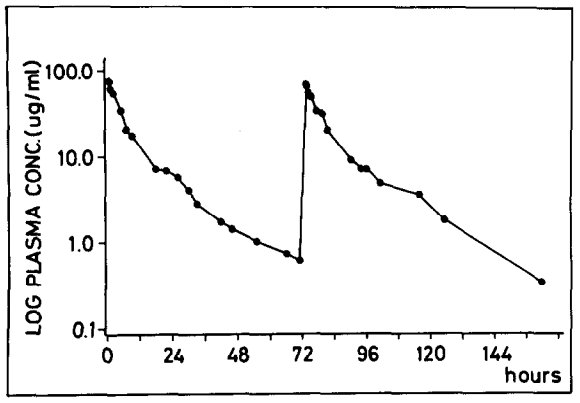

Fig. 5. Plasma concentrations of DUP 785 in patient No. 13 treated with 750 mg/m² × 2 weekly.

the transitional cell type, was found. Five other bladder cancer patients did not show any significant response during treatment and all were discontinued because of progressive disease.

Another patient treated with 750 mg/m² on a biweekly schedule had complete remission of an unknown primary tumor located in an axillary lymph node. This tumor was initially suspected to be a non-Hodgkin malignant lymphoma, but further histopathologic investigations did not confirm this diagnosis. In spite of special immunohistochemical tests, it was not possible to characterize the tumor further than as a low differentiated large cell carcinoma. Nor was any primary tumor found after extensive search. Conclusively, the patient was treated on an experimental basis with DUP 785. After 2 weeks of treatment, the tumor was reduced to 1/3 in size and after a further 4–8 weeks it could not be detected. Because of stomatitis and recurrent fungus infections related to therapy the schedule was interrupted for a period of 2 months, giving a total treatment duration of 8 months. The patient is still alive without signs of recurrent disease, having received no antineoplastic treatment in the last year.

DISCUSSION

The present study reveals that the main dose-limiting toxicity of Brequinar, DUP 785, is myelosuppression, primarily thrombocytopenia and less pronounced leucopenia and anemia. A toxic skin rash was a major problem in some patients although it did not constitute a life-threatening side-effect. Mucositis of mild to moderate degree occurred frequently. The data indicate that the recommended doses for further clinical trials range from 1500 to 2000 mg/m² on a weekly schedule depending on the degree of prior treatment. When DUP 785 was given twice weekly, toxicity was observed at a lower dose level compared with the weekly administration, although the toxicity pattern was similar. The recommended doses are 500–750 mg/m² on a biweekly schedule.

A safe starting dose with DUP 785 should be 1/10 of LD_{10} in mice based on preclinical toxicity studies [9, 10], but as this dose was still toxic to dogs, a dose close to 1/3 of MTD in dogs was chosen, which was about 5–6 times lower. The difference in toxicity may be explained by differences in pharmacokinetic properties showing an elimination half life about 5 times longer in dogs than in mice. Since no human pharmacokinetic data were available, a cautious and very limited escalation scheme was followed in the present study. Consequently, a large number of patients were included in the study on a weekly schedule. Guided by clinical experience as well as pharmacokinetic data obtained from the first patients, a Fibonacci escalation scheme was applied without difficulty in the last part of the study. During the parallel phase I studies performed in Europe, an attempt was made to use comparative pharmacokinetics between mice and man to guide dose escalation prospectively [12]. The $T_{1/2}$ in man was found in these studies to be a median 10 h vs. 4.5 in mice and 24 in dogs. It has been shown previously [13] that the ratio between AUCs at LD_{10} in mice and the MTD in man is closer to unity than the ratio between doses, a finding which was also observed in these studies [14].

At dose levels above 1000 mg/m² a concentration above 10 μg/ml was noted 48 h after drug administration. These concentrations are sufficient to cause antitumor activity *in vitro* and in animal models [1–4]. As the enzymatic inhibition of DHO-DH is reversible within hours to days, either more frequent administrations or continuous infusion should be rational in order to maintain activity. In two patients, pharmacokinetic data at a biweekly schedule showed a consistent concentration >10 μg/ml (Fig. 3).

The toxicity pattern observed in this study is comparable with that observed in other phase I studies of DUP 785 using different dose schedules [5–7]. Compared with other antimetabolites, such as methotrexate and 5-fluororacil, the organ-specific side-effects of Brequinar seem very similar to methotrexate toxicity, with the exception that there were no signs of hepatic and renal toxicity [15]. Similarly, a considerable individual dose and schedule variation was observed.

Antitumor activity was observed in one patient with bladder cancer and complete remission was obtained in a patient with a tumor of unknown origin. Ongoing phase II trials will reveal the degree of clinical effectiveness of this compound against human malignancies.

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