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Phase I Study of the Antifolate N^{10}-Propargyl-5,8-dideazafolic Acid, CB 3717

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Abstract—*The thymidylate synthase (TS) inhibitor CB 3717 was administered intravenously to 24 adult patients as a single bolus repeated every 3–4 weeks. The doses were escalated from 50 to 400 mg/m². At the highest level, hydration and urinary alkalinization were routinely performed. A >20% decrease of the creatinine clearance value occurred in 35% of the cycles performed at 400 mg/m², a dose which could be recommended for phase II studies. Hepatic toxicity, represented by an increase of the glutamic pyruvic transaminase (GPT) plasma levels, occurred in 70% of the patients after the first cycle. GPT peak levels were neither related to the dose nor to the peak drug plasma concentrations or AUC values in the dose range from 225 to 400 mg/m². Malaise, reported after 46% of the cycles, was the most disturbing side-effect and its occurrence was statistically correlated with the degree of elevation of GPT. A suggestion of antitumor activity was reported for dosages of 300 and 400 mg/m² in three patients with ovarian cancer refractory to cisplatin. Further clinical evaluations of TS inhibitors rely on the development of more water soluble and less hepato- and nephrotoxic agents.*

INTRODUCTION

THE QUINAZOLINE FOLATE analog N^{10}-propargyl-5,8-dideazafolic acid (CB 3717; ICI 155387; NSC 327182 and NSC 373233) (Fig. 1) is an antifolate whose cytotoxic activity is solely related to a tight inhibition of thymidylate synthase [1], the rate-limiting enzyme in the synthesis of the only nucleotide required exclusively for DNA synthesis.

In mice, the compound was not shown to be toxic to normal proliferating tissues, such as gut and bone marrow [2]. An 80–100% cure rate was observed in the L1210 ascites tumor used at the Institute of Cancer Research, London. The antitumor activity observed against other murine models was limited.

An interesting feature of this quinazoline analog was the antitumor activity shown in some rodent and human cell lines resistant to methotrexate either by virtue of dihydrofolate reductase overproduction or a membrane transport defect [2, 3].

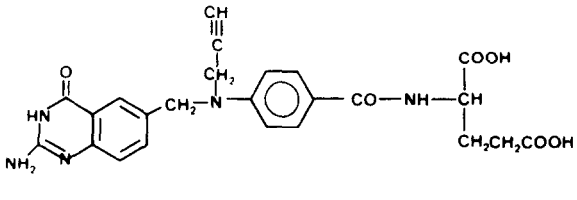

Fig. 1. Structure of N-(4-(N-((2-*amino*-4-*hydroxy*-6-*quinazolinyl*)-*methyl*)*prop*-2-*ynylamino*)*benzoyl*)-L-*glutamic acid* (*CB*3717).

The human toxicity of single intermittent administrations of CB 3717 was initially evaluated at the Institute of Cancer Research and Royal Marsden Hospital, Sutton and London [4]. The drug was given intravenously (i.v.) over 1 h every 3 weeks, from a starting dose of 140 mg/m² up to a maximum of 600 mg/m².

The variability in incidence of all the side-effects did not allow one to define the maximum tolerated dose (MTD). A decrease in the [^{51}Cr]EDTA clearance, possibly due to a precipitation of the drug in the renal tubules, was the only dose-related toxicity for doses of ≥450 mg/m² and was considered to be dose-limiting. The recommended dose for phase II studies was 400 mg/m².

The most troublesome side-effect was malaise with symptoms of fatigue, anorexia and lethargy occurring in about 70% of the patients, which appeared to be correlated with elevations in plasma glutamic pyruvic transaminase (GPT, ALT) levels.

The objective responses observed in heavily pretreated patients with breast or ovary carcinoma, together with the complete lack of myelotoxicity and mucositis at effective antitumor doses, prompted further clinical evaluations of the drug. Before start-

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ing phase II studies, the Early Clinical Trials Group of the EORTC decided to better define the toxicity of different schedules of treatment.

The clinical and the pharmacokinetic results of the phase I study of CB 3717 given on a single intermittent schedule are reported in this paper.

PATIENTS AND METHODS

Eligibility criteria were: histologically confirmed diagnosis of malignant disease refractory to conventional therapies or for which no treatment of established effectiveness existed; age ≤75 years; WHO performance status ≤2; life expectancy ≥8 weeks; no chemotherapy or radiotherapy in the previous 4 or 6 weeks; adequate hematologic (WBC count $\geq 3.0 \times 10^3/\mu l$; platelet count $\geq 100 \times 10^3/\mu l$), hepatic (serum alkaline phosphatase and GPT values $\leq 1.25 \times N$) and renal (serum creatinine value $\leq 1.25 \times N$; creatinine clearance ≥60 ml/min) function tests. Oral informed consent according to local regulations was obtained from each patient.

CB 3717 was manufactured and supplied by ICI Pharmaceuticals (Alderley Park, Cheshire, U.K.) in vials containing 5 ml of a solution of the disodium salt in bicarbonate buffer, pH 9.5, at a concentration equivalent to 10 mg/ml as free acid.

The drug was administered i.v. at an injection rate of 100 mg/min every 3–4 weeks. The starting dose was 50 mg/m^2.

The patients treated at 400 mg/m^2 were routinely hydrated with 1000 ml of a solution of 1:1 normal saline and 5% dextrose administered every 6 h starting 12 h prior to treatment up to 48 h after it. A urine pH ≥8 was achieved through the i.v. administration of 167 mmol of sodium bicarbonate and of acetazolamide 500 mg PO every 12 h in the pre-hydration period. Additional acetazolamide or sodium bicarbonate were administered i.v. as required in the post-hydration period.

Physical examination, complete blood count, blood chemistries, including urea, creatinine, uric acid, bilirubin, alkaline phosphatase, GOT, GPT, gamma-GT, LDH and protein, were performed weekly. Twenty-four-hour creatinine clearance was repeated before each drug administration and at shorter intervals in patients receiving doses of ≥300 mg/m^2.

Clinical response and toxicity grade were defined according WHO criteria [5]. The grades of malaise and creatinine clearance reduction were defined according to the criteria applied in the previous phase I trial [4].

At least two courses of treatment were given unless there were clear contraindications to the treatment.

A minimum of two evaluable patients and three courses of therapy were required at each non-toxic or sub-toxic dose level. At least five evaluable patients had to be treated at each toxic level. Retreatments at higher levels were allowed if no significant toxicity had occurred at the lower ones.

Considering the lack of hematologic toxicity and the available results, the doses were increased by 50% increments from 100 to 225 mg/m^2. The two highest levels of 300 and 400 mg/m^2, which had been recommended for phase II studies, were selected to define further their toxic effects.

SAMPLE COLLECTION AND DRUG ASSAY

Blood samples (5 ml) were taken before and 5, 15, 30, 60, 120, 150, 180, 240, 360, 460, 720 and 1440 min after the injection. Urine samples were collected for 24 h after the injection, adjusted at pH 9 with NaOH 1 M (to allow a good CB 3717 solubility) and stored at −20°C until analyzed. In patient 8 10 ml of ascites were aspirated at 360, 720 and 1440 min after the first injection of the drug.

CB 3717 was assayed by HPLC as follows: to 0.5 ml of plasma or urine or ascitic fluid 10 μg of dansylproline (internal standard) and 1 ml of methanol were added. The plasma–methanol mixture was mixed vigorously, then cooled at −60°C (acetone–dry ice) for 2–3 min and centrifuged at 3000 rpm in a refrigerated centrifuge. An aliquot (10–25 μl) of clear surnatant was injected directly into a Waters model 6000 A HPLC equipped with a 254 nm absorbance detector. Separation was achieved using an isocratic solvent system of water: acetonitrile:acetic acid (67:32:1) at a flow rate of 1 ml/min, using a 25 cm long μ Bondapack phenyl column purchased from Waters Assoc. New York, NY, U.S.A. The sensitivity of the method was 1 μg of CB 3717/ml of plasma, urine and ascites.

PHARMACOKINETIC ANALYSIS

The plasma concentrations of CB 3717 vs. time for each patient were fitted to the standard equation for a two-compartment model [6] using a non-linear fitting computer program [7]; data were weighted with the function $1/C_t^r$, where C_t^r is the plasma concentration at time t after the administration. AUC was determined by the trapezoidal rule. Terminal half-life ($T_{1/2}\beta$), plasma clearance (Cl_p) and volume of distribution ($V\beta$) were calculated using the equations:

$$T_{1/2}\beta = 0.693\ \beta \quad Cl_p = \text{dose}/AUC_{0-\infty}$$

$$V\beta = Cl_p/\beta.$$

Table 1. Characteristics of patients

Characteristics	No. of patients
Total	24
Men/women	14/10
Median age in years (range)	57 (35–70)
WHO performance status	
0–1	19
2	5*
Previous treatment	
chemotherapy only	12
chemo- and radiotherapy	12
Site of malignancy	
ovary	8
unknown origin	3
colon	2
head and neck	2
kidney	2
urinary tract	2
other	5

*Treated at 50 mg/m^2 (two patients), 300 mg/m^2 (two patients), 400 mg/m^2 (one patient).

clearance value of <60 ml/min did not produce a further reduction of the renal function. One patient, with a pretreatment creatinine clearance of 56 ml/min and a performance status of 2, showed within 24 h from the first cycle at 300 mg/m^2 a 60% reduction of the creatinine clearance. Intravenous fluids were then administered with recovery of the renal function within a week. The patient developed a severe leuko- and thrombocytopenia on day 13 (WBC 0.4 × 10^3/μl and platelet count 45 × 10^3/μl) and died of uncontrolled infection on day 15.

Among patients with a pretreatment creatinine clearance value of ≥60 ml/min, the reduction of the creatinine clearance did not seem to be dose related for doses <400 mg/m^2. A reduction of >20% was reported in four out of 29 cycles (14%) performed at dosages of <400 mg/m^2; in one patient treated at 50 mg/m^2 the >50% reduction of the creatinine clearance was due to the development of a tumor-related cardiac failure. Among 17 cycles performed at 400 mg/m^2 six were associated with >20% decrease of the creatinine clearance: all of them were reported in two patients with ovarian cancer

Table 2. Percentage of reduction of creatinine clearance according to the pretreatment value

Dosage (mg/m^2)	Pretreatment creatinine clearance >60 ml/min				Pretreatment creatinine clearance 41–60 ml/min			
	No. of evaluable patients/cycles	No. of cycles with % reduction of creatinine clearance			No. of evaluable patients/cycles	No. of cycles with % reduction of creatinine clearance		
		<20	21–50	>50		<20	21–50	>50
50	2/2	—	1	1	—/—	—	—	—
100	1/3	3	—	—	3/4	4	—	—
150	3/5	3	2*	—	1/2	2	—	—
225	4/4	4	—	—	1/1	1		—
300	4/15	15	—	—	1/1	—	—	1
400	6/17	11	5†	1	—/—	—	—	—

*One cycle in a patient previously treated at 100 mg/m^2.
†Two cycles in a patient previously treated at 300 mg/m^2.

RESULTS

Table 1 summarizes the characteristics of the 24 patients treated. Four patients were retreated at higher levels: the dosage was increased from 100 to 150 mg/m^2 in two patients and from 300 to 400 mg/m^2 in the remaining two patients.

Toxicity

Table 2 reports the number of patients evaluable for renal toxicity as well as the number of cycles associated with a decrease of the initial creatinine clearance value by dosage given.

The administration of 100, 150 and 225 mg/m^2 in five patients with a pretreatment creatinine pretreated with cisplatin. Their lowest creatinine clearance values occurred within 72 h from administration, and recovery of the renal function was complete by the time of the subsequent cycle.

GPT and GOT plasma levels started to rise the week following the first administration in 75% and 68% of the cases. The degree of elevation of both liver enzymes was similar in 57% of the cases, while the GPT levels rose proportionally more than GOT levels in 43% (Table 3). Similarly, 43% of the first cycles were associated with an elevation of alkaline phosphatase plasma levels which was of a lesser degree than elevation in GPT in all cases but one. Alterations in liver function tests were not dose-

*Table 3. Degree of elevation of GPT plasma levels after the first cycle**

Dosage (mg/m²)	No. of patients	No. of cycles with GPT toxicity grade†					
		0	1	2	3	4	
50	2		1		1		
100	4	2		1		1	(GOT_3)
150	5	1 (GOT_1)	2‡ ($1GOT_0$)	1	1		
225	5			1	2 ($1GOT_2$)	2	($1GOT_3$)
300	6	2	2 ($2GOT_0$)	1	1 (GOT_2)		
400	6	2	2§	2 ($2GOT_1$)			

*In parentheses, GOT toxicity grade if different from that of GPT.
†0, <1.25 × N; 1, 1.26–2.5 × N; 2, 2.6–5 × N; 3, 5.1–10 × N; 4, >10 × N.
‡Two patients previously treated at 100 mg/m².
§One patient previously treated at 300 mg/m².

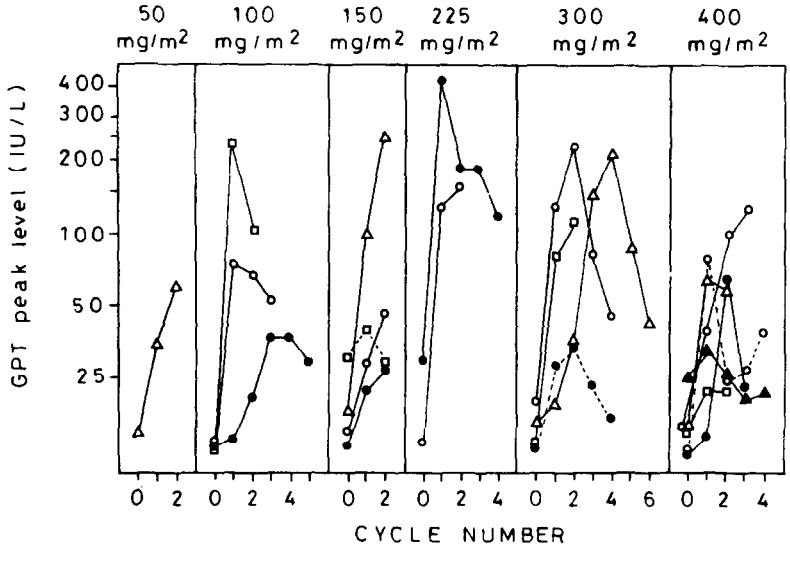

Fig. 2. GPT peak plasma levels after repeated administrations of CB3717 (semilog plot).

related. Bilirubin levels were never affected.

Figure 2 depicts the pattern of the GPT plasma levels among patients who received at least two cycles of treatment by dosage administered. The elevation of the GPT plasma levels showed a characteristic profile through the subsequent cycles, usually reaching the peak value after the second or third cycle and decreasing thereafter in spite of the repeated administrations of the drug.

With the exception of the patient who presented an early impairment of the renal function and subsequently developed severe leuko- and thrombocytopenia, hematologic toxicity was represented by four episodes of leukopenia grade 1 observed in the dose range of 150–400 mg/m².

Overall non-hematologic toxicity is reported in Table 4. Nausea and vomiting the day of treatment were almost exclusively observed at the highest dosage. One patient presented a cutaneous rash of the limbs and trunk, which appeared the week following the third administration and lasted approximately 2 weeks.

Malaise with fatigue, nausea, loss of appetite, lethargy and diffuse mild myalgia was reported following 46% of the cycles. The symptoms started the week after treatment and lasted for 1 up to 3 weeks. Three patients with no evidence of tumor progression refused further administrations of the

Table 4. Non-hematologic toxicity

	Dosage (mg/m²)					
	50	100	150	225	300	400
No. of patients/No. of cycles	2/3	4/11	5/9	5/8	6/18	6/18
No. of cycles with						
nausea/vomiting	0	0	0	0	1	9
malaise						
grade 1*	0	2	6†	4	9	8‡
grade 2	0	0	0	1	0	1
diarrhea	0	0	0	0	0	2
cutaneous rash	0	0	0	0	0	1

*0, no symptoms; 1, symptoms confined to bed <24 h; 2, symptoms confined to bed >24 h.
†Two cycles in a patient previously treated at 100 mg/m².
‡Four cycles in two patients previously treated at 300 mg/m².

Table 5. Distribution of patients by grade of malaise and GPT toxicity levels after the first cycle

Malaise grade*	GPT toxicity grade†				
	0	1	2	3	4
0	6	4‡	1	1	0
1	1	3§	4	3	3
2	0	0	1	1	0

*0, no symptoms; 1, symptoms confined to bed <24 h; 2, symptoms confined to bed >24 h.
†0, <1.25 × N; 1, 1.26–2.5 × N; 2, 2.6–5 × N; 3, 5.1–10 × N; 4, >10 × N.
‡One patient retreated at 150 mg/m².
§One patient retreated at 150 mg/m² and 1 at 400 mg/m².
Chi-squared test for homogeneity: $P < 0.01$.

drug because of this side-effect. Occurrence of malaise was statistically correlated with the degree of elevation of the GPT plasma levels after the first cycle (Table 5). Patients complained of malaise after 86% of the cycles associated with a GPT toxicity grade of 2 and after 28.5% of the cycles associated with a GPT toxicity grade of 1.

Antitumor activity

Twenty patients were evaluable for tumor response. A suggestion of antitumor activity was reported at 300 and 400 mg/m² in three among eight patients with cancer of the ovary pretreated with cisplatin and alkylating agents. The responses consisted in two cases of the disappearance of neoplastic effusions for 2 and 7 months and in one case of significant decrease of the CA 125 level for 2 months.

Pharmacokinetic studies

Figure 3 shows the plasma decay curves of drug concentrations in nine patients given CB 3717 at dosages of 225 mg/m² (panel A), 300 mg/m² (panel B), and 400 mg/m² (panel C). In panel C the ascitic levels following the first cycle of patient 8 are also reported. Ascitic drug concentrations were higher than the plasma ones; the maximal difference was found at 24 h when ascitic concentration was 4.2 μg/ml and plasma concentration was 1.3 μg/ml.

In all patients a two open compartment model gave a good representation of the pharmacokinetics. The mean (±SE) of $T_{1/2}\beta$, Cl_p and $V\beta$ after the injection and the CB 3717 urinary excretion are reported in Table 6. There was no consistent correlation between peak levels, and AUC values and elevation in liver or renal function tests after the first administration of the drug.

The 24 h urinary excretion varied between 10.2% and 47.9% of the administered dose.

DISCUSSION

The results of this study confirm those of the previous phase I trial with CB 3717 administered on a single intermittent schedule [4]. In the present study, the alkaline diuresis maintained in the patients receiving 400 mg/m² was associated with a reversible decrease of the creatinine clearance in 35% of the cycles. Considering only this side-effect, 400 mg/m² with urine alkalinization may therefore be recommended for phase II studies, at least for patients in good general condition.

The occurrence of a profound malaise, however, which was clearly associated with a higher elevation of the GPT peak plasma levels, represents for the patients the most disturbing side-effect, and seems to preclude a broader clinical evaluation of this

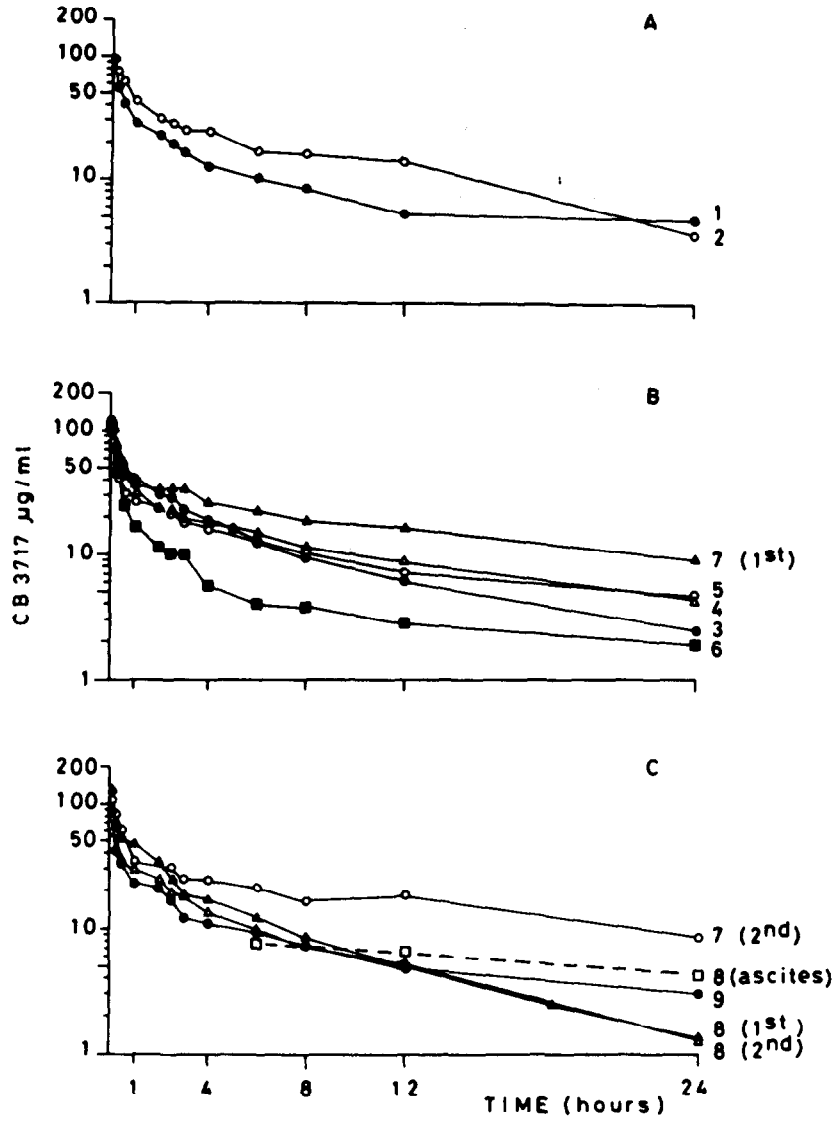

Fig. 3. CB 3717 plasma decay curves in nine patients given CB 3717 225 mg/m² (panel A), 300 mg/m² (panel B) and 400 mg/m² (panel C) by bolus injection. In patient 8 CB 3717 ascitic levels are also represented.

Table 6. Main pharmacokinetic parameters and 24 h urinary excretion of CB3717 in 9 cancer patients

Patient	Dose (mg/m²)	$T_{1/2}\beta$ (min)	Cl_p (ml/min/m²)	$V\beta$ (l/m²)	Peak plasma level* (µg/ml)	24 h urinary excretion (% dose administered)
1	225	929	10.7	14.4	115.4	41.2
2	225	514	8.3	6.3	95.8	25.2
3	300	553	16.1	11.8	152.7	47.9
4	300	596	13.6	11.7	120.9	10.2
5	300	852	13.3	16.4	94.9	22.1
6	300	993	11.2	30.4	126.2	36.8
7 (1st)	300	729	7.6	7.9	119.3	47.6
(2nd)	400	784	10.0	11.3	125.8	46.0
8 (1st)	400	377	30.6	16.7	156.2	32.9
(2nd)	400	384	25.6	14.2	97.8	42.8
9	400	825	25.9	30.0	113.9	41.2
$\bar{X}$		685	15.7	15.5		
± S.E.		64	2.4	2.4		

*Plasma level extrapolated at time 0 using the equation: $C_t = A\,e^{-\alpha t} + B\,e^{-\beta t}$ [6].

schedule. On the other hand, the more frequent administration of lower doses had to be abandoned because of the occurrence of severe renal or hematologic toxicity in patients treated at weekly intervals or daily for 5 consecutive days ([4], Hansen H, personal communication). The ECTG has therefore decided to abandon, at least for the time being, the clinical evaluation of this drug.

The pharmacokinetic data obtained in the present study are in agreement with those previously published by Alison *et al.* [8]. The CB 3717 plasma levels found—even at 24 h after a dose of 225 mg/m^2—were greater than those reported as cytotoxic *in vitro* against tumor cells [2, 3]. This suggests that the CB 3717 plasma levels are sufficiently high to exert an antitumoral effect. However, these extrapolations must be taken with caution, particularly considering the complexity of the pharmacodynamic aspects related to the CB 3717 antitumoral activity; for example, the levels of thymidine present *in vitro* in growth medium were not necessarily comparable to those present in the extracellular fluid surrounding tumor cells *in vivo*. As previously reported, the CB 3717 ascitic concentrations—higher than the plasmatic ones, particularly at longer times—may be effective against tumor cells floating in the peritoneal cavity.

Alison *et al.* [8] reported a weak correlation between CB 3717 peak plasma levels and GPT peak levels when the drug was given as a 1 h infusion. We failed to confirm this correlation. The CB 3717 peak levels found by us were about three times greater than those shown in the previous study and this was expected since we gave similar doses over shorter infusions. Nevertheless, the incidence of hepatic toxicity was similar in both studies, thus confirming the lack of correlation between the maximal plasma concentrations of CB 3717 and the drug-related hepatic toxicity.

The lack of myelosuppression and mucositis and the tumor responses observed in the early clinical studies with CB 3717 make thymidylate synthase inhibitors a new class of antimetabolites with great potential therapeutic interest. The development of non-hepatotoxic and non-nephrotoxic analogs appears to be possible, since the mechanism of hepatic and renal toxicity apparently differs from those leading to antitumoral activity.

Some novel, more water-soluble and less toxic analogs have already been synthesized and their preclinical data are very encouraging [9]. New clinical trials with this interesting class of antimetabolites should await the availability of these analogs.

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