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The clinical pharmacokinetics of the novel antifolate N^{10}-propargyl-5,8-dideazafolic acid (CB 3717)*

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Summary. *The pharmacokinetics of the new antifolate CB 3717 were studied in 20 patients during its phase-I clinical evaluation. The drug was administered at doses of 100–550 mg/m² in 1-h and 12-h infusions, resulting in peak plasma concentrations of CB 3717 of 40–200 μM. There was a linear relationship between the dose and both CB 3717 AUC and peak plasma levels. Following a 1-h infusion, drug levels in the plasma decayed biphasically ($t_{1/2\alpha}$ = 49 ± 9 min, $t_{1/2\beta}$ = 739 ± 209 min). 27% ± 2% of the dose was excreted in urine in the 24-h period after treatment, suggesting that the major route of elimination was via the bile. Furthermore, the parent compound CB 3717 and its desglutamyl metabolite, CB 3751, were found in a faecal collection although the metabolite was not detected in plasma or urine samples. Plasma protein binding of CB 3717 was extensive (97.6% ± 0.1%). Significant quantities of CB 3717 penetrated into ascitic fluid but not into cerebrospinal fluid.*

Residual drug was detected in postmortem kidney tissue from a patient who died of progressive disease 8 days after treatment with 330 mg/m² CB 3717. Thus, dose-limiting renal toxicity (maximum tolerated dose 600 mg/m²) may be due to drug precipitation in the renal tubules. Elevation of liver enzymes, in particular transaminases, occurred frequently as a toxic manifestation of CB 3717 therapy. In 11 patients studied after their first treatment there was a positive correlation between the rise in serum alanine transaminase and peak drug levels (r = 0.69, P = 0.02).

These pharmacokinetic studies have shown that, by analogy with experimental systems, cytotoxic plasma levels of CB 3717 are archieved in man. In addition, they have been valuable in interpreting toxicities observed during phase-I clinical studies.

Introduction

The quinazoline folate analogue CB 3717, or N^{10}-propargyl-5,8-dideazafolic acid [*N*-(4-(N-((2-amino-4-hydroxy-6-quinazolinyl) methyl) prop-2-ynylamino) benzoyl) -L- glutamic acid, NSC-327182] (Fig. 1), is a tight binding inhibitor of the enzyme thymidylate synthetase [8, 9]. CB 3717 was selected on the basis of its superior inhibitory activity against thymidylate synthetase purified from L1210 leukemia cells and its in vitro cytotoxicity [12]. The activity of CB 3717 against the L1210 tumor was confirmed in tumor-bearing mice and subsequently a phase-I clinical trial with this agent began in September 1981 at the Royal Marsden Hospital. Initially the drug was administered either as a single 1-h IV, infusion or as one 1-h infusion given daily for 5 days. Both schedules started at a dose of 100 mg/m²/day, and treatments were repeated every 3 weeks. Although renal toxicity became dose-limiting at 600 mg/m² with the single-infusion schedule, the toxicity most frequently observed was hepatic. Mild reversible hepatic dysfunction with associated malaise was experienced by approximately 80% of patients and was typified by a rise in serum alanine transaminase levels to two to 15 times normal values beginning 2–3 days after treatment and lasting 10–20 days [1]. These changes were generally paralleled by slight rises in alkaline phosphatase levels.

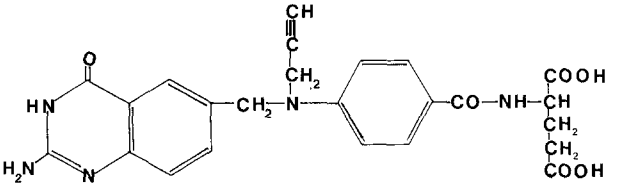

Fig. 1. The structure of CB 3717 [*N*-(4-(*N*-((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamic acid]

In rats, following high doses of CB 3717, a precipitate in the bile and decreased bile flow rates were observed. However, this effect could be reduced by extending the period of administration (D. R. Newell and Z. H. Siddik, personal communication). By extrapolation to patients this mechanism was invoked as a possible cause of the liver toxicity, although the biochemical lesion in man was not typical of cholestasis. Accordingly the infusion time was extended to 12 h in an attempt to ameliorate this problem.

Pharmacokinetic studies were performed in conjunction with the phase-I trial of CB 3717, and attempts have been made to correlate the pharmacokinetic parameters with the toxicities observed.

Materials and methods

Chemicals

CB 3717 (NSC-327182) was supplied by ICI Pharmaceuticals (Alderley Park, Macclesfield, Cheshire) and CB 3751 [4-(*N*-

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((2-amino-4-hydroxy-6-quinazolinyl)methyl)prop-2-ynylamino)-benzoic acid], by Dr T. R. Jones (Institute of Cancer Research, Sutton, Surrey). All other reagents were analytical grade and were obtained from Fisons (Loughborough, Leics.), BDH Chemicals (Poole, Dorset,) or East Anglia Chemicals (Hadleigh, Ipswich, Suffolk).

Patients, protocol and sample collection

Verbal consent was obtained from patients selected for study, whose characteristics are presented in Table 1. Antiemetics were not routinely used and analgesic preparations were avoided where possible for the 24-h period of study. Plasma levels of CB 3717 were measured in 20 different patients (16 following 1-h infusion, 3 following 12-h infusion, 1 following both 1- and 12-h infusion). Blood samples (5–10 ml) were collected into heparinized tubes from an IV cannula sited in the contralateral arm from that receiving the drug infusion. Patency was maintained by the injection of 1 ml heparin (10 U/ml) after each sampling. The diacid of CB 3717 was dissolved in 250 ml or 1 l 0.15 *M* $NaHCO_3$ adjusted to pH 9.0 when infused over 1 or 12 h, respectively. Sample collection times were as follows: 1-h infusion – before mid and end of infusion, and then 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, and 23 h after infusion; 12-h infusion – before mid and end of infusion, and then 0.5, 1.0, 6, 12, and 24 h after infusion. Plasma was separated immediately by centrifugation and stored frozen (< – 20° C).

Urine samples were taken from random pretreatment specimens and collections were made for 24 h following the start of treatment on 57 occasions (31 following 1-h infusions in 25 patients and 26 following 12-h infusions in 21 patients). Aliquots (20 ml) from the 24-h urine samples were stored frozen (< – 20° C). An incomplete collection of feces was made from one patient (no. 4) during a 5-day treatment schedule. Cerebrospinal fluid was also acquired from this patient by lumbar puncture 1 h after completion of his drug infusion on day 1. Postmortem tissue and bile samples were retained for analysis from a patient who died of progressive disease 8 days after his second dose of CB 3717 at 330 mg/m^2. In addition, 15-ml volumes of ascitic fluid were aspirated from a patient with ovarian carcinoma at 1, 2, 4, 6, and 23 h after a 1-h IV infusion and were stored frozen (< – 20° C).

Sample preparation. All samples were thawed at room temperature.

Urine. The samples were adjusted to pH 9.0 by dropwise addition of 1 *M* NaOH to ensure the complete dissolution of any CB 3717 present.

Feces. The total fecal collection was weighed, diluted to a 20% (w/v) mixture with 0.1 *M* trishydroxymethylammonium-HCL buffer pH 9.0, left for 24 h at 4° C, and then stirred for 30 min.

Tissues. The tissues available form the post mortem (primary tumor, metastatic tumor, small bowel, spleen, pancreas, kidney, bone marrow, liver, thyroid, cerebellum, cerebrum, myocardium, skin, adipose tissue, large bowel) were homogenized in a teflon-glass homogenizer in 0.1 *M* trishydroxy-

Table 1. Patient characteristics

Patient	Dose (mg/m^2)	IV infusion schedule (h × days)	Treatment course	Pretreatment			Posttreatment (peak rise in ALT IU/l[b]
				GFR (ml/min)	Liver function tests		
					Alanine[a] transaminase	Alkaline[a] phosphatase	
1	100	1 × 5	1	138	↑[c]	–	–
2	100	1 × 5	1	99	↑	N	–
3	140	1 × 5	1	66	N[c]	N	–
4	140	1 × 5	1	–	N	N	–
5	300	1	1	128	↑	↑	133
6	300	1	1	72	N	N	21
7	300	1	1	89	N	↑	55
8	300	1	3	48	↑[d]	↑	–
9	300	1	1	49	N	N	157
10	330	1	1	124	N	N	116
11	450	1	1	61	N	N	4
12	500	1	8	76	N	N	–
13	500	1	1	100	N	↑	152
14	500	1	1	142	N	↑	57
15	500	1	1	76	N	N	35
16	550	1	1	65	N	↑	221
17	550	1	1	117	N	↑	355
18	300	12	1	172	N	↑	435
10	330	12	2	107	↑[d]	↑	–
19	330	12	7	31	↑[d]	N	–
20	330	12	4	64	↑[d]	N	–

[a] Upper limit of normal ranges: alanine transaminase 22 IU/l; alkaline phosphatase 95 IU/l
[b] Data shown only for patients receiving their first course of CB 3717 by a single infusion
[c] N, normal; ↑, raised
[d] Alanine transaminase elevated after previous CB 3717 treatment

methylammonium-HCL buffer pH 9.0 as 5% (w/v) homogenates.

Two volumes of methanol were added to one volume (routinely 1 ml) of plasma, CSF, urine, fecal and tissue homogenates, bile, and ascitic fluid. These were mixed thoroughly and centrifuged at 1,000 *g* for 5 min at 4° C. The supernatants were decanted into glass vials for analysis by HPLC within 12 h.

HPLC analysis

All HPLC analyses were performed on a Waters Associates Chromatograph (Waters Associates, Northwich, Cheshire), which consisted of two model 6000A HPLC pumps, a model 660 solvent programmer, a model 440 fixed-wavelength detector (254 nm and 280 nm), and a model 710 (A or B) Waters Intelligent Sample Processor. The outputs from the detectors were recorded on a standard chart recorder and, for some analyses, on an automatic integrator (Trivector Ltd, Sandy, Beds.). Peaks were measured either by peak height, determined manually, or by peak area, determined by automatic integration. HPLC solvents were filtered and degassed prior to use by passage through a 0.45 μm filter: polypropylene-backed PTFE for organic solutions and cellulose nitrate for aqueous solutions (Whatman Ltd, Maidstone, Kent).

Separations were performed on a μ-Bondapak C18 column (25 × 0.46 cm, Water Associates) fitted with a CO: Pell ODS precolumn (6.5 × 0.21 cm, Whatman Ltd). Samples were eluted isocratically at a flow rate of 2 ml/min with a solvent mixture containing, by volume, one part glacial acetic acid, 64 parts water, and 35 parts methanol. Sample volumes were within the range 10–50 μl.

Recovery of CB 3717 from human plasma was essentially complete (94.4% ± 3.4%, $\bar{x}$ ± SD) and linear (r = >0.999) over the concentration range 2–400 μ*M* (coefficients of variation: 20 μ*M*, 3.5%; 100 μ*M* 5.3%; 200 μ*M*, 2.2%). Similarly, CB 3717 estimation in urine was linear over the range 2–200 μ*M* (r = >0.999; coefficients of variation: 20 μ*M*, 4.1%; 100 μ*M*, 1.0%; 200 μ*M*, 1.0%) Quantification of CB 3717 and CB 3751 in CSF, ascites, kidney, and fecal homogenates was achieved by comparison with standard solutions dissolved in 0.15 *M* $NaHCO_3$ pH 9.0 and recovery taken as 100%. Chromatograms illustrating the analysis of CB 3717 in plasma are shown in Fig. 2. Peaks present in the HPLC effluent were identified by co-chromatography with CB 3717 and CB 3751 standards and by their 254 nm/280 nm absorbance ratios.

Protein binding estimation

Plasma (20 ml) was separated from blood venesected from a normal volunteer. Standard solutions of CB 3717 in 0.15 *M* sodium bicarbonate adjusted to pH 9.0 were further diluted in 1-ml aliquots of 0.15 *M* sodium bicarbonate and 1-ml aliquots of plasma to give final concentrations of 1–200 μ*M*. These bicarbonate and plasma dilutions were ultrafiltered using an Amicon micropartition system with YMT membranes (Amicon House, Woking, Surrey). Centrifugation was at 1,500 *g* for 10 min at 15° C. The ultrafiltrates were then analyzed by HPLC.

Pharmacokinetic analyses

Following the end of the infusion plasma levels of CB 3717 were fitted to a two-compartment open model using a nonlinear least-squares analysis [20]. The data were weighted either with the function $1/(\hat{y} + yi)^2$ or with the function $1/(\hat{y})^2$ [17, 21]. The values obtained for the two-compartment model were corrected for the infusion period as described by Loo and Riegelman [13]. From the corrected values the alpha and beta phase half-lives, volume of the central compartment, steady-state volume of distribution, total plasma clearance, area under the plasma concentration versus time curve (AUC), and microscopic rate constants (k_{12}, k_{21}, k_{el}) were calculated using the equations described by Wagner [22]. In two patients, following a 12-h infusion a one-compartment model was fitted to the data.

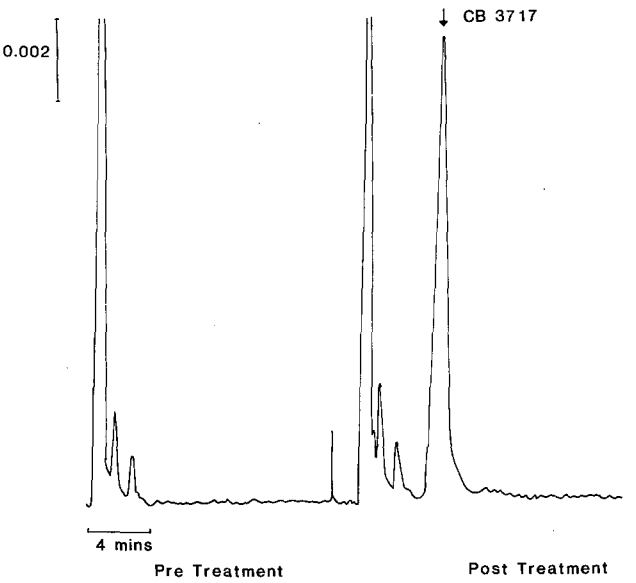

Fig. 2. HPLC trace of plasma samples before and immediately after CB 3717 treatment with a 1-h IV infusion of 400 mg/m^2 (wavelength 280 nm)

Results

CB 3717 Plasma pharmacokinetics

Following a 1-h infusion of CB 3717, plasma levels decayed biphasically with first-order kinetics as shown in Fig. 3, where the computer fits of the plasma decay curves from three patients are depicted. The pharmacokinetic parameters determined for 17 patients are given in Table 2. Peak plasma CB 3717 concentration correlated strongly with dose (r = 0.839, P = 0.01) but only a weak correlation with plasma AUC was found (r = 0.473, P = 0.05). Other parameters were independent of dose. In the 11 patients who were studied during their first course of CB 3717 and who received only a single 1-h infusion, hepatic toxicity, as measured by the peak rise in alanine transaminase level after treatment (see Table 1), showed some correlation with peak plasma CB 3717 concentration (Fig. 4) (r = 0.690, P = 0.02). There was no correlation between hepatic toxicity and any of the other parameters determined, including k_{21}, k_{12}, and k_{el}. An example of the plasma levels of CB 3717 in one of the four patients studied following a 12-h infusion is shown in Fig. 5. Comparison of Tables 2 and 3 shows that the beta phase half-life and total plasma clearance are similar despite the prolonged infusion time. In patient 10 extending the drug infusion time from 1 to 12 h and using the same total dose decreased the peak plasma concentration from 81 μ*M* to 32 μ*M*.

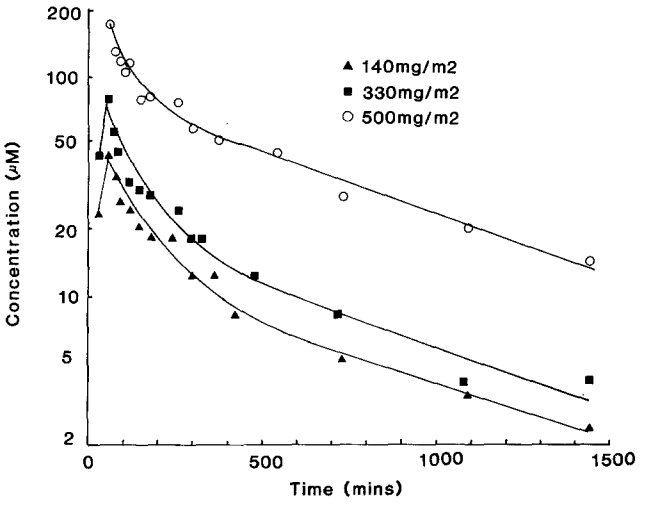

Fig. 3. Plasma levels of CB 3717 in three patients following a 60-min infusion. *Lines* are the computer-generated fits

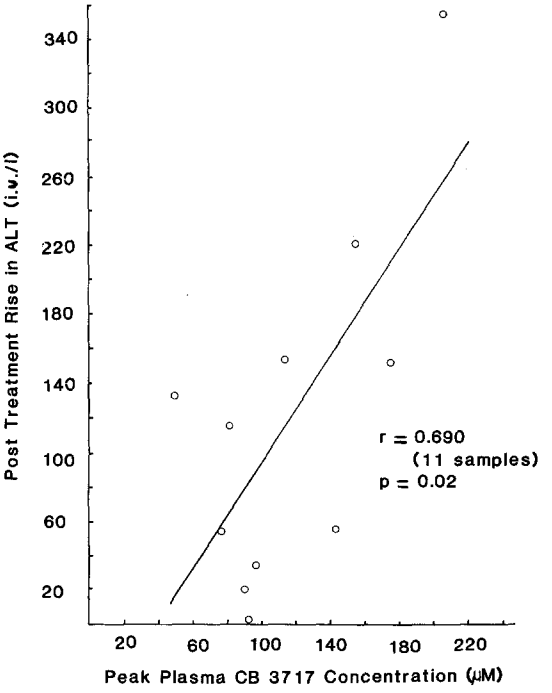

Fig. 4. Correlation between peak plasma concentration of CB 3717 and rise in serum alanine transaminase levels ($r = 0.690$, $P = 0.02$)

Table 2. CB 3717 plasma pharmacokinetic parameters in man following a 1-h infusion

Patient	Dose (mg/m^2)	$t^{1}/_{2}\,\alpha$ (min)	$t^{1}/_{2}\,\beta$ (min)	V_1[a] (l/m^2)	Vdss[a] (l/m^2)	CLR[a] ($ml/min/m^2$)	AUC[a] ($uM \times h/m^2$)	Peak plasma conc. (uM)
1	100	32	301	3.4	6.7	17.8	98	42
2	100	58	770	4.6	12.6	13.7	128	39
3	140	43	770	3.8	14.7	17.0	192	53
4	140	96	658	6.7	13.9	19.8	144	45
5	300 (285)	131	767	13.0	23.8	26.6	235	50
6	300	109	1,188	7.5	16.4	11.1	533	90
7	300	88	3,850	7.2	49.3	10.6	541	76
8	300	15	747	2.8	9.9	9.7	855	128
9	300	20	385	3.1	7.9	15.9	361	114
10	330	63	533	8.9	19.8	32.7	177	81
11	450	13	341	4.6	19.4	46.4	215	92
12	500	15	289	4.1	9.3	23.9	201	123
13	500	36	577	4.7	10.2	13.5	924	175
14	500	33	289	5.7	11.2	31.2	304	142
15	500	8	383	4.3	12.8	24.3	429	96
16	550	50	364	6.1	10.3	22.3	581	155
17	550	23	350	3.4	8.2	18.3	543	203
$\bar{x} \pm SE$		49 ± 9	739 ± 203	5.5 ± 0.6	15.1 ± 2.4	20.9 ± 6.2		

[a] V_1, volume of central compartment; Vdss, volume of distribution at steady state; CLR, total plasma clearance for $t = 0-\infty$; AUC, area under the plasma concentration versus time curve for $t = 0-\infty$

Table 3. CB 3717 plasma pharmacokinetic parameters in man following a 12-h infusion

Patient	Dose (mg/m^2)	$t^{1}/_{2}\,\alpha$ (min)	$t^{1}/_{2}\,\beta$ (min)	V_1[a] (l/m^2)	Vdss[a] (l/m^2)	CLR[a] ($ml/min/m^2$)	AUC[a] ($uM \times h/m^2$)	Peak plasma conc. (uM)
18	300	121	990	10.2	26.2	23.4	165	17
10	330	–	323	–	12.1	26.1	221	32
19	330	63	866	1.9	6.6	7.3	928	75
20	330	–	770	–	8.5	7.7	691	59
$\bar{x} \pm SE$		–	737 ± 145	–	13.3 ± 4.4	18.6 ± 6.6	–	–

[a] Parameters as defined for Table 2

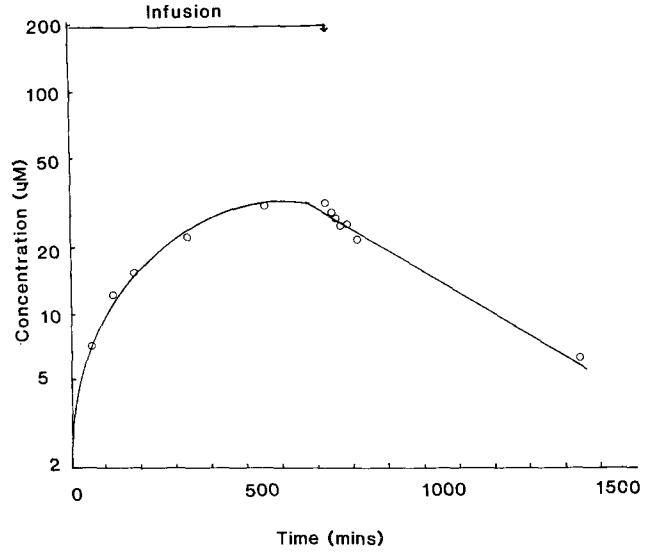

Fig. 5. Plasma levels of CB 3717 during and after a 12-h infusion (330 mg/m^2). The postinfusion line is the computer-generated fit

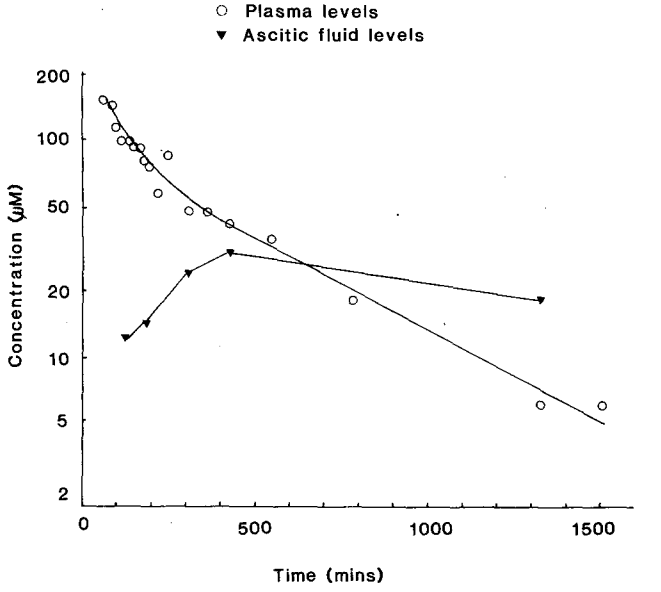

Fig. 6. Plasma levels and ascitic fluid levels of CB 3717 following a 60-min infusion (550 mg/m^2). The postinfusion line for plasma levels is the computer-generated fit

Table 4. Cumulative 24-h urinary excretion of CB 3717

Dose (mg/m^2)	Urinary excretion of CB 3717 following 1-h infusion (% dose administered ± SEM)	Urinary excretion of CB 3717 following 12-h infusion (% dose administered ± SEM)
100	23 ± 11 (n = 3)	–
140	27 ± 11 (n = 3)	–
300	34 ± 8 (n = 9)	26 ± 5 (n = 13)
330	–	27 ± 6 (n = 6)
360	32 ± 4 (n = 4)	24 ± 2 (n = 5)
400	26 ± 8 (n = 4)	38
450	10,46	–
500	30 ± 4 (n = 4)	–
550	39,26	–

CB 3717 Excretion and tissue distribution

The cumulative urinary excretion data (Table 4) implied that renal excretion was not the major elimination route for CB 3717, with only approximately 30% excreted over the first 24-h period regardless of dose. Strict correlation of CB 3717 renal clearance with pretreatment glomerular filtration rate (GFR) was not possible as no analysis was made of CB 3717 plasma protein binding in individual patients. However the 24-h urinary excretion of CB 3717 after 57 doses was weakly correlated with pretreatment GFR (r = 0.374; P = 0.01).

Fecal elimination was investigated in one patient, and 15% of the total dose of drug administered was detected as unchanged CB 3717 in an incomplete fecal collection. In addition, a compound was observed in the fecal extract which co-chromatographed with CB 3751, the desglutamyl metabolite of CB 3717.

The only postmortem tissue found to contain CB 3717 was the kidney, in which the concentration of 140 μg/g wet weight represented 5% of the total dose administered 8 days prior to the patient's death, assuming even drug distribution throughout the kidneys.

Figure 6 displays the data concerning the distribution of CB 3717 into the ascitic fluid of a patient with ascites secondary to ovarian carcinoma. Equilibration between plasma and ascitic fluid was observed 7 h after the end of the infusion. CB 3717 was not detected in the sample of CSF taken 1 h after the infusion from patient 4, and hence the plasma-to-CSF ratio at this time point was at least 45 : 1.

Protein binding

CB 3717 was 97.6% ± 0.1% protein bound in human plasma. The free drug concentration was linear over the range 20–200 μM CB 3717 in plasma (τ = 0.99, $P < 0.001$). Below a concentration of 20 μM, CB 3717 was undetectable in plasma ultrafiltrates (< 1 μM).

Discussion

The pharmacokinetic studies of CB 3717 in man have been pertinent to its appraisal in early clinical studies. The peak drug plasma concentrations observed lie within the range that is cytotoxic to cultured cells in vitro (I_{50} 1–40 μM [6, 12] and are similar to those seen in mice at doses that cure the L1210 leukemia; [15]. In this respect antitumor effects have been observed in man during the phase-I trial [1].

With regard to toxicity, CB 3717-induced hepatotoxicity, although relatively mild and reversible, has been a frequent problem. The commonly associated malaise is troublesome to patients, and methods of amelioration have been sought. Prolongation of the infusion time to 12 h at doses of 200–400 mg/m^2 did not significantly alter either the incidence or the severity of hepatitis [4]. However, the peak plasma CB 3717 levels observed at 300–330 mg/m^2 following a 12-h-IV infusion were still within the concentration range associated with hepatotoxicity after 1-h infusions.

When CB 3717 was administered as a 1-h infusion there was a weak positive correlation between peak plasma CB 3717 concentrations and the subsequent elevation of serum transaminase levels. This reinforces the hypothesis, which is suggested by the frequency of hepatic disturbance, that the phenomenon is a direct toxic manifestation and not due to some idiosyncratic reaction. Although a dose-hepatotoxicity relationship could not be demonstrated with CB 3717 in a

larger series of patients [4], this may have been a reflection of interpatient variation in peak CB 3717 plasma levels. For example, in the present study there was no significant difference between the peak CB 3717 plasma levels achieved following 300 and 500 mg/m^2 (Table 2, t-test $P = 0.16$).

When considering the mechanism of hepatotoxicity it is of interest to compare CB 3717 with methotrexate (MTX). In high-dose regimens (1–20 g/m^2) MTX has been shown to produce transient rises in alanine transaminase values, in some cases up to >200 IU/l, which return to normal by day 7–10, with an incidence which varies between 50% and 100% [11, 16, 18, 19]. Further, Pratt et al. report an increasing incidence of liver function test abnormality with dose escalation [18]. At the top of the dose range, with 500 mg/kg (~17.5 g/m^2), the peak plasma concentration of MTX was approximately 1,600 μ*M*, whilst following 100 mg/kg (3.5 g/m^2) it was approximately 160–250 μ*M*. By comparison, peak plasma CB3717 concentrations of 20 μ*M* have been associated with hepatic dysfunction. Despite this difference in hepatotoxic potency there is some consolation to be gained from the fact that in 13 patients receiving more than four courses of CB 3717 the transaminase values normalized after five or six courses (unpublished observations). Similarly, liver autopsy specimens available from four patients surviving 6–18 months after high-dose MTX/citrovorum factor treatment who had developed abnormal liver function did not show any histopathological abnormalities [11].

The urinary excretion of CB 3717 is approximately 30% of the administered dose within 24 h of treatment, whereas a larger proportion of a MTX dose (up to 90%) can be recovered in the urine within a similar time period [2, 3, 5, 7]. Significant quantities of 7-hydroxyMTX are also found in the urine of patients receiving high-dose MTX [2, 10], and this is considered relevant to the renal toxicity seen in such regimens. MTX and 7-hydroxyMTX are both relatively insoluble at the acid pH generally present in the distal renal tubule, and may precipitate, giving rise to crystalluric tubular damage [10]. Although in the case of CB 3717 metabolites have not as yet been found in plasma or urine, the parent compound is a weak acid and insoluble at acid pH. Studies in mice showed that nephrotoxicity was caused by precipitation of CB 3717 in the renal tubules, which could be prevented by an alkalinization regimen [14]. Significantly, the autopsy kidney specimen from a patient who died 8 days after treatment at 330 mg/m^2 contained approximately 5% of the total dose although microscopically no crystalluria was seen and there was no evidence of renal failure. If further dose escalation in man were desired, concurrent alkalinization or hydration regimens would be recommended.

CB 3717 is highly plasma protein bound, and consideration should therefore be given to the co-administration of other drugs with this property to avoid displacement of CB 3717 and consequent increases in the circulating level of free drug. As a reflection of the substantial protein binding the volume of distribution of total plasma CB 3717 is roughly equivalent to the extracellular fluid space. In one patient good distribution of the drug into the ascitic fluid was seen, an observation which may be related to the activity of CB 3717 against ovarian cancer [1; S. B. Kaye, personal communication]. In contrast, CB 3717 did not partition readily into the CSF, being similar to MTX in this respect.

In conclusion, the results of this study have demonstrated that cytotoxic levels of CB 3717 can be achieved in human plasma. In conjunction with the phase-I clinical trial they have shown that extensive retention of the drug in the kidney can occur, which is probably related to the dose-limiting renal toxicity. In addition, a tentative correlation between the incidence of hepatic toxicity and peak CB 3717 plasma levels has been shown.

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