Cancer Chemother Pharmacol (2000) 45: 284–290

ORIGINAL ARTICLE

Huw D. Thomas · Michael J. Lind · Judith Ford
Norman Bleehen · A. Hilary Calvert · Alan V. Boddy

Pharmacokinetics of carboplatin administered in combination with the bradykinin agonist Cereport (RMP-7) for the treatment of brain tumours

Received: 12 April 1999 / Accepted: 9 September 1999

Abstract *Introduction*: Cereport (RMP-7) is a novel bradykinin agonist which is being developed as a modulator of the blood–brain barrier (BBB). In order to investigate the pharmacokinetics of carboplatin in combination with Cereport, we performed pharmacological studies in conjunction with early clinical trials. *Methods*: Pharmacokinetic samples were collected from eight patients in a phase I study (Cereport 100–300 ng/kg) and ten patients in a phase II study (Cereport 300 ng/kg). Pharmacokinetic parameters for carboplatin were compared with respect to the dose of Cereport and with historical controls. *Results*: Cereport combined with carboplatin was well-tolerated, with mild haematological toxicities consistent with the target area under the concentration–time curve (AUC) of 7 mg/ml*min. Although the clearance of carboplatin was within the range reported for this drug alone, the addition of Cereport resulted in a higher than expected carboplatin AUC. This effect was related to the dose of Cereport in the phase I study (AUC values 104–133% of target, Spearman rank correlation coefficient = 0.71, $P < 0.001$). The higher than expected AUC value was confirmed in the phase II study (AUC values 106–189% of target). *Conclusions*: Co-administration of Cereport with carboplatin may result in a greater than predicted AUC. The mechanism of this possible interaction remains to be determined, although this did not result in any increased toxicity. Thus, the clinical potential of this combination in the treatment of brain tumours warrants further investigation.

H.D. Thomas · M.J. Lind · A.H. Calvert · A.V. Boddy (✉)
Cancer Research Unit, Medical School,
University of Newcastle upon Tyne,
Newcastle upon Tyne NE2 4HH, UK
e-mail: alan.boddy@ncl.ac.uk
Tel.: +44-191-2228233; Fax: +44-191-2227556

J. Ford · N. Bleehen
Addenbrookes Hospital, Cambridge, UK

Key words Carboplatin · Pharmacokinetics · Cereport · Clinical · Brain tumour

Introduction

The treatment of glioma with carboplatin at present has limited success, partly due to the highly polar nature of the drug. Carboplatin does not easily cross plasma membranes of any type and so it is not surprising that its ability to cross the blood–brain barrier (BBB), a membrane structured so as to prevent access of drugs to the brain, is limited. Indeed in studies in both adults [6] and children, the area under the concentration–time curve (AUC) of free carboplatin in the cerebrospinal fluid (CSF) accounted for only 20% of the plasma AUC.

Bradykinin is the naturally occurring active nonapeptide of the kallikrein-kinin system. It has several physiological actions (for an overview of the pharmacology of bradykinin see [26]), including increasing capillary permeability, constriction of smooth muscle, stimulation of pain receptors, and nitric oxide dependent vasodilatation; it may also reduce cerebral blood flow [7, 24]. In addition, it is proposed that bradykinin may increase the permeability of the blood–brain and blood–tumour barriers [3].

Other mediators of inflammation, including histamine [7], thromboxanes [3] and 5-hydroxytryptamine (5-HT) [24, 26], have been reported to increase the permeability of the BBB. The mechanism by which bradykinin may act in this way is not fully understood. In the normal brain, bradykinin appears to increase BBB permeability to small ionic species such as Na^+-fluorescein, which has a molecular mass of 376 [30]. Experiments with specific kininergic receptor antagonists has led to the characterisation of the BBB permeabilisation as a bradykinin B2 effect [28], which is the same class of receptor responsible for dilatation of blood vessels. This may be due to a direct effect on smooth muscle or to the release of some endothelium-derived relaxing factor [30].

For example, nitric oxide, which is released in response to stimulation of bradykinin receptors, induces disruption of the BBB that leads to an increase in ionic permeability [21].

The blood–brain-tumour barrier (BTB), although similar to the BBB, is not as robust in its ability to resist the passage of molecules. The endothelium of the capillaries of a tumour is often abnormal, containing vesicles, open junctions and a fragmented basal lamina, all of which contribute to an overall increase in permeability [18]. There have been reports of cultured neoplastic glial cells producing a permeability factor [23]. In spite of this increase in permeability compared with that of the normal BBB, the BTB can and does still limit the access of polar molecules, including anti-tumour agents, to the tumour. Recent studies have demonstrated that bradykinin can increase the permeability of the BTB. However, the dose of bradykinin required to open the BTB appears to be much less than that needed to open the BBB [20]. Thus, bradykinin can be used to selectively increase permeability of the BTB whilst leaving the BBB relatively intact. Cereport (RMP-7) is an analogue of bradykinin that was developed to increase the permeability of the BBB to drugs. It acts by interacting with a B_2 kininergic receptor on the endothelial cells which comprise the BBB [1, 9].

Following a number of experiments in pre-clinical models, it was proposed that co-administration of Cereport would increase the effectiveness of carboplatin in the treatment of brain tumours (Reviewed in [5]). Uptake of carboplatin into rat glial tumours was increased, following intracarotid [1, 9] or intravenous [10, 11] administration of Cereport. Survival of animals treated with a combination of Cereport and carboplatin was greater than those treated with carboplatin alone.

Based on this evidence phase I and II clinical trials of Cereport and carboplatin were performed on patients with glioblastoma multiforme or anaplastic astrocytoma. The dose of carboplatin was individualised, based on the renal function of the patient, and dose escalation of Cereport was carried out in the phase I trial, after which further patients were studied at the phase II dose-level. Pharmacokinetic data on carboplatin were obtained in both studies to determine any possible effect of Cereport administration on the elimination of carboplatin. Safety data on the combination were also collected for comparison with that from the original Calvert analysis and more recent experience with carboplatin.

Methods

Protocol, and patient eligibility

In both phase I and II studies patients were aged between 18 and 75 years, and had recurrent or primary, poor prognosis glioma, and a diagnosis of recurrent anaplastic astrocytoma or glioblastoma multiforme, WHO grade III or IV, and had received no chemotherapy for at least 6 weeks prior to study (4 weeks in phase II), and no radiotherapy for at least 2 months (3 months phase II) (Table 1). Patients were also required to have a WHO performance status of less than 2 (Karnofsky performance status of at least 60, phase II), adequate hepatic, renal and haematopoietic function and a life expectancy of more than 8 weeks. In total, 15 patients were recruited to the phase I trial and 87 patients to the two phase II trials. The latter were split between chemotherapy-naive patients ($n = 45$) (who had received prior surgery and radiotherapy), and a separate trial of patients ($n = 42$) who had received one prior course of chemotherapy. Both studies were approved by the relevant local ethics committee, and written, informed consent was obtained.

The dose of carboplatin was individualised to a target AUC as calculated by the Calvert formula [8], in which a measure of the patient's renal function is used for the calculation of carboplatin clearance and consequent by the dose:

$$\text{Dose} = \text{AUC} \times (\text{GFR} + 25)$$

where Dose is dose in milligrams, AUC is the target AUC in mg/ml*min, GFR is glomerular filtration rate in ml/min, determined by ^{51}Cr EDTA clearance, and 25 (ml/min) is a constant that accounts for non-renal clearance i.e. protein binding [8]. The target AUC in most patients' courses of treatment was 7 mg/ml*min, although in some patients a lower AUC was used, as indicated by initial dose-escalation or toxicity. Details of patients in the pharmacological studies, GFR, and Cereport and carboplatin doses are given in Table 2.

Table 1 Summary of patients treated with Cereport in phase I and phase II studies, and clinical outcome details (*V* vasodilatation, *N and V* nausea and vomiting, *M*/*F* male/female)

Study	Number studied	M/F	Age (years)	Cycles of treatment	Grade 3 or 4 toxicities	Incidence of adverse events		
						V	N and V	Other
Phase I	8 (15 total)	5/3	47 (33–72)	2 (1–7)	1 (WBC) 1 (Lymphopenia) 2 (Hyperglycaemia)	5	2	ECG change (2) Malaise (2) Tachycardia (2)
Phase II	10 (87 total)	4/6	39 (24–56)	8 (1–16)	2 (Anaemia) 4 (Neutropenia) 6 (Platelets) 2 (Leucopenia) 7 (Lymphopenia)	9	9	Abdominal pain (4) Convulsion (2) Dizziness (2) Somnolence (2) Aching eyes (2)

Number in pharmacokinetic studies
Age and cycles are given as median (range)
Figures for toxicities and adverse events are numbers of patients reporting that symptom

Table 2 Dosing information on patients in phase I (patients 1–8) and phase II (patients 9–18) trials with Cereport and carboplatin. Patient 17 was incorrectly dosed with relation to glomerular filtration rate (GFR)

Patient number	Cereport dose (ng/kg)	Surface area (m^2)	GFR (ml/min)	Calculated CL (ml/min)	Carboplatin dose (mg)	Target AUC (mg/ml*min)
1	100	2.20	177	202	1000	5
2	100	1.98	128	153	1050	7
3	100	1.83	106	131	900	7
4	200	1.37	104	129	900	7
5	200	1.89	144	169	1180	7
6	300	1.57	115	140	980	7
7	300	1.75	91	116	810	7
8	300	1.93	133	158	1100	7
9	300	2.00	113	138	965	7
10	300	1.69	150	175	1225	7
11	300	1.96	90	115	805	7
12	300	1.89	139	164	1145	7
13	300	1.58	95	120	840	7
14	300	1.86	73	98	400	4
15	300	1.64	96	121	847	7
16	300	1.98	218	243	1700	7
17[a]	300	1.86	176	201	847	4.2
18	300	1.84	140	165	1155	7

[a] Patient 17 was dosed to a target AUC of only 4.2 mg/ml*min, as GFR was divided by surface area in calculating the dose

Administration and sampling

The carboplatin was administered as a 15 min intravenous infusion. The infusion of Cereport was timed to coincide with the last 5 min of the carboplatin administration and continued for a further 5 min (total time 10 min, Fig. 1). In the phase I study, the dose of Cereport was escalated from 100 to 300 ng/kg, toxicity permitting. No intra-patient dose escalation was allowed. In the phase II studies all patients received 300 ng/kg Cereport.

Blood samples were obtained by venepuncture at 5, 15, 20, 30 and 60 min, and at 2, 3, 6, 8 and 24 h after the start of carboplatin infusion. The samples were placed into tubes containing EDTA, and immediately centrifuged at 1000 *g* for 10 min, after which the plasma was removed. Of the resultant plasma, 1 ml was placed in a Centrifree micropartition unit (Amicon Beverly, USA) and centrifuged at 3000 *g* for 10 min. The ultrafiltrate was frozen and stored at −20 °C until required for analysis. The remaining plasma was placed into a clean tube and stored frozen at −20 °C. Carboplatin was determined by flameless atomic absorption spectrophotometry (AAS) in both plasma and plasma-ultrafiltrate. Details of the assay have been previously described [19].

Urine sampling

Complete urine samples for 24 hours after the start of carboplatin administration were obtained in patients on the phase II study and assayed for platinum.

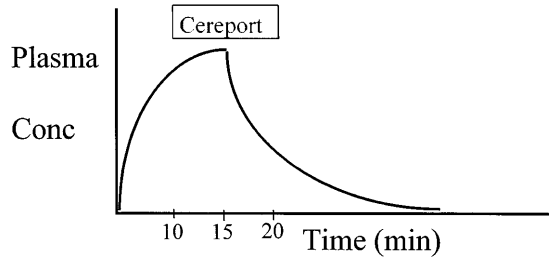

Fig. 1 Simulation of carboplatin plasma ultrafiltrate concentration–time curve. The timing of Cereport administration coincides with peak carboplatin levels

Binding of carboplatin to plasma protein, in vitro

In order to determine if Cereport had any effect on the binding of carboplatin to plasma protein, we incubated carboplatin in plasma in vitro in the presence and absence of Cereport. Three solutions at 5 μg/ml of carboplatin in human plasma were prepared as controls, one containing placebo (formulation without Cereport), one containing a high concentration of Cereport (30 ng/ml), and one containing normal concentrations of Cereport (3 ng/ml). Normal was estimated from the maximum dose (300 ng/kg) multiplied by average body weight (70 kg) divided by average human blood volume. The solutions were then incubated at 37 °C.

Aliquots of 1.5 ml were removed at timed intervals. From each aliquot 1 ml was immediately ultrafiltered using Amicon Centrifree micropartition units, by centrifugation at 1000 *g* for 10 min. The remaining solution was stored frozen until analysis.

Samples were analysed for free (ultrafiltered) and total (whole plasma) platinum content using AAS.

Pharmacokinetic analysis

Pharmacokinetics were evaluated by model-independent methods, with AUC determined by a combination of trapezoidal and log-trapezoidal rules with extrapolation to infinity. Clearance of carboplatin in the ultrafiltrate was estimated as dose/AUC. In addition, a two compartment model with first order pharmacokinetics was fitted to the data from the phase I study, by the use of ADAPT II release 3 (Biomedical Simulations Resource). The maximum likelihood estimator and a proportional error model were used. Parameters derived from the model were total volume of distribution (Vdss) and half-lives of the two phases of disposition. Urine recovery was expressed as a percentage of the dose administered.

Results

Phase I study

Pharmacokinetic parameters for the 8 patients investigated in the phase I study are given in Table 3. Only one patient was dosed to a target AUC of 5 mg/ml*min, and the measured AUC was 5.2 mg/ml*min. Of the other

Table 3 Carboplatin pharmacokinetics expressed as concentrations of free and total platinum in patients on the phase I study ($T_{1/2}\lambda_1$ initial half-life, $T_{1/2}\lambda_2$ terminal half-life, *Vdss* volume of distribution at steady state, *CL* plasma clearance based on measures of unbound platinum)

Patient number	$T_{1/2}\lambda_1$ (min)	$T_{1/2}\lambda_2$ (min)	Vdss ($1/m^2$)	Free Pt AUC (mg/ml*min)	CL (mg/min)	AUC % of target	Total Pt AUC (mg/ml*min)	Total/Free AUC ratio
1	43	208	11	5.2	191	104	6.7	1.3
2	58	690	24	7.3	144	104	8.5	1.2
3	23	164	13	7.5	113	107	11.4	1.5
4	10	116	11	8.5	105	121	12.7	1.5
5	48	471	18	8.4	141	120	11.2	1.3
6	33	115	9	8.6	114	123	11.4	1.3
7	76	563	9	7.9	103	113	11.0	1.4
8	75	668	16	9.3	118	133	13.1	1.4

AUC values calculated on data to 8 h for free and to 24 h for total. Extrapolated AUC was less than 20% of the total in each case

patients, dosed to a target AUC of 7 mg/ml*min, the observed AUCs ranged from 7.3 to 9.3 mg/ml*min. The AUC expressed as a percent of the target, for all 8 patients, ranged from 104 to 133%. The degree to which the AUC deviated from that anticipated appeared to be related to the dose of Cereport administered (Fig. 2). The Spearman rank correlation coefficient was 0.71 ($P < 0.001$) for the relationship between AUC (% of target) and dose of Cereport. The $T_{1/2}\lambda_1$ was 46 (SD = 24, $n = 8$) min and the $T_{1/2}\lambda_2$ was 374 (SD = 250, $n = 8$) min for free platinum (Table 3), and neither was related to Cereport dose. Also, Vdss was independent of dose and was consistent with previous reports for this parameter for carboplatin alone.

Phase II study

Pharmacokinetic data were available on ten of the 87 patients entered into the phase II studies. Patient 17 was dosed incorrectly, in that the GFR was corrected for surface area before the dose was calculated. This resulted in a "predicted" AUC of 4.2 mg/ml*min. Patient 14 had a target AUC of only 4 mg/ml*min on the course of treatment studied (course 4) due to haematological toxicity. The mean AUC value for the 8 patients dosed to a target AUC of 7 mg/ml*min was 9.0 mg/ml*min (SD = 1.15, $n = 8$). The observed AUC exceeded the predicted or target value by 6 to 89%, in keeping with the results of the phase I study. A plot of predicted and observed carboplatin AUCs for the combined data set from phase I and II studies is shown in Fig. 3, together with historical data and data from other studies in our laboratory on single agent carboplatin [8, 15]. This plot clearly shows the consistent deviation from the anticipated AUC values in the patients receiving Cereport.

Reliable and complete 24 h urine collections were available for only five of the patients studied (Table 4). The percentage of the dose recovered in the urine was 77 ± 10%.

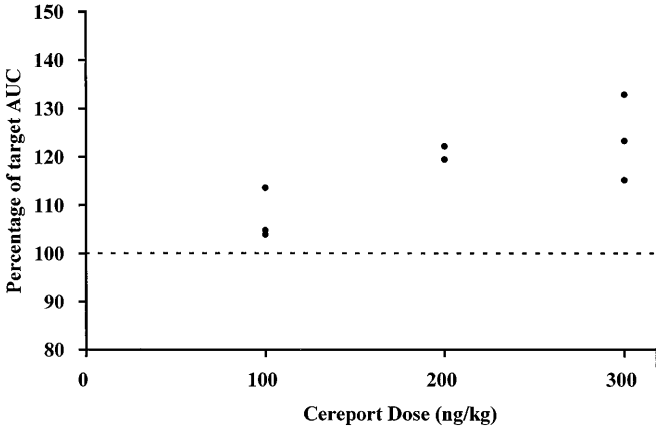

Fig. 2 The relationship between the dose of Cereport (ng/kg) and the percentage of the target AUC of carboplatin achieved in the phase I study ($n = 8$)

In vitro carboplatin binding study

The presence of Cereport did not affect carboplatin protein binding over a period of 48 h. The rate constants for carboplatin protein binding from 8 to 48 h were 0.049 (SE = 0.007) h^{-1} for carboplatin alone, 0.055 (SE = 0.007) h^{-1} for carboplatin and 3 ng/ml Cereport, and 0.054 (SE = 0.010) h^{-1} for carboplatin and 30 ng/ml Cereport. The corresponding half-lives of free carboplatin were 14.0 h for carboplatin alone, 12.7 h for carboplatin and 3 ng/ml Cereport, and 12.8 h for carboplatin and 30 ng/ml Cereport.

Clinical summary of patients studied

In the 8 patients studied in the phase I investigation, haematological toxicity was minor, with vasodilatation being the most common drug-related effect. There were no incidents of thrombocytopenia of Grade III or IV. Six patients were withdrawn due to progressive disease, and the two others due to either disease-related death or adverse event (ECG change). In the phase II trials, the incidence of haematological toxicity was somewhat higher, with 6 of 10 patients studied experiencing Grade III or IV thrombocytopenia. However, this did not present any problems clinically and was consistent with

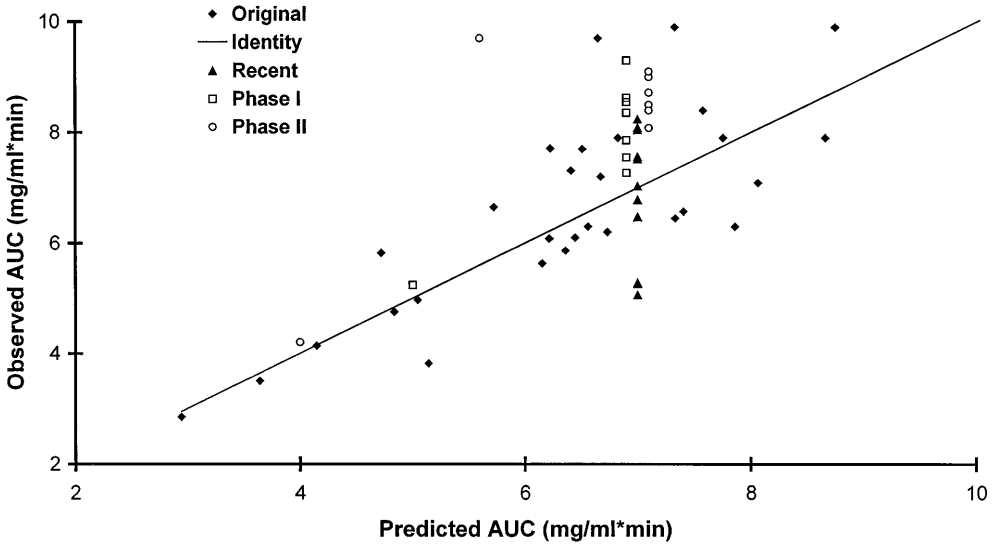

Fig. 3 Comparison of data from phase I and II studies with Cereport plus carboplatin, with historical and recent data for carboplatin pharmacokinetics. Original data from Calvert et al. 1989 [8]. Recent data from Ghazal-Aswad et al. 1996 [15]

Table 4 Carboplatin pharmacokinetics expressed as concentration of free and total platinum in patients on the phase II study

Patient number	Free Pt AUC (mg/ml*min)	CL (ml/min)	AUC % of target	Total Pt AUC (mg/ml*min)	Total/Free AUC ratio	Urine recovery (% dose)
9	8.5	114	121	14.1	1.7	68
10	8.1	152	116	9.9	1.2	68
11	9.1	88	130	9.7	1.1	
12	8.9	129	127	15.8	1.8	
13	8.7	96	125	8.1	0.9	
14	4.2	95	106	6.5	1.5	
15	8.9	101	126	9.4	1.1	
16	11.5	154	189	14.9	1.3	82
17	6.3	135	152	8.5	1.3	74
18	9.0	128	129	12.0	1.3	91

For abbreviations see Table 3

the AUC of carboplatin achieved in these patients. Again, vasodilatation was the major non-haematological adverse effect. Four patients were withdrawn from study due to progressive disease, five at the request of the patient and one due to an increase in neurological problems.

Discussion

In these early clinical studies of the combination of Cereport with carboplatin, the main aims were to determine a safe, effective dose of the bradykinin analogue and to demonstrate the safety of the combination in the treatment of patients with glioma. A significant component of this assessment of safety was the investigation of the pharmacokinetics of carboplatin in the presence of Cereport. This was also important from the point of view of assessing the activity of this combination, because modulators of drug distribution, such as MDR inhibitors, may also produce an improvement in anti-tumour effect by changing systemic pharmacokinetics [2].

The short administration time for Cereport was based on the observation that its effects are transient and rapidly reversed as the drug is eliminated [1]. Since Cereport has a half-life in vivo in humans of only a few minutes [22], the effect on the BBB or BTB should last only for a short period while carboplatin concentrations are high. The closure of the BBB (or BTB) thereafter traps the drug in the brain where it can exert an anti-tumour effect, while systemic exposure is limited by rapid drug elimination.

The safety and toxicity data from the phase I and II trials indicated that carboplatin can be given in combination with doses of Cereport of up to 300 ng/kg [4, 16, 17]. Thrombocytopenia was mild and only two patients from a total of 97 in phase II studies had to be withdrawn from treatment for this reason. Overall, the clinical data from these early studies are promising, with a significant proportion of patients having clinical improvement or measurable response [16, 17, 25].

The pharmacokinetic data from both the phase I and phase II studies indicated that there was a higher than expected AUC of carboplatin when administered with Cereport. In the phase I study, with escalating doses of Cereport, the effect seemed to be dependent on the dose of the BBB-modulator. In the phase II study where all patients received 300 ng/kg of Cereport, the higher AUC

of carboplatin was maintained. Nevertheless, the observed clearance values were within the range of those reported previously in the absence of Cereport [29] and this pharmacokinetic interaction was not accompanied by an increase in toxicity. The patients studied here represented a subset of those participating in the clinical trials, however, the range of GFR, carboplatin doses and other patient-characteristics were not different from those of the overall patient-population.

The clearance of carboplatin is determined by the sum of renal clearance (glomerular filtration) and non-renal clearance (mostly protein binding). Either one or both of these two factors might have been affected by Cereport to produce the interaction observed. However, the ratios of total platinum to ultrafiltered carboplatin were not dependent on the dose of Cereport and were not different from those observed previously [29]. The absence of an effect on protein binding was supported by the results of the in vitro study, showing no effect of Cereport on the kinetics or magnitude of carboplatin reacting with plasma protein.

The other possible mechanism by which the carboplatin clearance could have been altered is by a change in the renal clearance. This may be due to a change in GFR, which in turn could be affected by renal blood flow. Considering the vasodilator properties expected of a bradykinin agonist it is unlikely that a decrease in blood flow to the kidney would have been observed. In fact in animal models, bradykinin caused an increase in renal blood flow [13, 14] and thus would have been expected to produce an increase in GFR. An alternative explanation involves tubular reabsorption. Many drugs that are subject to glomerular filtration have low renal clearances due to reabsorption in the distal tubule of the nephron. Normally, the reabsorption of carboplatin is negligible, due to its polar nature. However, if Cereport caused an increase in the permeability of the distal tubule epithelium, reabsorption of carboplatin could have occurred. Bradykinin does affect secretion and reabsorption in the kidney tubule via receptors on the basolateral surface of the tubule cells [12]. However, there is no evidence that Cereport has any effect on renal function in humans (Dr N.J. Brown, Vanderbilt University, Nashville Tenn., USA) and, where measured, the urinary recovery of platinum in the patients in the current study was similar to that observed previously.

Although this study was not designed to evaluate the effectiveness of Cereport as a modulator of carboplatin access to the brain, the pharmacokinetic data presented do support the notion that any therapeutic gain is due to increased distribution to the central nervous system (CNS), rather than to altered systemic pharmacokinetics. The observed AUCs were higher than expected. However, given the normally limited dispersion of carboplatin into the CNS, an increase in therapeutic effect would require a larger increase in drug concentration in the tumour than that provided by the 20–30% increase in systemic concentrations. An increase in the relative amount of carboplatin crossing into the CSF would be associated with an increase in the volume of distribution, however this was not observed, possibly because the CSF has a small volume and so would contribute relatively little to the overall distribution of carboplatin in the body.

In conclusion, these studies demonstrated that carboplatin can be administered safely with Cereport, at doses that are consistent with intended therapeutic effects. Although a small change in the pharmacokinetics of carboplatin was noted, this was not associated with an increase in toxicity.

Acknowledgements This work was supported by the Cancer Research Campaign and by Alkermes Europe.

References

1. Bartus RT, Elliott P, Hayward N, Dean R, McEwen EL, Fisher SK (1996) Permeability of the BBB by the bradykinin agonist, RMP-7: Evidence for a sensitive, auto-regulated, receptor-mediated system. Immunopharmacology 33: 270–278
2. Berg SL, Tolcher A, O'Shaughnessy JA, et al (1995) Effect of R-verapamil on the pharmacokinetics of paclitaxel in women with breast cancer. J Clin Oncol 13: 2039–2042
3. Black KL, King WA, Ikezaki K (1990) Selective opening of the blood-tumor barrier by intracarotid infusion of leukotriene C4. J Neurosurg 72: 912–916
4. Bleehen NM, Ford JM, Miles KA, Barton T, Osborn C (1996) A Phase I dose rising study of RMP-7 and carboplatin in patients with recurrent malignant glioma. Second Congress of the European Association for Neuro-oncology. Würzburg, Germany
5. Boddy AV, Thomas HD (1997) RMP-7 – Potential as an adjuvant to the drug treatment of brain tumours. CNS Drugs 7: 257–263
6. Brunner V, Houyau P, Chatelut E, Roche H, Canal P (1995) Cerebrospinal fluid concentrations of carboplatin in a patient without blood-brain barrier disruption. Cancer Chemother Pharmacol 35: 352–353
7. Butt AM (1995) Effect of inflammatory agents on electrical resistance across the blood-brain barrier in pial microvessels of anaesthetized rats. Brain Res 696: 145–150
8. Calvert AH, Newell DR, Gumbrell LA et al (1989) Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol 7: 1748–1756
9. Elliot PJ, Hayward NJ, Huff MR, Nagle TL, Black KL, Bartus RT (1996) Unlocking the blood-brain barrier: a role for RMP-7 in brain tumor therapy. Exp Neurol 141: 214–224
10. Elliott PJ, Hayward NJ, Dean RL, Blunt DG, Bartus RT (1996) Intravenous RMP-7 selectively increases uptake of carboplatin into rat-brain tumors. Cancer Res 56: 3998–4005
11. Emerich DF, Snodgrass P, Pink M, Bloom F, Bartus RT (1998) Central analgesic actions of loperamide following transient permeation of the blood-brain barrier with Cereport(TM) (RMP-7). Brain Res 801: 259–266
12. Fenoy FJ, Roman RJ (1992) Effect of kinin receptor antagonists on renal hemodynamic and natriuretic responses to volume expansion. Am J Physiol 263: R1136–R1140
13. Gardiner SM, Compton AM, Bennet T (1989) Regional haemodynamic effects of bradykinin or substance P in conscious Long Evans rats. Br J Pharmacol 96: 119P
14. Gardiner SM, Compton AM, Kemp PA, Bennet T (1990) Regional and cardiac haemodynamic responses to glyceryltrinitrate, acetlycholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. Br J Pharmacol, 101: 632–639
15. Ghazal-Aswad S, Calvert AH, Newell DR (1996) A single sample assay for the calculation of the area under the free carboplatin plasma concentration versus time curve. Cancer Chemother Pharmacol 37: 429–434

16. Gregor A, Lebrun-Frenay C, Osborn C (1997) Phase II study of IV RMP-7 + carboplatin in recurrent malignant glioma WHO grade III-IV previously treated with chemotherapy. (Proc ECCO, Hamburg Sept 1997) Eur J Cancer 33 [Suppl 8]: 201 (abstract 908)
17. Gregor A, Lind M, Osborn C (1997) Phase II study of intravenous RMP-7 and carboplatin for chemotherapy naive recurrent malignant glioma. (Proc ECCO, Hamburg Sept 1997) Eur J Cancer 33 [Suppl 8]: 199 (abstract 901)
18. Greig NH (1987) Optimizing drug delivery to brain tumors. Cancer Treat Rev 14: 1–28
19. Harland SJ, Newell DR, Siddik ZH, Chadwick R, Calvert AH, Harrap KR (1984) Pharmacokinetics of cis-diammine-1,1-cyclobutane dicarboxylate platinum(II) in patients with normal and impaired renal function. Cancer Res 44: 1693–1697
20. Inamura T, Black KL (1994) Bradykinin selectively opens blood-tumor barrier in experimental brain tumors. J Cereb Blood Flow Metab 14: 862–870
21. Janigro D, West GA, Nguyen TS, Winn HR (1994) Regulation of blood-brain barrier endothelial-cells by nitric oxide. Circ Res 75: 528–538
22. Jordan T, Walus L, Velickovic A, Last T, Doctrow S, Liu H (1996) A competitive chemiluminescent ELISA for the determination of RMP-7 in human blood. J Pharmaceut Biomed Anal 12: 163–1662
23. Ohnishi T, Sher PB, Posner JB, Shapiro WR (1990) Capillary-permeability factor secreted by malignant brain-tumor – role in peritumoral brain edema and possible mechanism for anti-edema effect of glucocorticoids. J Neurosurg 72: 245–251
24. Olesen SP, Crone C (1986) Substances that rapidly augment ionic conductance of endothelium in cerebral venules. Acta Physiol Scand 127: 233–241
25. Osborn C, Stenning S, Downing G (1997) Survival following treatment with RMP-7 and carboplatin in malignant glioma WHO grade III-IV: comparisons with matched controls. (Proc ECCO, Hamburg Sept 1997) Eur J Cancer 33 [Suppl 8]: 200 (abstract 902)
26. Regoli D, Barabe J (1980) Pharmacology of bradykinin and related kinins. Pharmacol Rev 32: 1–46
27. Riccardi R, Riccardi A, Di Rocco C, et al (1992) Cerebrospinal fluid pharmacokinetics of carboplatin in children with brain tumors. Cancer Chemother Pharmacol 30: 21–24
28. Unterberg A, Wahl M, Baethmann A (1984) Effects of bradykinin on permeability and diameter of pial vessels in vivo. J Cereb Blood Flow Metab 4: 574–585
29. van der Vijgh WJM (1991) Clinical Pharmacokinetics of Carboplatin. Clin Pharmacokinet 21: 242–261
30. Wahl M, Unterberg A, Baethmann A, Schilling L (1988) Mediators of blood-brain barrier dysfunction and formation of vasogenic brain edema. J Cereb Blood Flow Metab 8: 621–634