Cancer Chemother Pharmacol (2001) 48: 275–282
DOI 10.1007/s002800100356

ORIGINAL ARTICLE

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Phase I trial of lobradimil (RMP-7) and carboplatin in children with brain tumors

Received: 24 January 2001 / Accepted: 15 June 2001 / Published online: 28 July 2001

Abstract *Purpose*: To determine the maximum tolerated dose (MTD), the incidence and severity of toxicities, and the pharmacokinetics of lobradimil administered intravenously over 10 min in combination with carboplatin in children with refractory brain tumors. *Methods*: A group of 25 children with primary brain tumors received carboplatin and lobradimil on two consecutive days every 28 days. The 10-min lobradimil infusion began 5 min before the end of the carboplatin infusion. Four lobradimil dose levels (100, 300, 450 and 600 ng/kg ideal body weight, IBW) were studied in cohorts of 4 to 13 patients. Carboplatin was adaptively dosed based on the glomerular filtration rate to achieve a target plasma area under the concentration-time curve (AUC) of 7.0 mg·min/ml per course (5.0 mg·min/ml for patients who had previously received craniospinal radiation or myeloablative chemotherapy). *Results*: Lobradimil toxicity was immediate, tolerable and rapidly reversible. The most frequent toxicities were hypotension, flushing, headache and gastrointestinal complaints. One patient on the 600 ng/kg dose level had a seizure during the lobradimil infusion. The incidence and severity of lobradimil toxicities were not dose-related and the lobradimil dose was not escalated beyond the 600 ng/kg IBW dose level. Two patients had partial responses and ten patients had stable disease. Myelosuppression (thrombocytopenia more prominent than neutropenia) was the primary toxicity attributed to carboplatin. Lobradimil pharmacokinetics were characterized by rapid clearance from the plasma compartment and substantial interpatient variability. *Conclusions*: The combination of carboplatin and lobradimil is safe and tolerable. An MTD for lobradimil was not defined because toxicity was not dose-related. The recommended pediatric phase II dose of lobradimil is 600 ng/kg IBW.

Keywords Pediatric · Blood:brain barrier · lobradimil · Carboplatin

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Introduction

The blood-brain barrier (BBB), which is a specialized layer of endothelial cells in brain capillaries, restricts entry of most hydrophilic and large lipophilic compounds into the brain [15]. Brain tumor capillaries have a similar barrier that limits the access of anticancer drugs to these tumors [7, 13]. Strategies to circumvent the BBB and enhance drug delivery to brain tumors have included the development of small lipophilic drugs that can penetrate the BBB [10] and osmotic disruption of the BBB by infusion of hyperosmolar mannitol solutions into the carotid artery [14].

The synthetic bradykinin analog, lobradimil (Cereport, RMP-7), is a potent and specific bradykinin B_2 receptor agonist. The B_2 receptor is expressed on the luminal and abluminal surface of brain capillary endothelial cells, and the interaction of bradykinin or lobradimil with the B_2 receptor rapidly and reversibly increases the permeability of the BBB [6, 8, 12, 16]. Lobradimil has enhanced B_2 receptor selectivity and potency, and greater plasma stability and a longer half-life compared to bradykinin [2, 12]. The effect of lobradimil on the BBB is rapid, autoregulated and transient [1, 8].

In rats implanted with RG2 gliomas, intracarotid infusion of lobradimil selectively increases the permeability of the BBB to tracer molecules within brain tumors [9]. This effect on permeability diminishes as the distance from the tumor increases [6]. Similarly, the delivery of carboplatin, which is active against a variety of childhood brain tumors, to tumor and brain surrounding tumor in this glioma model is enhanced with intracarotid lobradimil [12]. Tumor-bearing animals that receive the combination of lobradimil and carboplatin survive longer than animals that receive carboplatin alone [12]. Intravenous lobradimil also significantly increases uptake of carboplatin into brain tumors and brain tissue surrounding the tumor in the animal model [5].

Lobradimil is well tolerated in adults when it is administered intravenously over 10 min or intraarterially at doses of up to 300 ng/kg. Toxicities attributed to lobradimil include flushing, tachycardia, blood pressure changes, headache, abdominal pain and emesis. All toxicities are rapid in onset and rapidly reversible. A maximum tolerated dose (MTD) of lobradimil has not been defined in dose-finding studies in adults and the current recommended dose is 300 ng/kg [3].

The objectives of our pediatric phase I trial were to determine the MTD of intravenous lobradimil administered over 10 min in combination with carboplatin, to describe the incidence and severity of lobradimil toxicities in children with refractory brain tumors, and to define the pharmacokinetic profile of lobradimil in children.

Methods

Patient eligibility

Patients ≤ 21 years of age with histologically confirmed primary brain tumors refractory to standard therapy were eligible for this trial. Histological confirmation was not required for brain stem gliomas. Patients had to have recovered from the toxic effects of prior therapy and have an ECOG performance score of ≤ 2. All patients were required to have a serum bilirubin and SGPT no more than twice the upper limit of normal. Normal renal function was not required for study entry because carboplatin was adaptively dosed based on renal function, but patients were required to have a serum creatinine of ≤ 1.2 mg/dl if aged under 5 years, ≤ 1.5 mg/dl if aged 5–10 years, ≤ 1.8 mg/dl if aged 10–15 years, and ≤ 2.4 mg/dl if aged over 15 years. Patients who had not previously received craniospinal irradiation or myeloablative therapy with bone marrow or stem cell rescue were considered to be evaluable for hematological toxicity and were required to have a granulocyte count > 1500/μl, hemoglobin > 8.0 g%, and a platelet count > 100,000/μl.

Patients treated with carboplatin within the 6 months prior to study entry were ineligible. Patients who were pregnant or breastfeeding or who had a significant systemic illness were also excluded from the study. This trial was approved by our Institutional Review Board. Before study entry, informed consent was obtained from the patient and his/her guardian.

Trial design

Lobradimil was provided by Alkermes (Cambridge, Mass.) as a sterile solution at a concentration of 0.02 mg/ml. The calculated dose was diluted in 0.9% sterile sodium chloride to a total infusion volume of 20 ml for all patients. Lobradimil was administered intravenously over 10 min without carboplatin on day 1 of the first treatment cycle to evaluate its toxicity and perform pharmacokinetic sampling. On days 2 and 3 of cycle 1, and days 1 and 2 of each subsequent cycle, lobradimil was administered intravenously over 10 min, beginning 5 min before the end of the carboplatin infusion. The starting dose of lobradimil was 100 ng/kg ideal body weight (IBW) with escalations to 300, 450 and 600 ng/kg IBW. IBW was used in place of actual weight, because children with recurrent brain tumors frequently require chronic dexamethasone therapy to control symptoms, and this can result in a rapid and marked increase in body weight. The half-life of lobradimil is short and therefore the drug is most likely distributed primarily within the vascular system. Intrapatient dose escalation of lobradimil was not allowed.

Carboplatin was adaptively dosed based on the radioisotopically (^{99m}Tc-DTPA) measured glomerular filtration rate (GFR) to achieve a target area under the concentration-time curve (plasma AUC) of 3.5 mg·min/ml per dose (7.0 mg·min/ml per cycle). The target plasma AUC was 2.5 mg·min/ml per dose (5.0 mg·min/ml per cycle) for patients who had previously received craniospinal radiation or myeloablative therapy. The adaptive dosing formula developed by Marina et al. [11] was used to determine the carboplatin dose:

$$\text{Carboplatin daily dose (mg/m}^2\text{)} = \text{Target plasma AUC (mg·min/ml)} \times [0.93 \times \text{GFR (ml/min/m}^2\text{)} + 15]$$

Body surface area was calculated using the actual body weight. In the first 18 patients, carboplatin was infused intravenously over 60 min. A second cohort of 7 patients subsequently received the carboplatin infusion intravenously over 15 min after the recommended dose of lobradimil had been determined. The shorter carboplatin infusion was designed to achieve higher peak plasma concentrations of carboplatin during the period of BBB opening produced by lobradimil.

The MTD of lobradimil was defined as that dose level immediately below the dose level at which two or more patients in a cohort of three to six patients experienced a dose-limiting toxicity (grade 3 or more). Toxicities were graded according to the NCI/CTEP common toxicity criteria (http://ctep.info.nih.gov), except for hematological toxicity and hypotension. Hematological dose-limiting toxicity was assumed to be from carboplatin and did not influence the dose escalation of lobradimil.

Patients who had been previously treated with myeloablative therapy followed by bone marrow or stem cell transplantation or patients who had received craniospinal radiation were not considered evaluable for hematological toxicity. Hematological toxicity was considered dose-limiting if the neutrophil count was < 500/μl for ≥7 days, the platelet count was < 25,000/μl on two separate days of a treatment cycle, or if the neutrophil count had not returned to ≥1500/μl and the platelet count had not returned to ≥100,000/μl by 6 weeks posttherapy. Patients who experienced dose-limiting toxicity attributed to carboplatin had subsequent doses recalculated with the target plasma AUC reduced by 25%.

Dose-limiting hypotension was defined as (1) a drop in systolic blood pressure to < 60 mmHg or a drop in diastolic blood pressure to < 25 mmHg lasting for ≥5 min, or (2) a > 25% drop in blood pressure associated with syncope or an increase in the heart rate to > 180 bpm for children ≤ 2 years of age, > 160 bpm for children aged 3 to 12 years, and > 140 bpm for children > 12 years. Blood pressure was monitored using an automated sphygmomanometer (Dynamap, Critikon, Tampa, Fl.) every 15 min for at least 2 h prior to the start of, and during, the carboplatin infusion to determine the baseline blood pressure. Vital signs, including blood pressure, heart rate, and oxygen saturation, were monitored and recorded every 1 to 2 min for at least 30 min starting with the lobradimil infusion, and then at 15-min intervals for at least 2 h until the blood pressure had returned to the baseline range. The vital sign data were acquired and downloaded onto a portable laptop computer.

Pharmacokinetics

Lobradimil pharmacokinetics were studied on the first day of cycle 1, after a dose of lobradimil had been administered alone as a 10-min intravenous infusion. Blood was collected into tubes containing EDTA as an anticoagulant immediately prior to the start of the infusion, and at 5, 10 (end of infusion), 12, 15, 20 and 25 min after the start of the infusion. Samples were chilled on ice for 5–15 min prior to centrifugation. The plasma was transferred to cryotubes and stored at –70°C until analysis.

Lobradimil was quantified in plasma with a chemiluminescent ELISA method. Plasma was extracted with acidified ethanol to separate lobradimil from endogenous plasma proteins. The extract was evaporated to dryness and reconstituted with 0.25 ml assay buffer consisting of 1.0 g I-BLOCK (Tropix, Bedford, Mass.) in 500 ml PBS and 0.25 ml Tween 20. A protein-A-purified rabbit polyclonal anti-lobradimil antibody (Cocalico Biologicals, Reamstown, Pa.) was immobilized on a 96-well microwell plate. Extracted plasma samples and standards and lobradimil tagged with biotin at the amino acid terminus were added to the plates and incubated for 12 to 24 h at 2–8°C. The biotin-labeled lobradimil with an avidin-biotin-peroxidase complex and 3,3′,5,5′-tetramethylbenzidine as the substrate was detected at 450 nm with a reference wavelength of 630 nm on a BioTek Elx808 plate reader (Bio-Tek Instruments, Winooski, Vt.). In addition to lobradimil, the assay detects C-terminal fragments of the nonapeptide (amino acids 2–9, 3–9, and 4–9). Therefore, the results are expressed as picograms of lobradimil equivalents per milliliter (pg eq/ml). Lobradimil equivalents in plasma samples were quantified by comparison with standards prepared by adding known amounts of lobradimil to human plasma. The limit of quantification was 153 pg eq/ml. The intraassay variability was approximately 20% and the interassay variability was approximately 40%.

A two-compartment model with first-order elimination from the central compartment was fitted to the plasma concentration-time data using MLAB (Civilized Software, Bethesda, Md.). The differential equations describing the concentration of drug in the central compartment (C_c) and the amount of drug in the peripheral compartment (X_p) were as follows:

$$\frac{dC_c}{dt} = \frac{k_0}{V_c} - k_{cel} \bullet C_c - k_{cp} \bullet C_c + \frac{k_{pc} \bullet X_p}{V_c}$$

$$\frac{dX_p}{dt} = k_{cp} \bullet C_c \bullet V_c - k_{pc} \bullet X_p$$

where k_0 is the drug infusion rate, V_c is the volume of the central compartment, k_{cel} is the first-order elimination rate constant, and k_{cp} and k_{pc} are the first-order rate constants for the exchange of drug between the central and peripheral compartments. Values for V_c, k_{cel}, k_{cp}, and k_{pc} were obtained from the model fits to each patient's lobradimil concentration-time data. Clearance, volume of distribution at steady state (V_{ss}), half-lives ($t_{1/2}\alpha$, $t_{1/2}\beta$), and area under the lobradimil concentration-time curve (plasma AUC_∞) were derived from the fitted model parameters. The model-predicted end-infusion concentration was used as a measure of the maximum plasma concentration (C_{max}) because not all patients had blood drawn at the end of the infusion.

Evaluation of response

The size of measurable tumors was monitored by MRI. Complete response (CR) was defined as complete resolution of all measurable tumor and no new lesions for ≥4 weeks. Partial response (PR) was defined as a ≥50% reduction in the sum of the products of the two longest perpendicular diameters of all measurable tumors and no new lesions for ≥4 weeks. Progressive disease was defined as the appearance of new tumors or a >25% increase in the product of the two longest perpendicular diameters in any previously measurable lesion. Stable disease (SD) was a change in tumor size that was insufficient to meet the criteria for a CR, PR, or progressive disease.

Results

Patient characteristics

A group of 25 pediatric patients with recurrent or refractory primary brain tumors were entered on this phase I trial over a 2-year period from July 1996 to June 1998 and received a total of 89 cycles (median 2, range 1–19) of carboplatin and lobradimil. All 25 patients were considered evaluable for lobradimil toxicity. Patient characteristics and prior treatment are shown in Table 1.

The GFR, which was measured by a ^{99m}Tc-DTPA technique, ranged from 38 to 98 ml/min per m^2 (median 66 ml/min per m^2). Carboplatin was dosed adaptively based on the GFR, and the dose ranged from 125 to 372 mg/m^2 per day. Of the 25 patients, 13 were adaptively dosed to achieve a target plasma AUC of 3.5 mg·min/ml per day (7.0 mg·min/ml per cycle). The remaining 12 patients were adaptively dosed to achieve a target plasma AUC of 2.5 mg·min/ml per day (5.0 mg · min/ml per cycle) because of prior craniospinal radiation or myeloablative therapy with bone marrow or stem cell rescue.

Lobradimil toxicity

The toxicities attributed to lobradimil are listed in Table 2. The common toxicities, which were related to the cardiovascular, neurological and gastrointestinal effects of lobradimil, were not dose-limiting and were not clearly dose-related. The onset of all toxic effects was rapid, usually during the 10-min lobradimil infusion, and most resolved completely within 60 min after the end of the lobradimil infusion.

All patients experienced a decrease in blood pressure from the baseline at some time during or after the lobradimil infusion (Fig. 1), but no patient experienced

Table 1 Patient characteristics and prior treatment at study entry

Enrolled/evaluable for lobradimil toxicity	25/25
Age (years)	
Median	14
Range	4–20
Gender (M/F)	17/8
GFR (ml/min/m^2)	
Median	66
Range	38–98
Diagnosis	
High-grade glioma	10
Medulloblastoma/PNET	6
Brainstem glioma	5
Ependymoma	4
Prior therapy	
No. of treatment regimens, median (range)	2 (0–5)
Cisplatin	9
Carboplatin	1
Cisplatin and carboplatin	4
BMT/SCT	7
Local radiation	14
Craniospinal radiation	11

Table 2 Toxicities attributed to lobradimil during cycle 1, day 1 in 25 patients. The numbers represent the number of patients with the specified toxicity within a dose level. The numbers in parentheses represents the percentage of patients at that dose level with the specified toxicity

Toxicity	Dose level of lobradimil (ng/kg)			
	100	300	450	600
Hypotension	4 (100)	4 (100)	4 (100)	13 (100)
Hypertension	4 (100)	4 (100)	4 (100)	12 (92)
Abdominal pain	0 (0)	1 (25)	4 (100)	10 (77)
Headache	0 (0)	1 (25)	3 (75)	11 (85)
Vasodilatation	0 (0)	3 (75)	4 (100)	12 (92)
Nausea	0 (0)	1 (25)	3 (75)	9 (69)
Vomiting	0 (0)	1 (25)	2 (50)	8 (62)
Tachycardia	0 (0)	0 (0)	1 (25)	12 (92)
Fatigue/somnolence	0 (0)	0 (0)	1 (25)	4 (31)
Asthenia	0 (0)	1 (25)	1 (25)	3 (23)

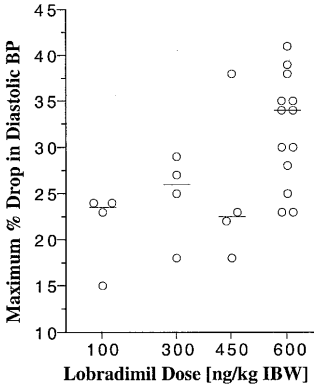

Fig. 1 Effect of lobradimil on diastolic blood pressure. Each point represents the mean of the maximum percentage drop in diastolic blood pressure from baseline for the three daily doses of lobradimil from the first cycle. The *bars* represents the median percentage drop in diastolic blood pressure for each dose level

syncope or hypotension-related tachycardia and no patient met the criteria for dose-limiting hypotension. The median percentage drop in diastolic blood pressure (calculated as the mean of the maximum percentage drop from baseline for the three daily doses of lobradimil from cycle 1) was 24% at 100 ng/kg IBW ($n=4$), 26% at 300 ng/kg IBW ($n=4$), 22% at 450 ng/kg IBW ($n=4$), and 33% at 600 ng/kg IBW ($n=13$). All patients also had a transient increase in blood pressure above baseline during or immediately after a lobradimil infusion. Symptoms related to vasodilatation, including flushing, injected sclera and a feeling of warmth when infused with lobradimil alone, were seen in 76% of patients.

The most prevalent neurological toxicity was headache, which ranged in severity from mild to severe, but was very transient and not dose-related (i.e. did not increase in severity or duration as the dose was escalated). Fatigue or drowsiness (48%), emotional lability (20%), and agitation (12%) were also observed. Three patients reported a visual change during the infusion, including transient (<30 s) loss of vision ($n=2$) and "seeing spots" ($n=1$). Abdominal pain, ranging in severity from mild discomfort to severe cramping, was experienced by 72% of patients, and was sometimes associated with nausea, vomiting, an urge to defecate, or diarrhea. The onset of these toxicities was immediately after the start of the lobradimil infusion, and they subsided within minutes after the completion of the infusion. Although at times severe, these cardiovascular, neurological and gastrointestinal toxicities from lobradimil were tolerable, because they usually persisted for only a few minutes.

One patient experienced dose-limiting toxicity that was attributed to lobradimil. This 9-year-old girl with a large diffuse pontine glioma experienced mild headache, nausea, vomiting, and hypotension with the first 600 ng/kg IBW dose of lobradimil, which was administered alone on the first day of cycle 1. On day 2 she received carboplatin over 60 min with lobradimil and had a partial complex seizure 4 min into her lobradimil infusion. She had no prior seizure history and her blood pressure had not changed significantly from baseline prior to the seizure. The drug infusion was immediately stopped and she was responsive to commands within 1 min. She complained of a severe headache which persisted for more than 5 h after the lobradimil infusion, and over the next 24 to 48 h she had intermittent confusion and disorientation, inability to follow commands, dysarthria, difficulty in moving her jaw, lethargy, incontinence, and greater upper extremity weakness. She had two additional seizures over the next 24 h, was started on anticonvulsants and mannitol, and her dexamethasone dose was increased. An EEG showed no abnormal electrical activity, a repeat MRI was unchanged, and electrolytes and glucose were normal. Her neurological symptoms gradually improved over the following 2 days, but she died within 3 weeks of progressive disease.

Lobradimil toxicity on the first day of cycle 1, when lobradimil was administered alone, was similar to the toxicity observed when the modulating agent was administered in combination with carboplatin on days 2 and 3 of cycle 1. In addition, the duration of the carboplatin infusion (15 or 60 min) did not have an impact on lobradimil toxicity.

Although the MTD was not defined for lobradimil, the dose was not escalated beyond the 600 ng/kg IBW dose level because the toxicities from lobradimil were not clearly dose-related and, at the time of this study, there was no clinical experience with doses of lobradimil >300 ng/kg in adults.

Carboplatin toxicity

Of the 13 patients who had not previously received craniospinal radiation or bone marrow/stem cell transplantation, ten were fully evaluable for hematological toxicity. Two inevaluable patients in this group had rapid tumor progression and were unable to complete a full 4 weeks of twice-weekly blood counts after the first

cycle of therapy. One patient received a higher dose of carboplatin than prescribed by the protocol because her dose was calculated from a misreported GFR value. The ten evaluable patients received a dose of carboplatin adapted to achieve a target plasma AUC of 3.5 mg·min/ml per dose × two doses. The 12 patients who were treated at the lower target carboplatin plasma AUC (2.5 mg·min/ml per dose × two doses), because of prior craniospinal radiation or bone marrow/stem cell transplantation, were also monitored with twice-weekly blood counts. Myelosuppression was the primary toxicity from carboplatin.

The nadir platelet and absolute neutrophil counts for the first cycle of therapy in the two carboplatin treatment groups are shown in Fig. 2. Neutropenia was less severe in the heavily pretreated group that was dosed based on the lower target carboplatin plasma AUC (Fig. 2A). Five of the ten patients in the hematologically evaluable group had an ANC nadir $< 500/\mu l$ during cycle 1, whereas only 1 of 12 patients in the heavily pretreated group had an ANC nadir $< 500/\mu l$. The median ANC nadirs in the two dosing groups were 525/μl for the higher target plasma AUC group and 1340/μl for the lower target plasma AUC. Five patients (three from the higher target plasma AUC group) required a carboplatin dose reduction at some point during therapy because of failure to recover their ANC to ≥1500/μl by 6 weeks after treatment. There were no episodes of febrile neutropenia in the 89 cycles of therapy.

Thrombocytopenia was more prominent than neutropenia. In the hematologically evaluable group treated at the higher target carboplatin plasma AUC, the median platelet count nadir from cycle 1 was 25,000/μl (Fig. 2B). Eight of the ten patients had a platelet nadir $< 50{,}000/\mu l$ and six experienced dose-limiting thrombocytopenia requiring a dose reduction. In the more heavily pretreated group treated at the lower target carboplatin plasma AUC, the median platelet count nadir on the first treatment cycle was 32,000/μl and 8 of 12 had a nadir platelet count $< 50{,}000/\mu l$. Three of 12 patients in this group experienced dose-limiting thrombocytopenia.

The duration of the carboplatin infusion (15 or 60 min) had no apparent impact on the incidence or severity of carboplatin hematological toxicity (Fig. 2).

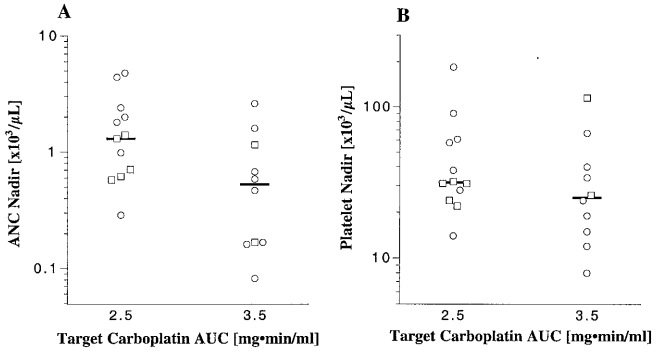

Fig. 2 Absolute neutrophil count (ANC) nadirs (**A**) and platelet count nadirs (**B**) after the first cycle of therapy for patients who were dosed adaptively to achieve a target carboplatin plasma AUC of 3.5 mg·min/ml per dose (7.0 mg·min/ml per cycle) or 2.5 mg·min/ml per dose (5.0 mg·min/ml per cycle) administered as a 60 min (○) or 15 min (□) intravenous infusion. Patients who were treated at the lower target carboplatin plasma AUC had previously received craniospinal radiation or myeloablative chemotherapy followed by bone marrow or stem cell rescue. Three of the 13 patients treated at the higher target carboplatin plasma AUC were not evaluable for hematological toxicity

Lobradimil pharmacokinetics

Of the 25 patients, 24 had lobradimil pharmacokinetic sampling performed, and pharmacokinetic modeling was performed in 16 patients. Pharmacokinetic modeling was not performed in the four patients who were treated at the 100 ng/kg IBW dose level because two of the four had too few measurable time points (plasma concentration was undetectable 2 and 5 min after the end of the infusion) and a third patient had plasma lobradimil concentrations that initially declined after the end of the infusion and then increased. The mean end-infusion lobradimil plasma concentration (C_{max}) in three of four patients who were treated at this lowest level and had a sample obtained at the end of the infusion was 381 pg eq/ml. There were also too few samples with measurable lobradimil concentrations to allow for modeling in one patient each at the 300 and 600 ng/kg IBW dose levels, one patient did not have the first three samples obtained, and one patient had a plasma concentration-time profile that could not be fitted by a two-compartment model.

The biexponential plasma concentration-time profile for lobradimil is illustrated in Fig. 3 and the pharmacokinetic parameters from the 16 evaluable patients at the 300, 450 and 600 ng/kg IBW dose levels are listed in Table 3. Lobradimil was rapidly eliminated from plasma with an initial (α) half-life of 0.9 min and a terminal (β) half-life of 10 min. The mean ($\pm$SD) clearance across the three dose levels was 0.0269 ± 0.0094 l/min per kg IBW.

The lobradimil plasma AUC_∞ and C_{max} did not increase in proportion to the dose over the 300 to 600 ng/kg IBW dose range (Fig. 4), and at the 600 ng/kg IBW dose level there was substantial variability in the lobradimil plasma AUC_∞ (range 13,900 to 34,900 pg eq·min/ml). There was also no correlation between lobradimil plasma AUC_∞ or C_{max} and the absolute dose, the dose normalized to body surface area, or the dose normalized to actual body weight. This lack of relationship between plasma lobradimil concentrations and dose could account for lack of relationship between the dose and the incidence and severity of lobradimil-related toxicities. The less than proportional increase in lobradimil plasma AUC_∞ with increases in dose from 300 to 600 ng/kg IBW appears to be accounted for by an increase in the volumes of distribution (V_c and V_{ss}) of the drug rather than an increase in the rate of elimination (k_{cel}) of the drug (Table 3).

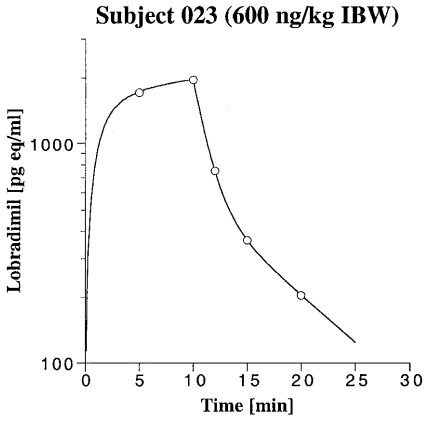

Fig. 3 Concentration-time profile for lobradimil in a representative patient during and after a 10-min infusion of 600 ng/kg IBW. Points represent the measured values, which included lobradimil and its metabolites, and the line represents the two-compartment model fit to the concentration-time data

Responses

Of the 25 patients, 23 were evaluable for response. Two patients died from progressive disease prior to re-evaluation. Baseline and follow-up MRI scans were reviewed by a single neuroradiologist (N.P.) after the patients were taken off study. Two patients with medulloblastoma achieved a PR, including one patient who experienced a >90% reduction in the size of his tumor. Both patients were heavily pretreated, and had previously received high-dose chemotherapy and transplant. One had received prior cisplatin. Ten patients had SD of 2 to 40+ months (median 5.5 months) duration.

Discussion

Intravenous lobradimil, which we administered as a BBB-modulating agent in combination with carboplatin, is safe and tolerable at doses up to 600 ng/kg IBW in children with brain tumors. The rapid onset and intensity of lobradimil toxicities was tolerable because of the short duration (minutes) of these toxicities. The lobradimil toxicity profile in children was similar to the profile in adults and is related to the drug's vasoactive properties. Lobradimil toxicities that were observed in children and not previously reported in adults included visual changes and palmar/plantar erythema with dysesthesias. Hypotension was observed in all patients who were treated on our trial, but no patient developed symptoms related to blood pressure changes. The high incidence of hypotension on our trial was probably related in part to the detailed vital sign monitoring.

The MTD of lobradimil was not defined in children because the incidence and severity of toxic effects from lobradimil did not appear to increase in proportion to the dose over the dosage range studied and because of reservations about escalating the dose beyond 600 ng/kg IBW without prior experience in adults at higher doses. Preclinical models have demonstrated that the pharmacological effects of lobradimil are receptor-mediated [4]. In general, when doses of a drug above

Table 3 Lobradimil pharmacokinetic parameters from 16 patients treated at the 300, 450, and 600 ng/kg IBW dose levels

Subject	Dose level (ng/kg IBW)	Age (years)	Dose (ng)	Model-fitted parameters				Parameters derived from model-fitted parameters					
				V_c (l/kg IBW)	k_{cel} (min^{-1})	k_{cp} (min^{-1})	k_{pc} (min^{-1})	C_{max} (pg eq/ml)	Plasma AUC (pg eq·min/ml)	Clearance (l/min/kg IBW)	V_{ss} (l/kg IBW)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)
005	300	4	5,310	0.0346	0.447	0.318	0.0792	1360	19,400	0.0154	0.173	0.87	15.7
006	300	18	20,580	0.0281	0.400	0.236	0.107	2010	26,700	0.0112	0.0900	1.02	11.0
007	300	12	13,950	0.0284	0.534	0.490	0.146	1440	19,800	0.0152	0.124	0.63	9.8
Mean				0.0304	0.460	0.348	0.111	1600	22,000	0.0139	0.129	0.84*	12.1*
SD				0.0036	0.068	0.130	0.034	350	4,100	0.0023	0.042	0.20	3.1
009	450	14	24,480	0.0332	0.399	0.270	0.0993	2290	34,000	0.0132	0.123	0.97	12.5
010	450	15	21,600	0.0418	0.382	0.196	0.0936	2160	28,200	0.0160	0.129	1.13	11.9
011	450	9	33,795	0.0290	0.496	0.559	0.111	2180	31,300	0.0144	0.175	0.62	14.0
012	450	9	13,950	0.0567	0.339	0.236	0.0813	1620	23,400	0.0192	0.221	1.13	15.4
Mean				0.0402	0.404	0.315	0.0963	2060	29,200	0.0157	0.162	0.96*	13.4*
SD				0.0122	0.066	0.165	0.0124	300	4,500	0.0026	0.045	0.24	1.6
014	600	8	14,580	0.104	0.297	0.154	0.113	1480	19,500	0.0308	0.245	1.40	10.2
015	600	16	36,120	0.0737	0.235	0.282	0.0521	1800	34,600	0.0173	0.473	1.27	31
016	600	9	14,400	0.173	0.249	0.202	0.115	932	13,900	0.0431	0.477	1.36	12.3
018	600	5	8,880	0.0820	0.237	0.403	0.964	2480	30,900	0.0194	0.116	0.48	4.4
019	600	18	30,600	0.0459	0.375	0.230	0.292	2980	34,900	0.0172	0.0820	0.92	4.7
021	600	10	22,800	0.0660	0.417	0.102	0.0903	1740	21,800	0.0275	0.140	1.28	9.9
022	600	14	22,800	0.0867	0.242	0.278	0.0300	1450	28,600	0.0210	0.889	1.29	51.2
023	600	11	20,100	0.0509	0.516	0.208	0.149	1960	22,800	0.0263	0.122	0.90	7.0
024	600	16	33,000	0.0711	0.550	0.889	0.227	1050	15,300	0.0391	0.350	0.44	8.8
Mean				0.0837	0.346	0.305	0.226	1760	24,700	0.0269	0.322	1.04*	15.5*
SD				0.0378	0.124	0.235	0.289	650	7,900	0.0094	0.262	0.37	15.6

*Harmonic mean calculated for $t_{1/2}\alpha$ and $t_{1/2}\beta$

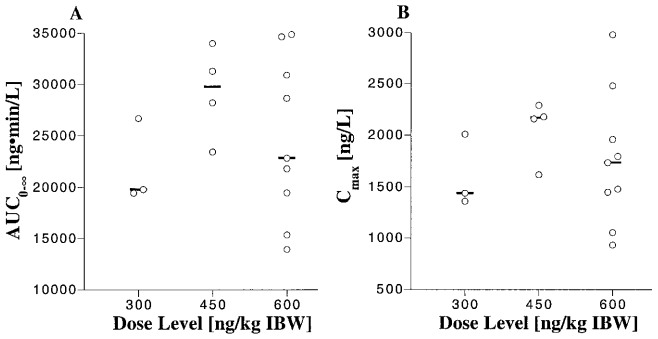

Fig. 4 Relationship between lobradimil dose and plasma $AUC_{0-\infty}$ (**A**) and C_{max} (**B**)

the level necessary to saturate receptors is given, no greater pharmacological effect is expected. In preclinical studies, the BBB permeability effects of lobradimil were dose-dependent, but the effect plateaued at higher dose levels [5]. At moderate to high doses, the relative increase in BBB permeability within normal brain tissue is similar to that in implanted brain tumors (i.e. the BBB modulating effects of lobradimil are less selective) [1]. Continuing to escalate the dose of lobradimil past the dose at which the B_2 receptors are presumably saturated could result in new toxic effects resulting from the interaction of lobradimil with other lower affinity receptors without an increase in the BBB-modulating effect of lobradimil. The substantial degree of interpatient variability in lobradimil pharmacokinetics and the lack of relationship between lobradimil dose and plasma drug exposure (plasma AUC) may provide an alternative explanation for the lack of relationship between lobradimil dose and the incidence and severity of toxic effects.

The pharmacokinetics of lobradimil in children were characterized by rapid plasma clearance and substantial interpatient variability. Some of the variability could have been related to technical issues in sampling and assaying the drug. With very short half-lives (e.g. $t_{1/2}\alpha$ of 1 min), small discrepancies in sampling times and differences in the duration of time over which the initial samples were drawn could have had a substantial impact on the results of the pharmacokinetic modeling. The assay that was used to measure lobradimil in this study detects the parent drug and its metabolites and the metabolites could have been contributing to the apparent variability in lobradimil disposition.

The short half-lives and duration of exposure to lobradimil are probably not a limiting factor for the BBB-modulating effects of the drug because of the rapid development of tachyphylaxis. In preclinical studies of more prolonged lobradimil infusions, restoration of the BBB after 15 min despite continuation of the lobradimil infusion has been found [1].

The plasma AUC_∞ and C_{max} values at the 300, 450 and 600 ng/kg IBW dose levels overlapped substantially and plasma AUC_∞ and C_{max} did not increase in proportion to the dose. This non-linearity appears to have been due to an increase in volumes of distribution at higher dose levels rather than an increase in the rate of elimination. The increase in V_c and V_{ss} may be a reflection of lobradimil-induced systemic vascular dilatation and permeability at higher dose levels.

Carboplatin was adaptively dosed in an effort to achieve more uniform drug exposure and more predictable toxicity. The target plasma AUC was based on the patient's prior treatment and predicted bone marrow reserve. Although the median platelet and neutrophil nadirs for the two target plasma AUC groups were in the desired ranged, there was considerable variability in the degree of neutropenia and thrombocytopenia (Fig. 2). This variable degree of hematological toxicity may be in part related to differences in the number and intensity of prior treatment regimens. The degree of thrombocytopenia in the more heavily pretreated group that received a carboplatin dose targeted to achieve a plasma AUC of 5.0 mg·min/ml per course was comparable to that in the less heavily pretreated group that was treated at the higher target plasma AUC (7.0 mg·min/ml per course). Although neutropenia (ANC $< 500/\mu l$) was common, there were no episodes of febrile neutropenia on the 89 cycles of therapy administered on this trial. The toxicity of carboplatin did not appear to be affected by lobradimil and the severity of carboplatin hematological toxicity was not related to the lobradimil dose.

Two cohorts of patients were treated with differing carboplatin infusion times (60 and 15 min). The rationale for the shorter infusion time was to achieve higher peak plasma carboplatin concentrations during the period of lobradimil-induced BBB opening. Lobradimil and carboplatin toxicities were not substantially altered by the shorter infusion regimen.

Two patients had objective responses, and ten patients had stable disease, including cases of prolonged stable disease of up to 40+ months duration. Although the contribution of lobradimil to this antitumor activity could not be quantified on this phase I trial, an objective response and stable disease were observed in patients who had previously received platinum therapy.

Lobradimil in combination with carboplatin is tolerable in children as a 10-min intravenous infusion at doses up to 600 ng/kg IBW, and the recommended dose of lobradimil in children for the phase II trials is 600 ng/kg IBW. This trial also suggests that higher doses of lobradimil should be tested in adults.

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