Virus-directed, Enzyme Prodrug Therapy with Nitroimidazole Reductase: A Phase I and Pharmacokinetic Study of its Prodrug, CB1954[1]

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ABSTRACT

CB1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide] is converted by the bacterial enzyme nitroimidazole reductase (NTR) into a potent cytotoxic bifunctional alkylating agent, which can be delivered to tumors in adenoviral vectors as virus-directed, enzyme prodrug therapy. This report summarizes a Phase I and pharmacokinetic study of the prodrug, CB1954. Thirty patients, ages 23–78 years (median 62 years), with predominantly gastrointestinal malignancies were treated. CB1954 was administered by i.v. injection every 3 weeks or i.p. followed by 3-weekly i.v. injections, toward a maximum of six cycles. The dose was escalated from 3 to 37.5 mg/m^2. No significant toxicity was seen until 24 mg/m^2 (recommended i.v. dose). Dose-limiting toxicities (DLT) were diarrhea and hepatic toxicity, seen at 37.5 mg/m^2. DLT has not been observed at the current i.p. dose of 24 mg/m^2. There was no alopecia, marrow suppression, or nephrotoxicity. Clearance data suggest hepatic metabolism, and <5% of CB1954 was renally excreted. There was a nonlinear relationship between i.v. dose and area under the curve (AUC). At the recommended i.v. dose of 24 mg/m^2, the AUC was 5.8 μM/h. Intraperitoneal administration (24 mg/m^2) achieved an AUC of 387 μM/h, giving a considerable regional advantage. *In vitro*, the AUC required to achieve the IC$_{50}$ for CB1954, in NTR-expressing cancer cells, ranges from 10–50 μM/h. Thus, CB1954 is well tolerated at a dose of 24 mg/m^2, and sufficient serum/peritoneal levels are achieved for an enzyme-prodrug approach to be feasible. We are now conducting a Phase I trial combining adenovirus-mediated NTR and i.v. CB1954 (24 mg/m^2) in patients with primary and secondary liver tumors.

INTRODUCTION

CB1954[3] is a weak monofunctional alkylating agent, which is converted by the *Escherichia coli* bacterial enzyme NTR to a cytotoxic species [5-(aziridin-1-yl)-4-N-acetoxy-2-nitrobenzamide], which induces cell death by forming interstrand DNA cross-links (Ref. 1; Fig. 1). *In vitro*, NTR-expressing cancer cell lines are up to 500-2000-fold more sensitive to CB1954 than parental cell lines (2, 3). Cell killing by activated CB1954 is cell cycle independent (4) and occurs in tumors, which have acquired resistance to other antineoplastic agents such as cisplatin (5). *In vivo*, CB1954 was found to be highly active against Walker rat 256 tumor xenografts because of high levels of endogenous DT-diaphorase (6). NTR is 100-fold more effective than DT-diaphorase in converting CB1954 to the activated species (7). There is no human homologue of NTR, and CB1954 is a poor substrate for human DT-diaphorase and is not readily converted to the activated species (8). Thus a VDEPT approach (Fig. 2), using adenovirus-mediated NTR combined with CB1954, may offer tumor-specific cell killing, because only cells expressing NTR should be directly susceptible to CB1954.

In a peritoneal pancreatic cancer model (SUIT-2), 80% of mice with NTR-expressing xenografts achieved long-term remissions when treated with CB1954 compared with none with wild-type tumors (5). Furthermore, in a peritoneal SUIT-2 model, which more closely resembles the clinical setting, NTR delivered i.p. by a replication-deficient adenovirus followed by i.p. CB1954 doubled the median survival ($P < 0.0001$; Ref. 3).

Only a small proportion of the target tumor cell population can be transduced to express the therapeutic gene by viral delivery methods. However, an important feature of the NTR/CB1954 combination is the "bystander effect," where surrounding cells not expressing the enzyme are also killed by a cell-permeable metabolite (9). *In vitro*, cell mixing experiments with wild-type and NTR-expressing human ovarian cancer cells exposed to 10 μM of CB1954 demonstrated 50% cell kill when only 10% of cells expressed NTR compared with no cell kill in wild-type cells (5). Moreover, there is a significant reduction in the tumor growth rate in a hepatoma xenograft model when only 5% of cells expressed NTR, thus demonstrating a strong by-

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[3] The abbreviations used are: CB1954, 5-(aziridin-1-yl)-2,4-dinitrobenzamide; G, grade; NTR, nitroimidazole reductase; VDEPT, virus directed enzyme-prodrug therapy; DLT, dose-limiting toxicities; AUC, area under the curve; i.p., intraperitoneal; i.v., intravenous; CTC, common toxicity criteria (National Cancer Institute, Bethesda, MD).

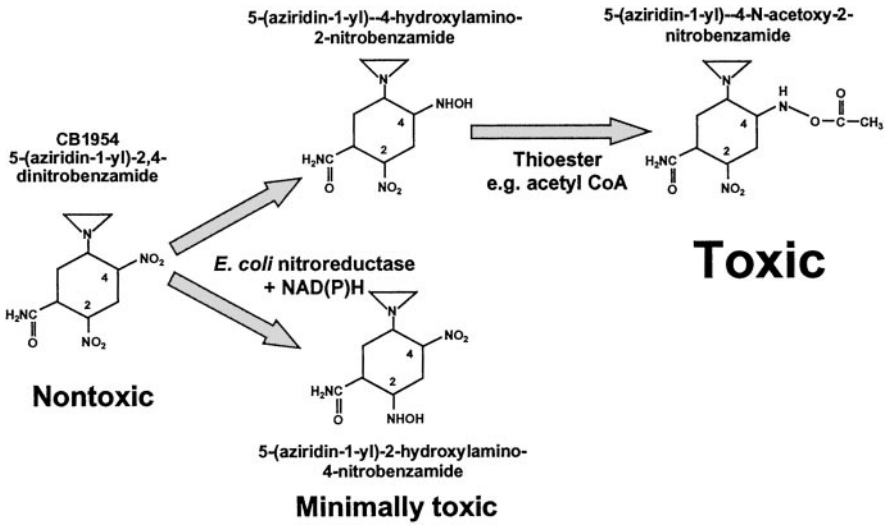

Fig. 1 Diagram showing the conversion of CB1954 to its active metabolite.

stander effect is generated with the NTR/CB1954 combination *in vivo* (10).

Formal evaluation of the toxicology of CB1954 in mice has shown that a single i.v. bolus at 88 mg/kg produced nonspecific systemic toxic effects and reduced testicular size. No systemic toxicity was observed after single or multiple weekly i.v. doses of 22 mg/kg. However, some lenticular changes (mild, diffuse lens opacification) were seen at a dose of 20 mg/kg, which were also present, to a lesser degree, in untreated mice. These effects were less severe in older mice ages >33 weeks, suggesting that lenticular changes are a spontaneous age-related occurrence, perhaps enhanced by CB1954. No lenticular changes were seen at a dose of 5 mg/kg. No lens abnormalities were detected in cynomolgus monkeys after CB1954 treatment.[4]

Because the VDEPT approach to cancer therapy has at least three potential sources of toxicity (adenoviral vector-encoding NTR, CB1954 alone, and the combination of the virus followed by CB1954), it was considered logical to assess the toxicity and pharmacokinetics of CB1954 as a single agent as a prelude to the combined virus/prodrug trial. Given the potential for compartmental administration of prodrug and virus to patients with peritoneal carcinomatosis, both the i.v. and i.p. routes of administration were investigated.

PATIENTS AND METHODS

Patient Selection. The study eligibility inclusion criteria were histologically confirmed cancers refractory to standard treatment or for which no conventional therapy existed. Although CB1954 is a weak substrate for other cellular reductases and has potential anticancer properties as an antipurine and inhibitor of ribonucleotide reductase, the likelihood of antitumor activity with CB1954 alone was low. This was carefully explained to the patient, and informed consent was obtained according to International Conference on Harmonization guidelines for good clinical practice. Other inclusion criteria were: age ≥18 years; WHO performance status of 0–2; serum levels of less than twice the upper limit of the normal range for creatinine, bilirubin, aspartate aminotransferase, and alanine aminotransferase (in the case of liver metastases, patients with aspartate aminotransferase and alanine aminotransferase $< \times 5$ the upper limit of the normal were eligible); hemoglobin ≥10 g/dl; white cell count $\geq 3 \times 10^9$/liter; platelets $\geq 150 \times 10^9$/liter; and the ability to give written, informed consent. For the i.p. arm of the study the additional inclusion criterion was malignant ascites requiring therapeutic drainage by paracentesis. The local research ethics committee approved the study protocol.

Exclusion criteria were as follows: previous malignancy; concurrent illness incompatible with the study protocol; active infection; chemotherapy within 4 weeks of study entry; and pregnancy or lactation. Additional exclusion criteria for the i.p. arm were: abdominal surgery within 4 weeks of study entry; extensive surgical adhesions; previous peritonitis; and previous or imminent bowel obstruction.

On trial entry and 1week before CB1954 administration, patients had a medical history and physical examination including appropriate tumor measurements. Blood samples were taken for hematological and biochemical indices including liver function and tumor markers, as appropriate. Patients also underwent chest X-ray, electrocardiograph, and radiological assessment of tumor size by ultrasound or computed tomography scanning. Because of concerns of possible cataract formation, an experienced ophthalmologist performed serial ophthalmological assessments including slit lamp examination, and patients on long-term corticosteroids were excluded from the trial. Patients were assessed weekly while on treatment and then 21 days after the final treatment or until treatment sequelae resolved.

CB1954 Administration. The initial i.v. and i.p. dose was 3 mg/m^2, which was less than one-tenth of the LD_{10} in mice. Dose escalation was calculated according to a modified Fibonacci scheme with a minimum of three patients per dose level but up to 6 patients if toxicity was observed. Dose-limiting toxicity was defined as grade 2 hepatic, renal, or neurological

[4] Toxicology report, Cobra Therapeutics, Ltd.

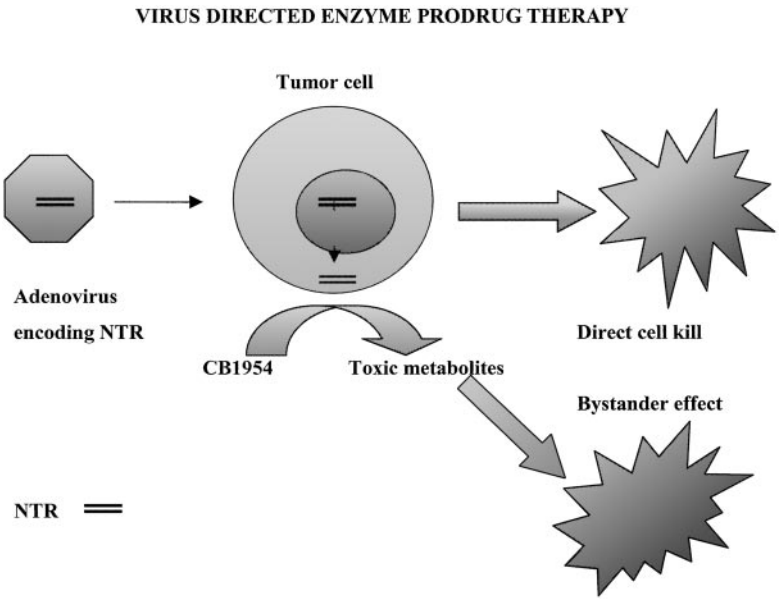

Fig. 2 Diagram outlining the VDEPT strategy.

toxicity; grade 3 mucositis, diarrhea, or ocular toxicity; and grade 4 hematological toxicity. The maximum tolerated dose was the dose level below which two of six or >33% of patients experienced DLT.

CB1954 was formulated in *N*-methyl pyrrollidone and polyethylene glycol 300. Intravenous CB1954 was given in 100 ml of normal saline over 5 min. CB1954 was administered every 3 weeks toward a maximum of six cycles. Intraperitoneal CB1954 was administered once only to patients with refractory ascites, via a paracentesis tube, after complete drainage of the ascites. Intraperitoneal CB1954 was administered over 15–20 min in 1 liter of a 4% icodextrin dialysate, which prolongs persistence of drug in the peritoneal cavity (11), and was left *in situ* for 24 h before removal. Patients subsequently received i.v. CB1954 3 weeks after i.p. CB1954, with a maximum of six cycles. Antiemetics (metoclopramide) and corticosteroids were administered as clinically indicated.

Pharmacokinetics. Peripheral venous blood samples were obtained for pharmacokinetic analysis of CB1954 at the following times relative to the end of the first infusion of CB1954: predose, 4, 10, 15, and 30 min and 1, 2, 4, 6, and 8 h after dose. Urine was collected for 24 h after CB1954 infusion. CB1954 concentration was assayed in urine produced between 0–4, 4–12, and 12–24 h after infusion. Intraperitoneal samples were taken after discarding the first 4 ml at the following time intervals: predose, 10, 20, 30, 60, 120, 240, and 360 min after i.p. infusion.

CB1954 was assayed using high-performance liquid chromatography using the following published protocol (12). Mitomycin C (500 ng/50 μl water) was added as an internal standard to 1 ml of plasma in a 15-ml polypropylene tube followed by the addition of 100 μl of 1 M phosphate buffer (1 M KH_2PO_4 and 1 M K_2HPO_4, titrated to pH 7). Extraction was accomplished by the introduction of ethanol (6 ml) followed by vortex mixing for 15 min. After mixing, the sample was centrifuged at 3,210 × *g* for 15 min. The supernatant was decanted into a fresh 15-ml polypropylene tube and dried by vortex evaporation (rapidvap; GRI, Essex, United Kingdom) settings (40°C, 85% speed, and 27 mm Hg). Once dry, the sample was resuspended in 200 μl of mobile phase A [Buffer A 17.5% Acetonitrile: 82.5% of 0.02 M phosphate buffer (0.02 M KH_2PO_4 and 0.02 M K_2HPO_4, titrated to pH 7)], and the resulting solution was centrifuged 13,000 × *g* for 15 min and 80 μl injected onto the high-performance liquid chromatography. Analysates were detected by UV absorption at 340 nm. The limit of detection by this method is 2.9 ng/ml. The concentration-time profiles were fitted using an in-house program based on the Marqhardt algorithm.

RESULTS

Patients. Patient characteristics are shown in Table 1. Thirty patients, ages between 23–78 years (median 62), were treated. Nineteen patients were male. There were 13 colorectal cancers, 4 gastric, 3 esophageal, and 3 mesotheliomas, with ovarian, pancreatic and unknown primaries accounting for the remainder. Twenty patients had received chemotherapy previously.

The dose range is summarized in Table 2, which shows that 22 patients received i.v. CB1954, and 8 patients had i.p. CB1954, of whom 4 received subsequent i.v. therapy. Patients received between one and six cycles (median two cycles) of i.v. treatment. The dose ranges achieved were 3–37.5 mg/m^2 and 3–24 mg/m^2 in the i.v. and i.p. arms, respectively.

Toxicity. Side effects are summarized in Table 3. No significant toxicity was seen until the fifth dose level of 24 mg/m^2, where 2/4 patients suffered CTC G3 nausea. Additionally, 8/9 patients at dose levels 24–30 mg/m^2 had mild G1–2 diarrhea. At a dose level of 30 mg/m^2, 2/5 patients had G2 and G3 transaminase elevations. However, one of these patients also had radiological evidence of progressive hepatic metastatic cancer, which may have contributed to the deterioration in liver function. At 37.5 mg/m^2, 3/3 patients suffered DLT. There was 1 G4 diarrhea, 1 G2 hyperbilirubinemia, and 1 G2 transaminase

Table 1 Patient characteristics

Characteristic	Number
Patient numbers	30
Male:female	19:11
Median age (range)/yr	62 (23–78)
Histological diagnosis	
Colorectal	13
Gastric	4
Esophagus	3
Mesothelioma	3
Ovary	2
Cholangiocarcinoma	1
Pancreatic cancer	1
Unknown primary	3
Previous chemotherapy	20
Previous radiotherapy	0

Table 2 Number of cycles of CB1954 administered

		Number of cycles	
Dose levels mg/m^2	No. of patients	Total	Median[a] (range)
i.v.			
3	3	5	2 (1–2)
6	4	4	1 (1)
12	5	9	2 (1–3)
18	3	5	1 (1–3)
24	4	10	2 (1–5)
30	5	14	2 (1–6)
37.5	3	3	1 (1)
i.p.			
3	1	1	1
6	4	4	1
12	1	1	1
18	1	1	1
24	1	1	1

[a] Median, 2 i.v. cycles of treatment.

Table 3 Summary of toxicities after i.v. administration

		Dose levels/mg/m^2 (No. of patients treated)		
Toxicity	CTC grade	24 (4)	30 (5)	37.5 (3)
Nausea	1	1	3	1
	2			
	3	2	1	1
	4			
Vomiting	1	2		1
	2	1	2	
	3			
	4			
Anorexia	1	1		1
	2	1		1
	3	1		1
	4			
Abdominal pain	1			
	2	1		1
	3			
	4			
Diarrhea	1	3	4	
	2	1		1
	3			
	4			1[a]
Fatigue	1	1	3	
	2	1		
	3	2		
	4			
Biochemical liver abnormalities	1		1	
	2		1[a]	2[a]
	3		1[a]	
	4			

[a] DLT.

elevation. All of the side effects had resolved 2 weeks after drug administration, and only 1 patient required hospitalization for treatment of diarrhea. The i.p. route of administration was well tolerated, and at the highest dose level explored, 24 mg/m^2, where only 1 patient has been treated, there was G1 diarrhea and G1 transaminase elevation. DLT has not been reached in the i.p. regime. No alopecia, marrow suppression, nephrotoxicity, or ocular toxicity was observed in either i.v.- or i.p.-treated groups. No deaths were attributable to CB1954.

Tumor Evaluation. In 8 patients with measurable disease, there was disease progression. Six patients came off study because of deteriorating performance status attributable to their underlying malignancy. One patient with hepatic metastatic colorectal cancer, who received i.v. CB1954 at 30 mg/m^2, showed a fall in carcino-embryonic antigen levels after the second cycle of treatment to <50% of baseline values (from 51 μg/liter to 11 μg/liter), which lasted for 68 days before increasing again toward the end of the course of treatment. However, radiological assessment 3 weeks after completing six cycles of treatment demonstrated progressive disease.

Pharmacokinetic Studies. Serum concentrations-time profiles for i.v. CB1954 are summarized in Fig. 3 and show biexponential decay. The first half-life ($t_{1/2}$ α) for i.v. CB1954 ranges from 3 to 17 min, and the second half-life ($t_{1/2}$ β), from 10 to 136 min (Table 4). At the recommended i.v. dose of 24 mg/m^2, the mean peak serum level was 6.3 μM and was maintained above 1 μM for 2 h, giving an AUC of 5.8 μM/h. Pharmacokinetic parameters are summarized in Table 4, whereas Fig. 4 indicates a nonlinear relationship between the i.v. dose and AUC for CB1954, with wide variances in AUC at higher CB1954 doses. Less than 5% of drug was detected in urine, and drug clearance ranged from 184 ml/min at 37.5 mg/m^2 to 958 ml/min at 3 mg/m^2. These data suggest that CB1954 undergoes predominantly hepatic metabolism.

Intraperitoneal administration at 24 mg/m^2 achieved peak peritoneal levels of 70 μM, which persisted above 1 μM for 18 h (Fig. 5) with a corresponding AUC of 387 μM/h. The "regional advantage" for any cytotoxic agent is characterized by the ratio of the $AUC_{i.p.}$:$AUC_{i.v.}$ sampled simultaneously in the peritoneum and serum. The mean regional advantage for CB1954 is 218 (SD 235).

DISCUSSION

The aims of this Phase I study were 2-fold: firstly to determine the safety and tolerability of the VDEPT prodrug CB1954; and secondly to describe its pharmacokinetics. The DLT for i.v. CB1954 was diarrhea and transaminase elevation, seen at 37.5 mg/m^2, where all 3 of the patients treated encoun-

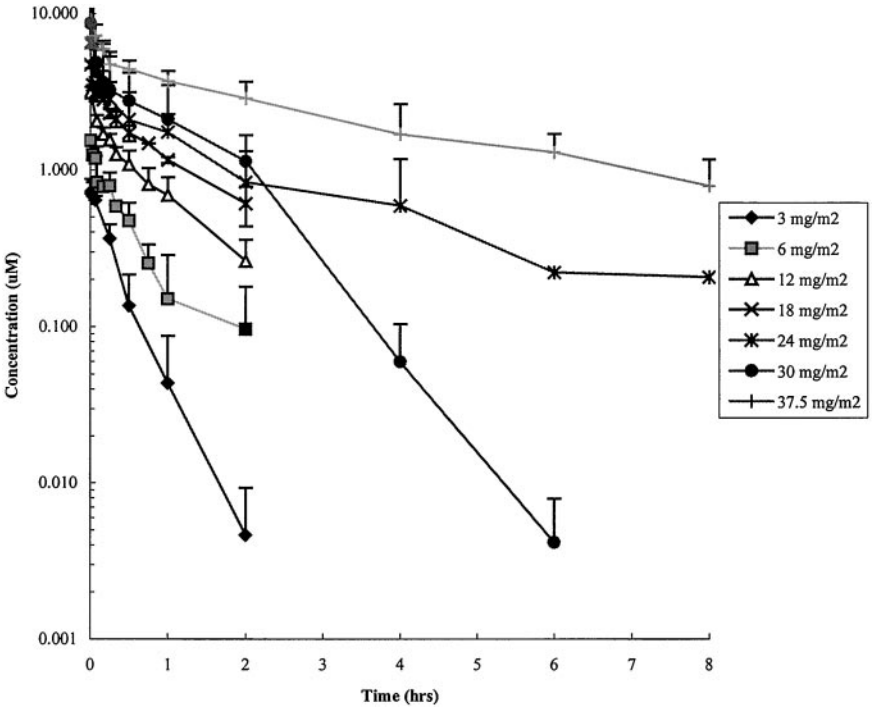

Fig. 3 Graph showing the mean dose-response curves after i.v. CB1954 administration at different dose levels (*bars*, means + SE of mean).

Table 4 Pharmacokinetic parameters after i.v. administration of CB1954

Dose (mg/m^2)	Mean peak concentration μM (SD)	Mean $t_{1/2}$ α mins (SD)	Mean $t_{1/2}$ β mins (SD)	Mean AUC μM hr (SD)	Clearance ml/min (SD)	Volume of distribution liter/m^2 (SD)
3	0.8 (0.2)	14 (8)	10 (4)	0.3 (0.2)	958 (443)	16.7 (6.3)
6	1.6 (0.2)	11 (11)	14 (2)	0.5 (0.2)	857 (251)	15.0 (1.5)
12	3.0 (0.8)	17 (19)	39 (19)	2.0 (0.9)	503 (328)	17.0 (5.9)
18	4.6 (0.4)	4 (2)	77 (53)	4.3 (1.7)	307 (100)	15.7 (1.3)
24	6.3 (2.8)	9 (13)	110 (100)	5.8 (3.6)	376 (250)	18.4 (9.7)
30	9.3 (5.3)	5 (7)	78 (56)	7.3 (2.6)	302 (106)	18.3 (12.5)
37.5	10.2 (4.3)	3 (1)	136 (78)	18.7 (12.2)	184 (129)	16.1 (5.8)

tered DLT. At the i.v. dose of 30 mg/m^2, hepatotoxicity was present in 2/5 patients (G2 and G3), one of whom also had progressive hepatic metastatic disease, which may have contributed to the deterioration in liver function. This consisted of asymptomatic, transaminase rises at day 8 after i.v. drug administration. The transaminases had normalized after 2 weeks. At 37.5 mg/m^2, hepatic DLT occurred in 2/3 patients with 1 transaminase elevation (G2) and 1 hyperbilirubinemia (G2). As at the previous dose level, these were asymptomatic and completely reversible. In view of the possible hepatotoxicity at 30 mg/m^2, the recommended i.v. dose was 24 mg/m^2. This degree of caution was warranted, because the ultimate aim of the gene therapy program is to combine CB1954 with adenoviral vectors, which also have a theoretical risk of hepatotoxicity. The most common side effect was mild diarrhea, which was seen in all 4 of the patients at the dose level of 24 mg/m^2 (G1/G2) and in 4/5 patients at 30 mg/m^2 (G1). More seriously, G4 diarrhea and ensuing dehydration occurred in 1 patient at 37.5 mg/m^2, which necessitated hospitalization for rehydration with subsequent full recovery. The mechanism for the diarrhea is unknown, but the most likely explanation is direct gastrointestinal epithelial toxicity. A plausible alternative mechanism may be the conversion of CB1954 to the activated form by NTR from *E. coli* present in colonic flora. It is not possible to detect the activated species of CB1954 in the bowel contents, because it is highly reactive and short-lived. However, it is possible to test the hypothesis by selective decontamination of the colonic flora by antibiotics before CB1954 administration to abrogate the diarrhea. Another gastrointestinal side effect noted was severe nausea, which occurred in 2/4 patients (G3) at 24 mg/m^2 but did not appear to be a prominent feature at higher dose levels (2 G3 in 8 patients) after antiemetics (metoclopramide) and corticosteroids were routinely administered.

In the i.p. regime at 24 mg/m^2, a mild reversible transaminase elevation (G1) and diarrhea (G1) were noted. No alopecia, marrow suppression, nephrotoxicity, or ocular toxicity was observed in either i.v.- or i.p.-treated groups. In particular, there were no lens abnormalities.

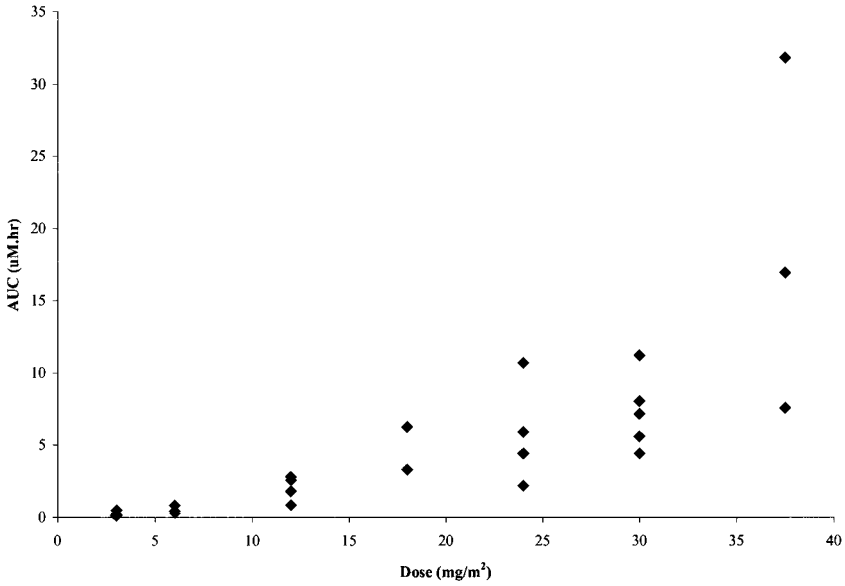

Fig. 4 Graph showing the relationship between i.v. dose and AUC at different dose levels.

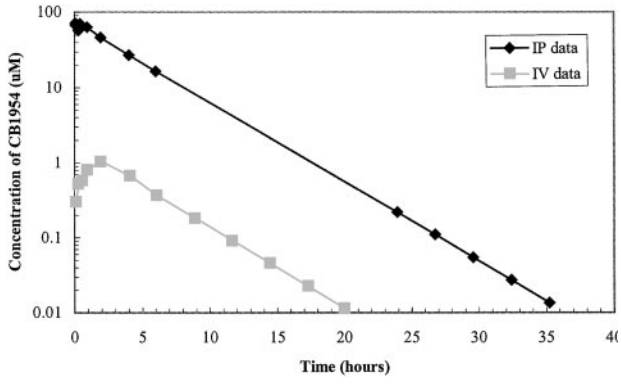

Fig. 5 CB1954 levels in the peritoneal cavity and serum sampled simultaneously in 1 patient receiving i.p. CB1954 at a dose of 24 mg/m^2.

Although, pharmacokinetic studies were not repeated in patients receiving multiple CB1954 doses, there was no evidence to suggest differing pharmacokinetics between the doses. The data show that CB1954 undergoes biexponential decay and is rapidly eliminated from the serum. At the i.v. dose of 24 mg/m^2, the serum concentration is reduced to ~1/30 of its peak value by 8 h (Fig. 3). As the repeat doses of CB1954 were given 3 weeks apart there was little reason to suggest drug accumulation, and no increased drug toxicity was seen clinically at the end of the courses of treatment.

After i.v. CB1954 administration, the mean AUC achieved increased in a nonlinear, dose-dependent manner from 0.3 to 18.7 μM/h (see Table 4). At the recommended i.v. dose of 24 mg/m^2, serum levels peaked at 6.3 μM and were maintained above 1 μM for 2 h, giving an AUC of 5.8 μM/h. In cancer cells expressing nitroreductase, the AUC required to achieve the IC_{50} for CB1954 ranges from 10 to 50 μM/h (3, 5). This suggests that serum levels attained may be adequate for a therapeutic response in the presence of sufficient NTR expression. Moreover, i.p. administration at 24 mg/m^2 achieved a peak peritoneal level of 70 μM, which was maintained above 1 μM for 18 h with an AUC of about 387 μM/h. The mean ratio of $AUD_{i.p.}$:$AUD_{i.v.}$, or the mean regional advantage in patients receiving i.p. CB1954 is 218 (SD 235). Thus, i.p. administration of CB1954 in combination with virus-mediated NTR delivery may provide a greater therapeutic benefit in the treatment of peritoneal carcinomatosis. Other possible routes of administration include hepatic arterial and intrapleural delivery. We recently received ethical approval for intrapleural delivery, and we will be commencing this shortly. Although hepatic arterial delivery is theoretically attractive there are some concerns regarding this route of administration in view of the hepatotoxicity of CB1954.

CB1954 metabolism is thought to be predominantly hepatic, as suggested by drug clearance, which ranges from 184 to 958 ml/min. This is also supported by the finding that <5% of drug was detected in urine. Intravenous injection of radioactive [^{14}C]CB1954 in mice showed activity in the liver, gall bladder, kidney, stomach, and tooth root. However, particularly high levels of radioactivity were found in the gall bladder, which again suggests hepatobiliary excretion as the main clearance mechanism.

The disposition kinetics and metabolism of CB1954 have been studied previously in mice and dogs (13). After i.v. bolus administration to mice (50 mg/kg) and dogs (25 mg/kg), respective peak concentrations of around 400 μM and 100 μM were found. Elimination half-lives were similar for mouse (1.4–2 h), dog (2.5–4 h), and man (0.1–2.2 h). Interestingly, tissue penetration in mice was generally good (tumor:plasma ratios of 50–90%). Preliminary pharmacokinetic studies in mice suggest that there is a slight regional advantage to i.p. administration, but it is not as marked as our clinical findings. Although there were detailed drug disposition studies in mice, a conventional rather than a pharmacokinetically guided dose escalation was undertaken, because there are difficulties applying this sort of scheme to drugs with nonlinear kinetics and wide interindividual variation in their handling.

Although CB1954 is a monofunctional alkylating agent, *in vitro* studies show that it forms interstrand DNA cross-links in cell lines on activation, demonstrating that it is converted to a bifunctional agent (14). Activation of CB1954 can increase its cytotoxic efficacy by up to 100,000-fold (15). This is greater than would be predicted by conversion of a monofunctional alkylating agent to a bifunctional one, which normally results in a 50–200-fold increase in cell killing (16). This increased sensitivity may be explained by the nature of the DNA lesion formed. Interstrand cross-inks are intrinsically more toxic than single strand or monofunctional lesions and are formed with much higher frequency by activated CB1954 than with other agents (~70% of the total lesions compared with ~2% with cisplatin; Ref. 15). These cross-links are poorly repaired compared with lesions induced by other alkylating agents, which may explain the increased cytotoxicity with activated CB1954. Although not yet fully identified, molecular modeling studies suggest that the lesion is a C8-O6 DNA interstrand cross-link, a lesion not induced by other alkylating agents, which may explain its unusual cytotoxicity (17).

Recent data has shown that activated CB1954, in common with a range of other cytotoxic agents with distinct cellular targets, kills cells predominantly by apoptosis. The mechanism by which activated CB1954 activate apoptotic pathways remains unclear but is thought to be p53-independent (18).

A VDEPT approach using the NTR/CB1954 combination appears promising for the treatment of cancer metastatic to the liver and the peritoneum. As a first step in the development of this system, we have conducted a Phase I trial of the prodrug CB1954. We have defined its toxicity profile and determined the maximum tolerated dose, and we conclude that it can be safely administered at 3-weekly intervals as a brief i.v. infusion at a dose of 24 mg/m^2. Peak levels of CB1954 are sufficiently high for a short time to allow conversion of CB1954 to the activated species. As a result of this data, we have now commenced a Phase I trial with a dose-escalating, NTR-encoded, adenovirus injection intratumorally, combined with i.v. CB1954 at the dose of 24 mg/m^2 in patients with primary and secondary liver tumors and plan a similar study in patients with peritoneal carcinomatosis with i.p. delivery of virus and prodrug.

ACKNOWLEDGMENTS

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