Cancer Chemother Pharmacol (1991) 28: 331–338
034457049100112Y

Cancer Chemotherapy and Pharmacology

The pharmacokinetics of the quinazoline antifolate ICI D 1694 in mice and rats*

D. I. Jodrell[1], D. R. Newell[1], W. Gibson[1], L. R. Hughes[2], and A. H. Calvert[1]****

[1] Drug Development Section, Institute of Cancer Research, Sutton, Surrey, UK
[2] ICI Pharmaceuticals, Macclesfield, Cheshire, UK

Summary. *N*-(5-[*N*-(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl)-L-glutamic acid (ICI D1694) is an analogue of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717). CB3717 was found to be an active anticancer agent in early clinical studies, but its use was limited by its relative insolubility at physiological pH. ICI D1694 has been shown to be a more active anticancer agent than CB3717 in model systems, and it is devoid of the acute renal toxicity associated with the administration of the latter drug to mice. In the present study, the pharmacokinetics of ICI D1694 were studied in both mice and rats using reverse-phase HPLC. In rats, ICI D1694 clearance (*CL*) conformed to a two-compartment open model and was rapid ($CL = 10.7$ ml min^{-1} kg^{-1}, $t_{1/2\beta} = 30$ min). Excretion was mainly biliary (65% of the delivered dose in 4 h vs 12% in urine) in the rat following a 100-mg/kg i.v. bolus. A high degree of protein binding was seen in rat plasma ($\geq 90\%$ over the range of 20–100 μM). In mice, ICI D1694 $CL = 27$ ml min^{-1} kg^{-1} and $t_{1/2\beta} = 30$ min following 100 mg/kg i.v., which was significantly faster than CB3717 clearance ($CL = 6$ ml min^{-1} kg^{-1}, $t_{1/2\beta} = 93$ min). ICI D1694 was fully bioavailable following i.p. administration (AUC = 3.73 mg ml^{-1} min i.v. 4.03 mg ml^{-1} min i.p.), but its bioavailability following oral administration appeared to be low (approximately 10%–20%). Tissue distribution and excretion studies in mice suggested that biliary excretion predominated, confirming the results obtained in rats. Following an i.v. dose of 500 mg/kg ICI D1694 in mice, drug was detectable at 24 h, suggesting the presence of a third phase of plasma clearance. The initial HPLC assay could not detect this third phase following a dose of 100 mg/kg; hence, a more sensitive assay was developed that includes a solid-phase extraction step. The latter assay was used to define the third phase of ICI D1694 clearance in mice, and preliminary studies demonstrated a terminal half-life of 6.5 ± 2.7 h.

Introduction

N-(5-[*N*-(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl)-L-glutamic acid (ICI D1694; Fig. 1, top) is an analogue of N^{10}-propargyl-5,8-dideazafolic acid (CB3717; Fig. 1, bottom). CB3717 is a potent folate-based inhibitor of the enzyme thymidylate synthase (TS, EC 2.1.1.45), with K_i values of <5 nM being reported for both murine (L1210) [9] and human (W1L2) enzyme [7]. CB3717 was found to be an active anticancer agent in early clinical trials, but its use was limited by renal and hepatic toxicities that were thought to be mediated in part by its poor solubility at physiological pH [2]. Removal of the amino group at the 2-position of CB3717 (see Fig. 1, bottom) has been shown to improve its solubility (>340 times at pH 7.4).

Desamino-CB3717 has also unexpectedly been found to be a 10-fold more potent inhibitor of L1210 cell growth, despite a 10-fold reduction in its affinity for TS [4, 10]. The 2-desamino-2-methyl analogue of CB3717 (ICI 198583) was found to show similar affinity for TS when compared with CB3717 but was more active against L1210 cells in vitro than was either CB3717 (34-fold difference in antitumour activity) or desamino-CB3717 (4-fold difference). In contrast to CB3717, which causes renal and hepatic toxicity in mice following a bolus dose of 100 mg/kg, both desamino-CB3717 and ICI 198583 were found to be nontoxic to the liver and kidneys at a dose of 500 mg/kg [3]. A

* These studies were supported by the UK Cancer Research Campaign and the British Technology Group

** *Present address:* Cancer Research Unit, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

Offprint requests to: D. I. Jodrell, Drug Development Section, E Block, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK

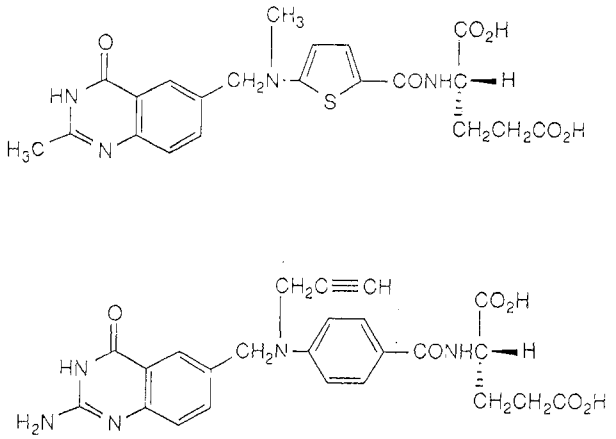

Fig. 1. The molecular structures of ICI D1694 (*top*) and CB3717 (*bottom*)

range of ICI 198 583 analogues have been synthesised, and following biochemical and antitumour testing, ICI D1694 has been shown to be an inhibitor of L1210-derived TS (K_i = 62 nM) and a highly active antitumour agent both in vitro [concentration of ICI D1694 required to inhibit L1210 cell growth by 50% (IC_{50}), 0.007 μM; CB3717 L1210 IC_{50}, 3.4 μM] [6] and in vivo. In vivo studies of ICI D1694 demonstrated antitumour activity in mice bearing the L1210:ICR cell line (>70% of the animals were cured at 0.4 mg/kg daily ×5 [5]) and the HX62 ovarian human-tumour xenograft (15-day growth delay at 1 mg/kg daily ×15 [17]). On the basis of these and other similar antitumour data and the demonstrated lack of toxicity of ICI D1694 to the liver and kidneys at 500 mg/kg [8], this drug was chosen for full preclinical evaluation prior to its entry into clinical trials.

ICI D1694 pharmacokinetics were studied in mice and rats and the data were compared with those previously obtained for CB3717 in similar investigations [12, 13]. Studies of ICI D1694 in mice were performed using a single dose (100 mg/kg) following intraperitoneal (i.p.), intravenous (i.v.) and oral administration. The pharmacokinetic studies defined the plasma concentration-time curves and the routes of excretion of ICI D1694. In addition, a comparison was made between the pharmacokinetics of ICI D1694 and those of CB3717 following i.v. administration. Studies in rats used a dose of 20 mg/kg with and without bile duct cannulation to investigate the potential influence of enterohepatic cycling on the plasma clearance of ICI D1694. The plasma clearance was also assessed in rats at 100 mg/kg. In initial studies, plasma levels of ICI D1694 were assessed using an HPLC assay system that had previously been employed to assay CB3717 [13]. However, pharmacokinetic results obtained in the mouse suggested the existence of a third phase of plasma clearance. Therefore, the existing assay was modified to include a solid-phase extraction step so as to increase its sensitivity. This modification of the assay and the results obtained following its use in the assessment of ICI D1694 clearance are also described.

Materials and methods

Materials

ICI D1694 was supplied by ICI Pharmaceuticals in the free acid form as a pale yellow powder; it was dissolved in 0.05 M $NaHCO_3$ and the pH was adjusted to 9–9.5 using NaOH. Solutions were prepared to enable the administration of a constant injection volume (10 ml/kg in mice, 2 ml/kg in rats). Injection solutions were protected from light and kept frozen until used, at which time they were thawed at room temperature. For oral administration, ICI D1694 was dissolved in phosphate-buffered saline (10 mM Na_2HPO_4, pH 7.5) and 10 ml/kg was given. CB3717 was supplied by ICI Pharmaceuticals as a clear solution of the disodium salt in 0.05 M $NaHCO_3$ at a concentration of 10 mg/ml. The molecular structure of ICI 212281, which was used as an internal standard in the more sensitive assay, is similar to that of ICI D1694 (the thiophene ring is replaced by thiazole and the N^{10}-substituent is ethyl); this drug was also supplied as a powder by ICI Pharmaceuticals. All other chemicals used were of analytical grade (when available) and were obtained from standard suppliers.

Methods

Pharmacokinetic studies in mice. Experiments were performed using C57/DBA2 F1 hybrid male mice aged between 6 and 10 weeks (National Medical Research Institute, NMRI, London). Drugs were given i.v. via the tail vein following whole-animal warming to ≤40° C. Oral administration was carried out using a 2-in. × 18-G curved needle with a bulbous tip. At selected time points, mice were killed by CO_2 asphyxiation and exsanguinated by open cardiac puncture. Blood was placed in heparinised microfuge tubes and immediately centrifuged. Plasma was separated and frozen for subsequent analysis. Samples were obtained at 5, 15, 30, 60, 90 and 120 min following parenteral administration and at 15, 30, 60, 90, 120, 240, 360, 480 and 1,440 min following oral dosing. At each time point the liver and kidneys were removed; the gall bladder was carefully excised, and the organs were thoroughly washed with deionised water (particularly following i.p. dosing) and then frozen for subsequent quantification of their ICI D1694 content.

Additional studies were performed using ICI D1694 at 500 mg/kg (i.v.) followed by limited sampling at 1, 4 and 24 h; blood and tissue samples were collected and prepared as above. This ICI D1694 dose (500 mg/kg) was directly compared with that of CB3717 (100 mg/kg) using this sampling schedule. For the assessment of ICI D1694 excretion, mice were placed in individual metabolism cages (Metabowls; Jencons, Hemel Hempstead, UK) and urine and faeces were collected in preweighed pots. These were replaced after 24 h and collections were continued until 48 h; thereafter, the cages were washed with 10 m/0.1 M TRIS (pH 10) and the washings were collected. Samples and washings were frozen until HPLC analysis was performed. Excretion studies were carried out following the i.v., i.p. and oral administration of 100 mg/kg ICI D1694; these data were compared with those obtained for CB3717 after i.v. administration of 100 mg/kg.

In studies designed to identify a third phase of clearance, whereby sample analysis was performed using the more sensitive assay, including solid-phase extraction, 100 mg/kg ICI D1694 was injected i.p. at time zero. Five mice were killed at hourly intervals for 8 h and then at 10, 12 and 24 h after drug administration; the animals were killed by CO_2 asphyxiation and exsanguinated by open cardiac puncture. Following centrifugation (11,600 *g* for 3 min), plasma was pooled (0.25 ml/mouse) and frozen prior to subsequent analysis by HPLC.

Pharmacokinetic studies in rats. Female Wistar rats were anaesthetised with 60 mg/kg i.p. pentobarbitone, and the trachea, the left carotid and left femoral veins were cannulated with polyethylene tubing. Patency of the carotid and femoral cannulae was maintained using heparinised saline (50 units/ml). The urethra was ligated to prevent voiding during the study. In certain studies, the common bile duct was also cannulated with polyethylene tubing and bile was collected. Rectal temperature was

maintained at 37°–38° C using overhead lamps. When required, further doses of pentobarbital (12.5 mg/kg) were injected via the femoral venous cannula. ICI D1694 was given via the left femoral cannula, and 200-µl blood samples were collected through the left carotid cannula at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 and 240 min after the injection of ICI D1694 and were centrifuged immediately. Plasma was frozen for later analysis by HPLC. After 4 h the rat was killed with an overdose of pentobarbital. Urine was aspirated from the bladder, the liver and kidneys were removed and wet-weighed and all samples were stored at –20° C. Studies ($n = 3$ rats) were performed using ICI D1694 (20 mg/kg i. v.) with or without bile duct cannulation to ensure that bile collection did not affect the plasma clearance or the pattern of excretion. ICI D1694 was also studied at 100 mg/kg i. v. following bile duct cannulation.

Sample preparation prior to HPLC analysis. All samples were thawed at room temperature. Then, 200 µl methanol was added to 100 µl plasma and samples were centrifuged at 1,500 *g* at 4° C for 20 min. The supernatant was analysed by HPLC, and standard curves were constructed for heparinised mouse and rat plasma (10–1,500 µM). Further standards were prepared in 0.05 M $NaHCO_3$ to assess recovery of drug from plasma. Tissue and faecal samples were weighed, soaked in 0.1 M TRIS (pH 10, 9 ml/g tissue wet weight; rat liver, 3 ml/g) for 1 h and homogenised in a Teflon/glass homogeniser. Then 1 ml methanol was added to 0.5 ml homogenate and protein was precipitated; following centrifugation as described above, the supernatant was analysed using HPLC. Bile samples were weighed to quantify the rate of bile excretion and then diluted 1 : 10 (v/v) with 0.1 M TRIS (pH 10). A further 1 : 10 dilution was performed on the 0 to 60-min bile specimen following the administration of 100 mg/kg ICI 1694. Next, 200 µl methanol was added to 100 µl diluted bile and centrifuged prior to HPLC analysis as described above. Urine samples were also weighed and diluted 1 : 10 (v/v) with 0.1 M TRIS (pH 10); as noted above 2 vol. methanol were added, the samples were centrifuged and the supernatant was analysed by HPLC. Washings from the metabolism cage were also analysed using HPLC.

Solid-phase extraction technique. Solid-phase extractions from plasma were performed using commercially packed 3-ml polythene tubes (Bond Elut; Analytichem International, Harbor City, Calif., USA) containing 200 mg C18 packing. First, 1.1 ml 0.1 M CH_3COONa (pH 4) and 0.055 ml 100 µM internal standard solution was added to 1.1 ml plasma. Next, 2 ml of this mixture was applied to pre-conditioned (3 ml methanol, 3 ml H_2O and 3 ml CH_3COONa, pH 4) Bond Elut cartridges. The cartridges were washed twice with 3 ml H_2O (pH 4, CH_3COOH) and then ICI D1694 was eluted with 3 ml 90 : 10 (v/v) methanol: H_2O. The eluent was dried under nitrogen at 50° C and reconstituted in 200 µl HPLC mobile phase (see below).

HPLC system. All HPLC analyses were performed on a Waters Associates (Northwich, UK) chromatograph. Separation was carried out on a Spherisorb 5-µm C6 (Phase Separation Ltd, Deeside, UK) column (15 × 0.46 cm, Waters Associates) equipped with a CO: Pell ODS precolumn (6.5 × 0.21 cm; Whatman Ltd, Maidstone, UK). Samples were eluted isocratically at a flow rate of 1.5 ml/min. The HPLC mobile phase comprised 40 : 60 (v/v) methanol: acetic acid (0.175 M); 30-µl samples (150 µl following solid-phase extraction) were applied to the column and data were collected for 20 min. Standard concentrations of ICI D1694 were prepared in 0.1 M TRIS (pH 10, 100 mg/ml) and in mouse or rat plasma (100 µM) for use as external standards. ICI D1694 and ICI 212281 were detected by UV absorption at both 313 and 280 nm.

Pharmacokinetic analyses of plasma concentration vs time data. The pharmacokinetic data obtained were analysed using a non-linear regression technique based on the method of Jenrich and Sampson [7 a] and a weighting function of $1/(y+\hat{y})^2$. An open two-compartment linear model was fit to the data. This model is described by the biexponential equation $C = Ae^{-\alpha t} + Be^{-\beta t}$, where A and B represent concentration constants and α and β represent rate constants for the two phases. The area under the plasma concentration vs time curve (AUC), volume of distribution of the central compartment (V_1), volume of distribution at steady state (V_{dss}), total body clearance (CL) and half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$) were calculated using standard equations [19]. For the determination of plasma clearance following i. p. administration, data points from the elimination phase of the plasma concentration vs time curve were fit to a single-compartment model described by the equation $C = Ae^{-\alpha t}$.

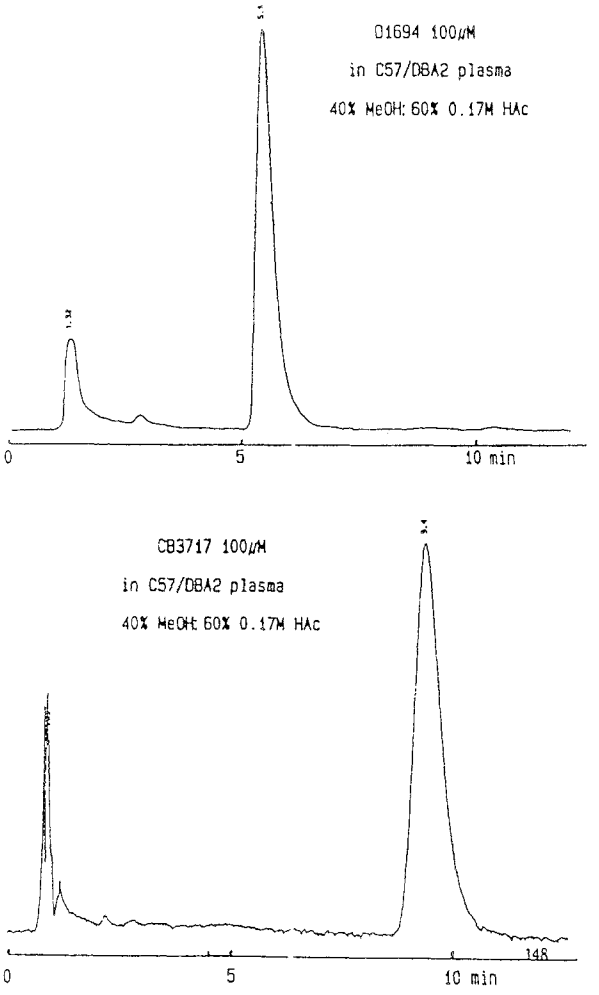

Fig. 2. Sample chromatograms from the HPLC assay for ICI D1694 (*top*) and CB3717 (*bottom*)

Protein-binding studies in rat plasma. For protein-binding studies, standard solutions of 20–1,500 µM ICI D1694 were prepared in Wistar rat plasma and incubated at 37° C for 30 min. Next, 0.5 ml was placed in the upper portion of an ultrafiltration tube (Centrifree Micropartition System; Amicon, Danvers, Mass., USA), which was centrifuged in a fixed-angle rotor (45°) at 3,000 *g* for 15 min. The concentration of drug in the ultrafiltrate, i. e. the free drug, was analysed by HPLC (see above). Similar standard concentrations were prepared in 0.1 M TRIS (pH 7.4) and added to the ultrafiltration tubes following incubation as described above. The drug concentration in the lower portion following centrifugation was compared with the standard concentration to assess non-specific binding to the filter. Results obtained using plasma standards were compared with those found for the filtered TRIS standards to correct for non-specific binding. The protein binding of ICI D1694 was studied to a concentration of 1,500 µM drug to encompass plasma concentrations found in rat pharmacokinetics studies at a dose of 100 mg/kg.

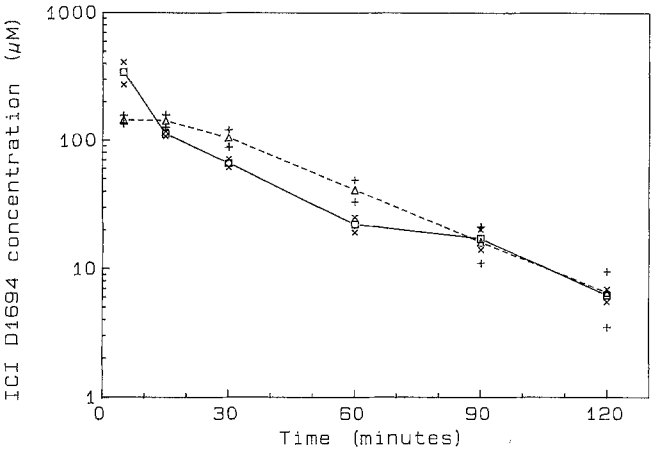

Fig. 3. The plasma clearance of ICI D1694 following a bolus dose of 100 mg/kg given i.v. (mean, □; SD, ×) and i.p. (mean, △; SD, +) to 3 mice each

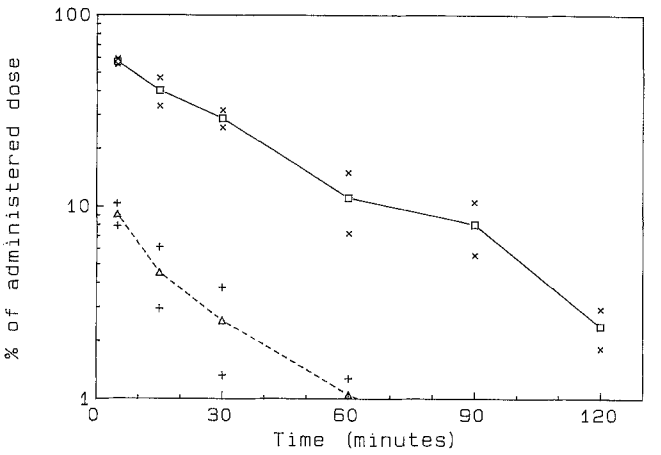

Fig. 4. The amount of ICI D1694 present in liver (mean, □; SD, ×) and kidney (mean, △; SD, +) tissue following an i.v. bolus dose of 100 mg/kg ICI D1694 in the mouse

Results

Validation of the HPLC assay

Standard curves were constructed for ICI D1694 and CB3717 in both mouse and rat plasma and 0.05 M $NaHCO_3$ over the concentration range of 10–1,500 μM. Linear relationships between peak area and concentration were demonstrated (CB3717, $r = 0.999$; ICI D1694, $r = 1$), and drug recovery from plasma was complete (CB3717, 99.5%±4.3%; ICI D1694, 105.3%±8.2%). The limit of detection for both compounds was 10 μM. Figure 2 shows sample HPLC chromatograms obtained for ICI D1694 (top) and CB3717 (bottom) in mouse plasma. The standard curve obtained using the Bond Elut assay for ICI D1694 demonstrated that the ratio of the peak areas for ICI D1694 and the internal standard was linear ($r = 0.999$) over the concentration range of 0.2–50 μM. The limit of detection of the assay system was 0.2 μM.

Pharmacokinetics of ICI D1694 in mice

The plasma levels of ICI D1694 following a bolus injection of 100 mg/kg are shown in Fig. 3, which compares i.v. and i.p. administration. A two-compartment open linear model was fit to data from the i.v. study and, after the initial absorption phase, a one-compartment model was fit to i.p. data. As can be seen in Table 1, after the initial distribution (i.v.) and absorption (i.p.) phases, the model fits and pharmacokinetic parameters were similar. Following i.v. administration, the terminal half-life was 30±3 min and the clearance was 26.8 ml min^{-1} kg^{-1}.

Table 1. Pharmacokinetic parameters for ICI D1694 given at 100 mg/kg by different routes of administration to mice

Route	C_{max} 5-min plasm[a] (μM)	$t_{1/2\beta}$ (min)	V_1 (ml/kg)	V_{dss} (ml/kg)	*CL* (ml min^{-1} kg^{-1})	AUC (mg ml^{-1} min)
i.v.	342 ±69[a]	30±3[b]	322	753	26.8	3.73
i.p.	145 ±11	22±2	–	781	24.8	4.03
p.o.	2.4[c]	–	–	–	–	–

[a] Data represent the mean ± SD ($n = 3$). C_{max}, Maximal plasma concentration

[b] Data represent the mean ± asymptotic error

[c] Detected only at 60 min in 2/3 mice

Table 2. Excretion of ICI D1694 after i.p., i.v., or p.o. administration of 100 mg/kg in mice

Route	Urine 0–24 h	Faeces 0–24 h	Total 0–24 h	Urine 24–48 h	Faeces 24–48 h	Total 0–48 h
i.v.	13±4	39± 4	52±3	2 ±1	1±3	54±2[a]
i.p.	12±8	33±11	46±8	3 ±4	1±2	50±7
p.o.	1±2	61±11	62±9	0.3 ±0.5	3±4	66±8

Data represent the percentage of the delivered dose expressed as the mean ± SD ($n = 4$)

[a] All cage washings accounted for <1% of the total dose

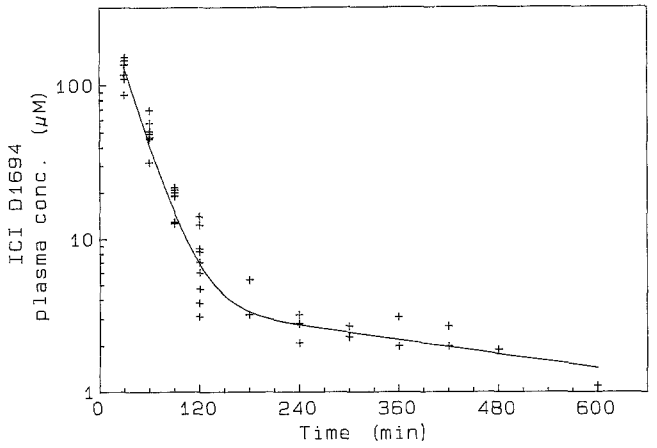

Fig. 5. The plasma clearance of ICI D1694 following an i.p. bolus dose of 100 mg/kg in the mouse. Later time points were measured using the modified assay incorporating solid-phase extraction

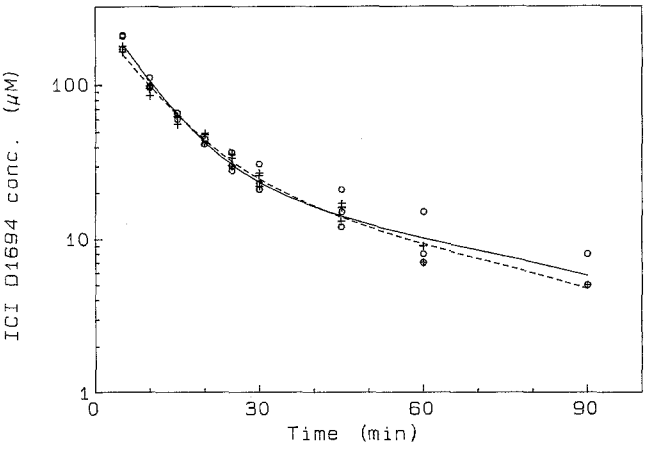

Fig. 6. The plasma clearance of ICI D1694 in the rat following an i.v. bolus dose of 20 mg/kg in studies carried out with (—O—) and without (– – + – –) bile collection ($n = 3$)

ICI D1694 levels detected in the liver and kidney (as a percentage of the delivered dose) over the same period are shown in Fig. 4. The high levels (peak, 57% at 5 min) seen in the liver following i.v. administration suggest that this is likely to be the major route of excretion. The proportions of the delivered dose of ICI D1694 found in urine and faeces at up to 48 h after i.p., i.v. and oral administration are shown in Table 2. These data were similar following i.v. and i.p. administration (i.v. 52% ± 3%; i.p., 46% ± 8% in the first 24 h), and the majority of the ICI D1694 dose was eliminated in the faeces (i.v., 39% ± 4% in the faeces vs 13% ± 4% in the urine), providing further evidence that the liver is the major route of excretion.

Following oral administration, ICI D1694 was detectable in the plasma at only one time point (60 min) and in only 2/3 animals; plasma concentrations in these two mice could only be estimated as being 2 and 4 μM. ICI D1694 was detectable in the liver at 30–120 min following oral administration but accounted for <1% of the delivered dose. Only 1%–3% of the ICI D1694 dose was found in the urine following oral administration, confirming that only a small percentage (possibly 10%–20%) of the delivered dose had been absorbed.

In all studies involving parenteral administration, the overall recovery of ICI D1694 from the urine and faeces was incomplete (i.v., 54% ± 2%, i.p., 50% ± 7%), and it

Table 3. Comparison in mice of plasma levels and organ content of ICI D1694 and CB3717 following doses of 500 and 100 mg/kg, respectively

Time	Plasma level (μM)		Liver content[a]		Kidney content[a]	
	CB3717	ICI D1694	CB3717	ICI D1694	CB3717	ICI D1694
1 h	155 ± 12[b]	145 ± 25	46 ± 2	5.7 ± 1	8.2 ± 3.5	0.7 ± 0.2
4 h	71 ± 12	c.4 ± 1[c]	11.4 ± 3.1	0.2 ± 0.1	13.1 ± 4.4	Trace in 1/4 mice
24 h	$c = 1$ in 4/4 mice [c]	Trace in 2/4 mice $c = 4$ and 2 μM[c]	3.3 ± 0.5	Trace in 2/4 mice <0.2%	5.1 ± 2.1	Not detected

[a] Data represent the percentage of the total delivered dose
[b] Data represent the mean ± SD ($n = 4$)
[c] Approximation at the limit of detection. c, Concentration

Table 4. Pharmacokinetic parameters for ICI D1694 in the rat

Parameter	Dose		
	20 mg/kg,bile duct intact	20 mg/kg,bile collection	100 mg/kg,bile collection
$t_{1/2}\alpha$ (min)	5.4 ± 1.5	4.5 ± 0.9	7.3 ± 0.5
$t_{1/2}\beta$ (min)	31 ± 14	29 ± 6	45 ± 3
V_1 (ml/kg)	152 ± 30	114 ± 28	191 ± 34
V_{dss} (ml/kg)	295 ± 90	240 ± 57	398 ± 62
C (ml min^{-1} kg^{-1})	11.9 ± 0.6	10.6 ± 1.5	11.4 ± 0.8
Plasma AUC (mg ml/min)	1.69 ± 0.09	1.91 ± 0.29	8.83 ± 0.58

Rats were anaesthetised using pentobarbitone and compounds were injected via i.v. bolus. Data represent the mean ± SD ($n = 3$)

was impossible to account for the remainder of the ICI D1694 dose used in these experiments. Samples were assayed for a range of potential ICI D1694 metabolites using characterised degradation products of the drug; these degradation products investigated included desglutamate-ICI D1694 and descarboxy-desglutamate-ICI D1694 (results not shown), but these compounds were not detected in the HPLC effluent.

Comparative pharmacokinetics of ICI D1694 and CB3717 in mice

Following the i.v. administration of 500 mg/kg ICI D1694 and 100 mg/kg CB3717, the plasma levels and the liver and kidney content at 1, 4 and 24 h were assayed and are listed in Table 3. These data show that the clearance of ICI D1694 was more rapid, with similar plasma levels being seen at 1 h (CB3717, 155 ± 12 μM; ICI D1694, 145 ± 25 μM) despite the 5-fold difference in the delivered dose; at 4 h the difference was even more apparent (CB3717, 71 ± 12 μM; ICI D1694, 4 ± 1 μM). Retention in the tissues was also clearly found for CB3717 but not for ICI D1694. Over 10% of the CB3717 dose persisted in the liver and kidneys at 4 h after dosing, at which time <1% of the delivered dose of ICI D1694 was detected. Despite its rapid early clearance, ICI D1694 could be detected in the plasma at 24 h in 2/4 mice. In excretion studies the more rapid clearance of ICI D1694 was confirmed. ICI D1694 was predominantly excreted during the first 24 h (0–24 h: urine, $30\% \pm 10\%$; faeces, $21\% \pm 7\%$ vs 24–28 h: urine, $3\% \pm 1\%$; faeces, $2\% \pm 1\%$ of the delivered dose). In all, 32% of the dose was accounted for in the urine. In contrast, $30\% \pm 11\%$ of the CB3717 dose was accounted for during the first 24 h and a further $18\% \pm 7\%$ was excreted at 24–48 h, highlighting the slow clearance of this compound.

Studies to identify a third phase of ICI D1694 clearance in the mouse

Data from all experiments using 100 mg/kg ICI D1694 (i.p.) in mice, including those obtained using the more sensitive solid-phase assay, are shown in Fig. 5. A third phase of plasma clearance was clearly demonstrated at time points of >180 min. The results generated by non-linear least-squares fitting of the biexponential equation were used to calculate the pharmacokinetic parameters. The volume of the first compartment (V_1) was 493 ml/kg and the volume of distribution at steady state (V_{dss}) was 2,000 ml/kg. The half-life for the second phase of ICI D1694 clearance was 17 ± 1 min, and that for the terminal phase was 390 ± 162 min. The total body clearance was calculated to be 16.3 ml min^{-1} kg^{-1}.

Pharmacokinetics of ICI D1694 in the rat

The plasma levels of ICI D1694 in rats treated at a dose of 20 mg/kg in the presence and absence of a bile cannula are shown in Fig. 6, and the pharmacokinetic parameters derived from this comparison are shown in Table 4 together with those obtained in studies using 100 mg/kg ICI D1694. No significant difference was found between any of the parameters listed following the administration of ICI D1694 (20 mg/kg) to rats with or without bile cannulae. Moreover, the 4-h urinary excretion was similar in both sets of animals, corresponding to $14\% \pm 1\%$ (intact bile duct) and $9\% \pm 5\%$ (bile collection) of the delivered dose.

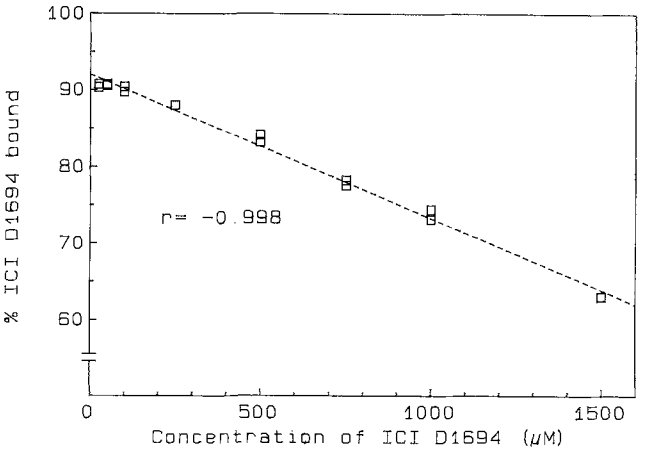

Fig. 7. The reversible protein binding of ICI D1694 at increasing concentrations in the plasma of female Wistar rats

Comparison of the parameters derived from studies using doses of 20 and 100 mg/kg revealed that the volume of distribution of ICI D1694 both in the central compartment and at steady state were significantly ($0.01 < P < 0.05$) greater at the higher dose. Biliary excretion following a dose of 100 mg/kg was significantly reduced, amounting to $58\% \pm 3\%$ in 1 h and $67\% \pm 2\%$ in 4 h as compared with $70\% \pm 3\%$ and $78\% \pm 4\%$, respectively, at 20 mg/kg. As a result, total excretion during the first 4 h was also lower at the higher dose, corresponding to $79\% \pm 1\%$ at 100 mg/kg as compared with $87\% \pm 4\%$ at 20 mg/kg. Following the administration of 20 mg/kg ICI D1694, the biliary flow rate was unaltered by drug administration, amounting to 65 ± 8 μl min^{-1} kg^{-1} during the 1st h vs 76 ± 10 μl min^{-1} kg^{-1} h^{-1} prior to drug administration. However, after a dose of 100 mg/kg, there was a clear increase in the biliary flow rate to 113 ± 14 μl min^{-1} kg^{-1} (pretreatment value, 70 ± 16 μl min^{-1} kg^{-1}) over the same period.

Protein-binding studies

In these studies, the results obtained using plasma standards were compared with those found for the filtered TRIS standards to correct for non-specific binding. However, the concentration in filtered standards was $99\% \pm 3.7\%$ of the original value, indicating the absence of non-specific binding. The protein binding of ICI D1694 appeared to be concentration-dependent in a linear manner over the range of 25–1,500 μM in rat plasma (Fig. 7). A linear regression (r) value of –0.998 was obtained. At

concentrations of up to 100 μM, 90% of the drug was protein-bound, whereas at the highest concentration tested (1,500 μM), only 61% of the drug remained bound.

Discussion

The present studies defined the pharmacokinetics of ICI D1694 following i.v. administration in rats and mice and i.p. and oral administration in mice; they also directly compared the pharmacokinetics of ICI D1694 and CB3717 in mice. In preclinical studies in mice, the i.p. route is generally used when repeated administration is required. In man, the i.v. route is most commonly used; therefore, it is important that bioavailability following i.p. administration be assessed. The AUC was similar after i.p. and i.v. administration (Table 1), demonstrating 100% bioavailability following an i.p. injection. The total body clearance of ICI D1694 was rapid (26.8 ml min^{-1} kg^{-1}) and the β-phase half-life was short (30 min) following i.v. administration. Maximal plasma levels (C_{max}) were seen at 5 min after i.p. administration, suggesting rapid absorption from the peritoneal cavity. This was clearly more rapid than the CB3717 absorption found in a previous study, in which C_{max} was noted at 120 min after i.p. administration [13].

Bioavailability following oral administration was low. ICI D1694 was detectable only at a single time point (60 min) in 2/3 mice. In excretion studies, 1%–3% of the delivered ICI D1694 dose was detected in the urine, suggesting a bioavailability of approximately 10%–20% (Table 3). ICI D1694 has been shown to be an active antitumour agent following oral administration in both the L5187Y (murine lymphoma) and HX62 (human ovarian xenograft) models (T. C. Stephens, personal communication). Therefore, plasma levels clearly associated with activity could not be detected by the HPLC system used initially.

Data from excretion studies in mice (Table 2) and the significantly higher ICI D1694 concentrations found in the liver as compared with the kidneys (Fig. 4) suggested that ICI D1694 is predominantly excreted in the bile. This was confirmed by studies in rats, in which 78% of the delivered dose of ICI D1694 (20 mg/kg) was excreted in the bile within 4 h. The low recovery of ICI D1694 (Table 2) seen in excretion studies in mice remains unexplained. It is possible that recovery of ICI D1694 from mouse faeces may be incomplete. Another explanation might be that ICI D1694 is metabolised within the gut lumen, as has been observed for both methotrexate (MTX) and CB3717 [13, 18]. However, comparison of the chromatograms obtained for synthetic desglutamate-ICI D1694 and descarboxy-desglutamate-ICI D1694 did not detect these potential metabolites in faecal samples (results not shown). These are likely metabolites of ICI D1694, by analogy with the formation of desglutamate-CB3717 from CB3717 in the mouse gut [13]. A third possible explanation would be that ICI D1694 is trapped in an enterohepatic cycle. Methotrexate has been shown to undergo enterohepatic circulation [16], and nuclear magnetic resonance studies using ^{19}F suggest that enterohepatic cycling occurs in rats following the administration of quinazoline antifolates [14]. However, enterohepatic cycling of ICI D1694 in rats was not apparent in the present study. In investigations in which the bile duct was either cannulated or left intact, ICI D1694 clearance was identical (Fig. 7). Finally, it is possible that the poor recovery of ICI D1694 in murine excretion studies is a result of rapid drug uptake into cells and subsequent metabolism to polyglutamate forms that are retained within the cell. Further explanation of this poor recovery will require the synthesis of radiolabelled ICI D1694.

The clearance of ICI D1694 was clearly more rapid than that of CB3717. Confirming the results of previous studies [12], CB3717 was detectable in both the liver and the kidney at 24 h after administration. Excretion studies also demonstrated the slow clearance of CB3717, whereby only 30% of the delivered dose was accounted for by 24 h and a further 18% was detected between 24 and 48 h (Table 4).

In rats, ICI D1694 pharmacokinetics following a dose of 20 mg/kg differed from those obtained using 100 mg/kg. The volume of distribution was higher at the latter dose (Table 6); the increase in distribution was probably a result of concentration-dependent protein binding. At peak plasma levels following the administration of 100 mg/kg (approx. 1,000 μM), the free ICI D1694 fraction was 25% as compared with a free fraction of 10% at the peak level following a dose of 20 mg/kg (approx. 200 μM). The increased proportion of free drug could facilitate distribution and cellular uptake, leading to the larger apparent volumes of distribution. Excretion of drugs in the bile increases the biliary flow rate [11, 15], and this was observed in the rat following the administration of ICI D1694 at 100 mg/kg but not at 20 mg/kg. In contrast, CB3717 (40 mg/kg) has been shown to reduce the biliary flow rate dramatically [12], an effect that is probably due to the precipitation of CB3717 in bile canaliculi, which may in turn be the cause of CB3717 hepatotoxicity. A comparison of ICI D1694 clearance in mice and rats as a function of body surface area produced similar results. Clearance in the rat was 64 ± 9 ml min^{-1} m^{-2} at 120 mg/m^2 ICI D1694 and 68 ± 5 ml min^{-1} m^{-2}, at 600 mg/m^2, and that in mice was 80 ml min^{-1} m^{-2} at 300 mg/m^2. Estimated α and β half-lives in mice and rats were also similar. It would be interesting to compare these data with results obtained in patients, when they become available.

The demonstration of a third phase of ICI D1694 clearance in mice was not unexpected, in the light of preliminary studies in which ICI D1694 was detected in plasma at 24 h after the administration of 500 mg/kg i.v. A third phase of clearance has been described for MTX [1], which may be attributable to enterohepatic cycling [16]. The question as to whether ICI D1694 undergoes enterohepatic cycling was addressed above.

In summary, ICI D1694 plasma clearance in mice was shown to conform to an open three compartment linear model, with the terminal half-life being approx. 6.5 h. ICI D1694 was found to be 100% bioavailable following i.p. administration in mice, but oral bioavailability was low (ca. 10%). ICI D1694 was primarily excreted in the bile (rats), with subsequent faecal elimination being observed (mice). In addition to its rapid plasma clearance, ICI D1694 was not retained in the liver or kidney as was

CB3717. As has previously been reported [8], the lack of hepatic and renal retention is likely to be related to the reduction in organ toxicity associated with ICI D1694 in comparison with CB3717.

References

1. Calvert AH, Bondy PK, Harrap KR (1977) Some observations on the human pharmacology of methotrexate. Cancer Treat Rep 61(9): 1647–1656
2. Calvert AH, Alison DL, Harland SJ, Robinson BA, Jackman AL, Jones TR, Newell DR, Siddick ZH, Wiltshaw E, McElwain TJ, Smith IE, Harrap KR (1986) A phase I evaluation of the quinazoline antifolate thymidylate synthase inhibitor, N^{10}-propargyl-5,8-dideazafolic acid, CB3717. J Clin Oncol 4 (8): 1245–1252
3. Harrap KR, Jackman AL, Newell DR, Taylor GA, Hughes LR, Calvert AH (1989) Thymidylate synthase, a target for anticancer drug design. Adv Enzyme Regul 29: 161–179
4. Jackman AL, Taylor GA, O'Connor BM, Bishop JA, Moran RG, Calvert AH (1990) Activity of the thymidylate synthase inhibitor 2-desamino-N^{10}-propargyl-5,8-dideazafolic acid and related compounds in murine (L1210) and human (W1L2) systems in vitro and in L1210 in vivo. Cancer Res 50: 5212–5218
5. Jackman AL, Taylor GA, Bishop JA, O'Connor BM, Bisset G, Hughes LR, Moran RG, Calvert AH (1990) Biochemical aspects of a new thymidylate synthase inhibitor, ICI D1694. Proc Am Assoc Cancer Res 31: 342
6. Jackman AL, Marsham PR, Moran RG, Kimbell R, O'Connor BM, Hughes LR, Calvert AH (1991) Thymidylate synthase inhibitors: the in vitro activity of a series of heterocyclic benzoyl ring modified 2-desamino-2-methyl-N^{10}-substituted-5,8-dideazafolates. Adv Enzyme Regul 31: 13–27
7. Jackson RC, Jackman AL, Calvert AH (1983) Biochemical effects of a quinazoline inhibitor of thymidylate synthetase. CB3717, on human lymphoblastoid cells. Biochem Pharmacol 32: 3783–3790
8. Jodrell DI, Newell DR, Calvete JA, Stephens TC, Calvert AH (1990) Pharmacokinetic and toxicity studies with the novel quinazoline thymidylate synthase inhibitor, ICI D1694. Proc Am Assoc Cancer Res 31: 341
9. Jones TR, Calvert AH, Jackman AL, Brown SJ, Jones M, Harrap KR (1981) A potent antitumour quinazoline inhibitor of thymidylate synthetase: synthesis, biological properties and therapeutic results in mice. Eur J Cancer 17: 11–19
10. Jones TR, Thornton TJ, Flinn A, Jackman AL, Newell DR, Calvert AH (1989) Quinazoline antifolates inhibiting thymidylate synthase: 2-desamino derivatives with enhanced solubility and potency. J Med Chem 32: 847–852
11. Macarol V, Morris TQ, Baker KJ, Bradley SE (1970) Hydrocortisone choleresis in the dog. J Clin Invest 49: 1714–1723
12. Newell DR (1984) The role of pharmacokinetic studies in the development of selective cancer chemotherapy. PhD Thesis, University of London
13. Newell DR, Alison DL, Calvert AH, Harrap KR, Jarman M, Jones TR, Manteuffel-Cymborowska M, O'Connor P (1986) Pharmacokinetics of the thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (CB3717) in the mouse. Cancer Treat Rep 70 (8): 971–979
14. Newell DR, Maxwell RJ, Bisset GMF, Jodrell DI, Griffiths JR (1990) Pharmacokinetic studies with the antifolate C^2-desamino-C^2-methyl-N^{10}-propargyl-2′-trifluoromethyl-5,8-dideazafolic acid (CB3988) in mice and rats using in vivo [^{19}F]-NMR spectroscopy. Br J Cancer 62 (5): 766–772
15. Shaw H, Capel I, Heath T (1972) Effect of ethacrynic acid on bile formation in sheep, dogs, rats, guinea pigs and rabbits. J Pharmacol Exp Ther 182: 27–33
16. Steinberg SE, Campbell CL, Bleyer WA, Hillman RS (1982) Enterohepatic circulation of methotrexate in rats in vivo. Cancer Res 42: 1279–1282
17. Stephens TC, Calvete JA, Janes D, Waterman SE, Valcaccia BE, Hughes LR, Calvert AH (1990) Antitumour activity of a new thymidylate synthase inhibitor, ICI D1694. Proc Am Assoc Cancer Res 31: 342
18. Valerino DM, Johns DG, Zaharko DS, Oliverio VT (1972) Studies of the metabolism of methotrexate by intestinal flora: 1. The identification and study of biological properties of the metabolite 4-amino-4-deoxy-N^{10}-methylpteroic acid. Biochem Pharmacol 21: 821–831
19. Wagner JG (1975) Fundamentals of clinical pharmacokinetics. Drug Intelligence Publications, pp 82–83