Cancer Chemother Pharmacol (2005) 56: 329–336
DOI 10.1007/s00280-004-0968-x

ORIGINAL ARTICLE

**Hiroyuki Kobayashi · S. Gail Eckhardt
Jennifer A. Lockridge · Mace L. Rothenberg
Alan B. Sandler · Cindy L. O'Bryant · Wendy Cooper
Scott N. Holden · Roger D. Aitchison · Nassim Usman
Maurice Wolin · Michele L. Basche**

Safety and pharmacokinetic study of RPI.4610 (ANGIOZYME), an anti-VEGFR-1 ribozyme, in combination with carboplatin and paclitaxel in patients with advanced solid tumors

Received: 9 June 2004 / Accepted: 14 September 2004 / Published online: 20 May 2005

Abstract *Purpose*: RPI.4610 (ANGIOZYME) is a chemically stabilized ribozyme targeting vascular endothelial growth factor receptor 1. The purpose of this study was to evaluate the safety and pharmacokinetics of RPI.4610 in combination with carboplatin and paclitaxel in patients with advanced solid tumors. *Methods*: The study used a sequential treatment design evaluating a single dose level for all three drugs: paclitaxel 175 mg m^{-2} and carboplatin AUC = 6 on day 1 of a 21-day cycle, and RPI.4610 100 mg m^{-2} day^{-1} beginning on day 8 and continuing daily thereafter. Pharmacokinetic samples were drawn on day 1 of courses 1 (chemotherapy alone) and 2 (chemotherapy + RPI.4610), and on day 8 of course 1 (RPI.4610 alone). Ratios were generated by comparing the pharmacokinetic parameters for the combination of carboplatin with paclitaxel when administered alone or together with RPI.4610. *Results*: Twelve patients were enrolled in this trial and received two to six courses of treatment each. The most common grade 3–4 toxicities were neutropenia (three patients), thrombocytopenia (three patients), pain (three patients), anemia (two patients) and fatigue (two patients). The ratio of the mean maximum plasma concentration (*C*max) for carboplatin when administered with paclitaxel alone versus when administered with paclitaxel and RPI.4610 was 1.07 (90% confidence interval, 0.77–1.37). Similarly, the ratio of the mean $AUC_{0\text{-last}}$ for carboplatin was 1.04 (0.73–1.35). For paclitaxel the ratio of the mean *C*max when administered with carboplatin alone versus with carboplatin and RPI.4610 was 1.17 (1.03–1.31), and the ratio of the mean $AUC_{0\text{–last}}$ was 1.17 (1.04–1.30). Objective tumor responses were observed and included one patient with a complete response (bladder cancer) and one patient with a partial response (esophageal cancer). *Conclusions*: These results indicate that RPI.4610, carboplatin, and paclitaxel can be administered safely in combination without substantial pharmacokinetic interactions.

Keywords ANGIOZYME · Carboplatin · Paclitaxel · Pharmacokinetics · Ribozyme · Vascular endothelial growth factor receptor · Angiogenesis inhibitors

H. Kobayashi · M. L. Rothenberg (✉)
A. B. Sandler · W. Cooper
Vanderbilt-Ingram Cancer Center,
777 Preston Research Building, Nashville,
TN 37232-6307, USA
E-mail: mace.rothenberg@vanderbilt.edu
Tel.: +1-615-9363831
Fax: +1-615-3437602

S. Gail Eckhardt · C. L. O'Bryant · S. N. Holden · M. L. Basche
Anschutz Cancer Pavilion,
University of Colorado Cancer Center,
1665 N Ursula St F 704, Aurora, CO 80010-0510, USA

J. A. Lockridge · R. D. Aitchison · N. Usman
Sirna Therapeutics, 2950 Wilderness Place,
Boulder, CO 80301, USA

M. Wolin
Chiron Corporation,
4560 Horton Street, Emeryville, CA 94608, USA

Introduction

Vascular endothelial growth factor (VEGF) is an angiogenesis-promoting molecule. Many of the effects of VEGF are mediated through multiple high-affinity receptors on endothelial cells, including VEGFR-1. One way in which inhibition of VEGF receptor activity can be accomplished is by use of catalytic RNA molecules known as ribozymes. Ribozymes can downregulate VEGF receptor function by specifically cleaving the mRNAs that encode for the primary VEGF receptor proteins. Because short RNA molecules are physiologi-

cally unstable, ribozymes must be chemically modified to provide in-vivo stability while preserving catalytic activity and target specificity. Chemically stabilized ribozymes which incorporate 2′-*O*-methyl sugar-modified nucleotides, 5′-phosphorothioate linkages, and 3′-inverted 2′-deoxyabasic residues provide improved resistance to nucleases without loss of catalytic activity [12]. In preclinical studies ribozymes directed against VEGFR-1 were more potent inhibitors of angiogenesis and tumor growth than a similar ribozyme directed against VEGFR-2 [13].

RPI.4610 (ANGIOZYME) is a stabilized ribozyme (Fig. 1) that specifically targets the preRNA for VEGFR-1 and its alternatively spliced, soluble form, sVEGFR-1. RPI.4610 is the first synthetic compound based on ribozyme technology to be developed and tested for clinical application.

In preclinical studies RPI.4610 inhibited in-vivo growth of a highly metastatic clone of the Lewis lung carcinoma in a dose-dependent manner, reducing the number of lung metastases [13]. RPI.4610 also reduced the number of peritumoral vessels in a murine intradermal model using Hey ovarian, SK-MEL-1 melanoma, ACHN renal and LNCap prostate cell lines. When combined with interferon, RPI.4610 was synergistic against Hey ovarian and SK-MEL-1 melanoma cell lines [5]. RPI.4610 reduced the number of metastases in a human KM12 colorectal cancer xenograft model and reduced the number of metastases and increased survival in a murine mammary 4T1 tumor model.

RPI.4610 is well absorbed by subcutaneous (s.c.) administration [13]. The only normal tissue changes observed in murine and primate toxicology studies were reversible accumulation of basophilic and cytoplasmic granules in the kidney and liver without renal or hepatic dysfunction and irritation at the s.c. injection site in 13-week studies at doses up to 300 mg m^{-2} day^{-1} [18].

A previous single agent clinical study of RPI.4610 revealed that agent was well tolerated with a dose-limiting toxicity of grade 3 s.c. injection site reactions at 300 mg m^{-2} dose. Specific localization of RPI.4610 and some decrease in immunohistochemical staining of VEGFR-1 were observed in endothelial cells [21]. The

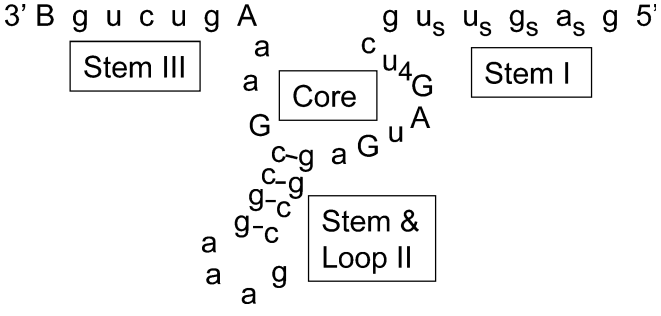

Fig. 1 Structure of RPI.4610. Nucleotide and backbone chemical modifications are: *uppercase*, ribonucleotide; *lowercase*, 2′-*O*-methyl sugar-modified ribonucleotide; *B*, inverted 2′-deoxyribose abasic; *s*, phosphorothioate linkage; u_4, 2′-C-allyl uridine

observation of dose-limiting toxicity at 300 mg m^{-2} and volume limitations of s.c. daily injection led to the determination of 100 mg m^{-2} as the recommended phase II dose. In a phase II monotherapy RPI.4610 trial, 45 late-stage metastatic breast cancer patients who had progressive disease despite chemotherapy received 100 mg m^{-2} RPI.4610 by daily s.c. injection. Although there were no objective responses, RPI.4610 monotherapy was associated with a significant decrease in sVEGFR-1 by week 6 (Gabriel N. Hortobagyi, unpublished data). It was hypothesized that inhibition of VEGFR-1 alone might be insufficient to induce clinically detectable antitumor activity and would require the combination of RPI.4610 with other agents for optimum antitumor effect. In fact, important additive or synergistic antitumor activity has been noted in preclinical models when angiogenesis inhibitors have been combined with classical cytotoxic agents, suggesting that this approach could provide superior clinical activity by complementary targeting of both the tumor and vascular compartments. Carboplatin and paclitaxel are cytotoxic agents commonly used in combination for treatment of a variety of diseases, including lung and ovarian cancers. An association between tumor angiogenesis, prognosis, and outcome has been reported for both of these tumors [4, 11]. In preclinical studies, carboplatin and paclitaxel resulted in enhanced tumor growth inhibition when combined with an antiangiogenic agent [7]. Therefore, addition of an angiogenesis-targeted agent to this combination could potentially result in increased antitumor efficacy.

RPI.4610 has multiple possible sites for platinum adduct formation and four phosphorothioate-linkages. Carboplatin has an extremely strong interaction with sulfur-containing compounds such as RPI.4610, because of platinum's electrophilic nature. Although no significant pharmacokinetic interactions between carboplatin and RPI.4610 were shown in a murine model [9], this study was designed to assess and potential pharmacokinetic interaction in humans.

The purpose of this study was to determine if full-dose RPI.4610 could be given safely in combination with standard doses of carboplatin and paclitaxel. The objectives were:

1 to characterize the toxicities of RPI.4610, carboplatin, and paclitaxel when administered over a 3 week cycle,
2 to characterize the pharmacokinetic behavior of the combination in addition to any drug interactions, and
3 to seek preliminary evidence of antitumor efficacy.

Methods

Patient selection

Patients with histologically or cytologically confirmed metastatic cancer of any histology appropriate for treatment with carboplatin and paclitaxel therapy were

eligible for this study. Other relevant eligibility criteria included:

1 Karnofsky performance status ≥70%,
2 age ≥18 years,
3 life expectancy ≥12 weeks, and
4 adequate hematopoietic function (absolute neutrophil count ≥1,500 μL^{-1}, platelet count ≥100,000 μL^{-1}), renal function [creatinine concentration ≤ 1.5 times the institutional upper limit of normal (ULN)], and hepatic function [total bilirubin level ≤ 1.5 times ULN and alanine aminotransferase (ALT) ≤ 2 times ULN].

Exclusion criteria included:

1 known central nervous system metastases;
2 chemotherapy, investigational drugs, hormonal therapy, surgery, or anticoagulant therapy within the preceding 21 days;
3 significant cardiac disease;
4 history of a bleeding disorder; or
5 the presence of a serious active infection or other underlying medical condition that would preclude full compliance with the study.

Informed consent was obtained according to federal and institutional guidelines. The institutional review boards for the Vanderbilt–Ingram and the University of Colorado cancer centers approved the protocol.

Drug administration

The study used a sequential treatment design exploring a single dose level for all drugs. Subjects received paclitaxel 175 mg m^{-2} and carboplatin targeting an area under the curve (AUC) of 6 mg mL^{-1} min on day 1 of a 21-day cycle. RPI.4610 100 mg m^{-2} was administered s.c. beginning day 8 and administered daily thereafter. Carboplatin could be discontinued at the discretion of the investigator after four courses of treatment.

Paclitaxel was administered intravenously (i.v.) over 3 h using standard premedications consisting of diphenhydramine 50 mg i.v., cimetidine 300 mg i.v., and antiemetics. Thirty minutes after the paclitaxel infusion was completed, carboplatin was administered i.v. over 30 min. The carboplatin dose was determined by the Calvert formula [the total dose of carboplatin (mg) = (target AUC)(GFR + 25)], where the glomerular filtration rate (GFR) was estimated by use of the Cockroft–Gault formula.

RPI.4610 was supplied by Sirna Therapeutics (Boulder, CO, USA) in 10-mL glass vials containing 180 mg or 225 mg of lyophilized drug. The appropriate vial strength was selected on the basis of subject body surface area. The subject (or the caregiver) reconstituted the drug with an appropriate volume of USP half-normal saline for injection to achieve a final drug concentration of 150 mg mL^{-1}. The total dose was then aspirated into a syringe and injected s.c. The dose of RPI.4610 was capped at 225 mg because of limitations to the volume of drug that could be daily administered subcutaneously.

Pretreatment and follow-up studies

Interval histories, physical examinations, and routine laboratory studies were conducted before treatment, weekly during course 1 and every 3 weeks thereafter. Routine laboratory studies included a complete blood count with differential white blood cell counts, prothrombin time (PT)/partial thromboplastin time (PTT), serum electrolytes, renal and liver function tests, urinalysis, and analysis of antibodies to RPI.4610. A 12-lead ECG was obtained before beginning treatment.

Plasma sampling and assay

To evaluate whether there was an interaction between RPI.4610 and the combination of carboplatin and paclitaxel, blood samples were drawn when carboplatin was administered with paclitaxel (day 1), when RPI.4610 was administered alone (day 8), and when all three agents were administered together (course 2, day 1) (Fig. 2). Pharmacokinetic samples were collected by drawing blood at the following times: course 1, day 1: baseline, 3, 4, 6, 8, 12, and 24 h after beginning of paclitaxel infusion; course 1, day 8: baseline, 2, 4, 6, 8, and 20 h after RPI.4610 injection; and course 2, day 1: baseline, 3, 4, 6, 8, 12, and 24 h after beginning of paclitaxel infusion. Samples were collected into sodium heparin tubes, placed on ice, and centrifuged at 3,000 rpm for 10 min at 4°C. The upper layer was transferred with a glass pipette to a cryovial and stored at −70°C until further processing.

Quantitation of RPI.4610 was performed by anion-exchange HPLC analysis as described previously [17]. The total platinum in plasma was assayed by inductively coupled plasma mass spectrometry as described previ-

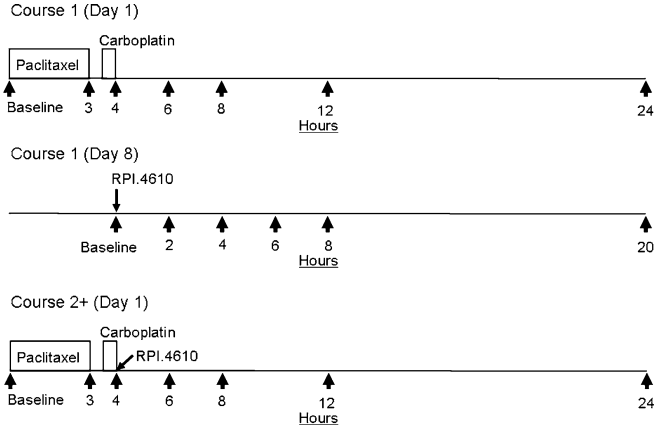

Fig. 2 Trial design and pharmacokinetic sampling schedule

ously [3]. Carboplatin plasma concentrations were recalculated on the basis of the molar ratio of platinum:carboplatin. Total paclitaxel in plasma was measured by liquid chromatography–mass spectroscopy as described previously [15].

Pharmacokinetic analyses

Pharmacokinetic parameters for RPI.4610, carboplatin, and paclitaxel were calculated from plasma concentration–time data using the noncompartmental approach. AUC was calculated using the linear trapezoidal method from time zero up to the last detectable concentration (AUC_{0-last}). The maximum plasma concentration (Cmax) and AUC_{0-last} values were calculated for each patient on each treatment. Mean pharmacokinetic parameters were compared using paired t-tests.

The primary statistical analysis assessed the effect of RPI.4610 on carboplatin and paclitaxel pharmacokinetic parameters. For each subject and each chemotherapy agent the difference between each pharmacokinetic parameter on day 1 (carboplatin and paclitaxel alone) and day 1 of course 2 (carboplatin and paclitaxel with RPI.4610) was determined. For each measure of exposure, ratios were calculated describing each parameter when given as monotherapy relative to the value obtained when the drug was administered as part of combination therapy. The mean and SD for each ratio was calculated, and 90% confidence intervals (CI) surrounding the mean of the ratios were calculated [19].

The secondary analysis assessed the effect of carboplatin and paclitaxel on RPI.4610 pharmacokinetic parameters. For each subject the difference in each RPI.4610 pharmacokinetic parameter on day 8 (RPI.4610 alone) and day 1 of course 2 (carboplatin and paclitaxel with RPI.4610) was obtained. For each measure of exposure, ratios were calculated describing each parameter when given as a monotherapy relative to the value obtained when the drug was administered as part of combination therapy. The mean and SD for each ratio were calculated, and 90% CIs surrounding the mean of the ratios were calculated.

Ratios near unity would be consistent with the lack of a pharmacokinetic interaction between these drugs under this treatment regimen [19].

Safety and tolerability assessments

Toxicities, graded according to the National Cancer Institute Common Toxicity Criteria (CTC version 2.0), were recorded as maximum grade/patient for all treatment courses. The relationship of the study treatment to an adverse event was determined by the investigator to be not related, possibly related, or probably related.

Statistical considerations

Descriptive statistics (mean ± SD) were calculated for all pharmacokinetic parameters. The statistical analyses were performed using SAS software (version 8.0 for Windows; SAS Institute, Cary, NC, USA). Statistical significance was considered to be demonstrated by a two-tailed P value of less than 0.05. With 12 patients we had 80% power to observe at least one patient experiencing an adverse event that occurred with a true frequency of 0.15 [6].

Results

Patient characteristics

Twelve patients were treated between August 2001 and November 2002. The characteristics of the patients are shown in Table 1. Three patients had received no prior therapy and received the study drugs as the first therapy for metastatic disease. Only one patient did not receive the combination therapy on day 1 of course 2 because of prolonged neutropenia. This patient is included in the safety analysis.

Safety

Table 2 lists all grade 2–4 toxicities observed during any course of treatment. Overall, the most common grade 3–

Table 1 Patient characteristics

Characteristic		No. of patients
Total patients		12
Male/female		7/5
Age (years)		
Median	53	
Range	35–70	
No. of carboplatin/paclitaxel courses per patient		
Median	3	
Range	2–6	
Days of exposure to RPI.4610		
Median	88	
Range	31–421	
Karnofsky performance status score		
Median	85	
Range	80–100	
Tumor types		
Non-small cell lung cancer		3
Esophageal cancer		4
Thymoma		1
Renal cell cancer		1
Renal pelvis and ureter		1
Cervical cancer		1
Bladder cancer		1
Prior therapy		
None		3
Surgery		7
Chemotherapy		7
Radiation		5

Table 2 Adverse events by patient in any cycle ($n = 12$)

Toxicity	Grade 2	Grade 3	Grade 4
Anemia	3	2	
Neutropenia	1	1	2
Thrombocytopenia	1	3	
Atrial fibrillation		1	
Blurred vision	1		
Abdominal pain	1		
Constipation	4		
Diarrhea	1		
Nausea/vomiting	4		
Stomatitis	1		
Asthenia		1	
Fatigue	3	2	
Pain	1	2	1[a]
Arthralgia	2	1[a]	
Myalgia	3		
Upper respiratory infection	2		
Bronchitis	1		
Pneumonia without neutropenia		1[b]	
Dyspnea	2	1[b]	
Hypoxia		1[b]	
Urinary tract infection	1		
Anorexia	3		
Dehydration	2		
Cerebral hemorrhage			1[c]
Peripheral neuropathy	2	1	
Somnolence	1		
Syncope		1	
Alopecia	5		
Rash	1	1	
Lymphoedema	1		

[a]These symptoms occurred in the same patient at the same time
[b]These respiratory system-related symptoms occurred in the same patient at the same time
[c]Bleeding occurred while thrombocytopenic at the site of previously resected brain metastases, although RPI.4610 was discontinued 13 days before the event

4 toxicities were neutropenia (three patients), thrombocytopenia (three patients), anemia (two patients), pain (three patients, although none of them localized to the injection site), and fatigue (two patients). No patient had severe infection or sepsis during neutropenia.

Four patients continued on RPI.4610 alone for more than 200 days following completion of four cycles of carboplatin, paclitaxel, and RPI.4610. Grade 2–4 toxicities observed during RPI.4610 monotherapy included fatigue (one patient, grade 2); pain (one patient, grade 4); and rash (one patient, grade 3). The patient who developed grade 4 pain on RPI.4610 monotherapy had transitional cell carcinoma of the renal pelvis and ureter. RPI.4610 was interrupted for 2 weeks during which time the pain resolved. RPI.4610 was reinitiated and the pain did not recur. RPI.4610 was interrupted for 10 days in one patient who developed grade 3 rash and, upon resolution, was resumed without problems.

Administration of RPI.4610 did not result in changes in PT/PTT. No patient developed a thromboembolic event during the study. One patient developed cerebral hemorrhage while thrombocytopenic at the site of previously resected brain metastases. RPI.4610 had been discontinued 13 days before the event. Because of timing of the event and the short half-life of RPI.4610 (209 min), this was considered not related to RPI.4610.

Pharmacokinetic studies

Protocol-specified pharmacokinetic samples were obtained from six patients and were used to determine pharmacokinetic interactions between RPI.4610 and the carboplatin/paclitaxel combination. Delays between chemotherapy and RPI.4610 administration on day 1 of course 2 rendered the data from the other six patients unreliable. The pharmacokinetic parameters *C*max and AUC_{0-last} (mean ± SD) for each of the drugs and the means for the ratio of monotherapy to combination therapy for *C*max and AUC_{0-last} are listed in Table 3.

Effect of RPI.4610 administration on plasma pharmacokinetics of carboplatin and paclitaxel

The carboplatin and paclitaxel concentration versus time curves are presented in Figs. 3 and 4, respectively. The pharmacokinetic analyses for carboplatin and paclitaxel (CP) are presented in Table 3. When carboplatin pharmacokinetic parameters were compared for the combination CP alone versus CP plus RPI.4610, no statistical difference was observed for *C*max ($P = 0.817$) and AUC_{0-last} ($P = 0.990$). Similarly, when paclitaxel pharmacokinetic parameters were compared for the combination CP alone versus CP plus RPI.4610, no significant difference was observed ($P = 0.101$ for *C*max,

Table 3 Pharmacokinetics of carboplatin, paclitaxel, and RPI.4610

Drug	Treatment	*C*max (μg mL^{-1} h; mean ± SD)	AUC_{0-last} (μg mL^{-1} h; mean ± SD)	Ratio of the means (90% CI)	
Carboplatin	(CP without RPI)	52.3 ± 17.4	149.0 ± 36.2	*C*max (without RPI)/*C*max (with RPI)	1.07 (0.77–1.37)
	(CP with RPI)	50.5 ± 11.3	149.2 ± 30.1	AUC (without RPI)/AUC (with RPI)	1.04 (0.73–1.35)
Paclitaxel	(CP without RPI)	4.6 ± 1.5	14.0 ± 4.8	*C*max (without RPI)/*C*max (with RPI)	1.17 (1.03–1.31)
	(CP with RPI)	3.9 ± 1.4	11.8 ± 3.4	AUC (without RPI)/AUC (with RPI)	1.17 (1.04–1.30)
RPI.4610	(RPI.4610 without CP)	3.4 ± 1.4	28.3 ± 13.2	*C*max (without CP)/*C*max (with CP)	1.19 (0.65–1.72)
	(RPI.4610 with CP)	3.2 ± 1.4	30.3 ± 9.8	AUC (without CP)/AUC (with CP)	0.90 (0.52–1.28)

RPI RPI.4610, *CP* carboplatin/paclitaxel

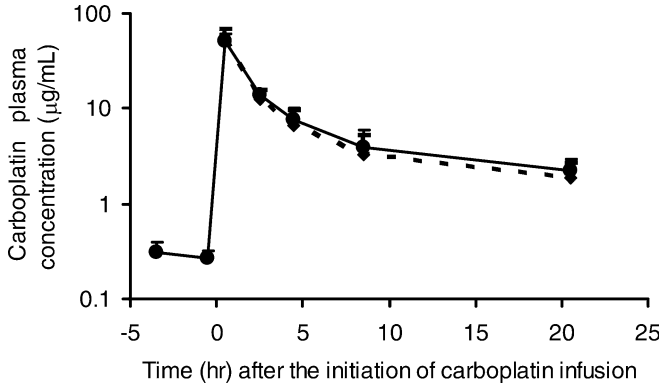

Fig. 3 Concentration of carboplatin in patients receiving carboplatin/paclitaxel monotherapy (*broken line*) or carboplatin/paclitaxel plus RPI.4610 combination therapy (*solid line*). Data presented are mean ± SD of results from six patients

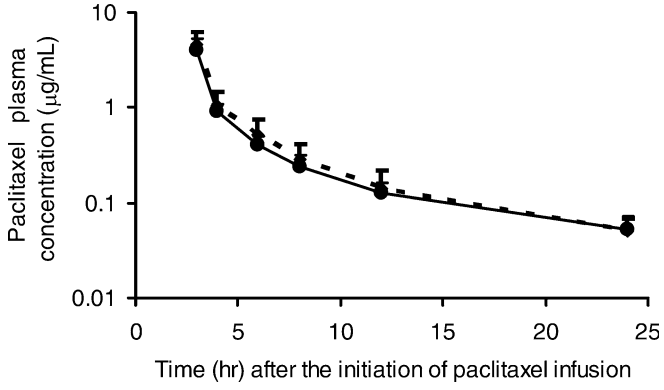

Fig. 4 Concentration of paclitaxel in patients receiving carboplatin/paclitaxel monotherapy (*broken line*) or carboplatin/paclitaxel plus RPI.4610 combination therapy (*solid line*). Data presented are mean ± SD of results from six patients

and $P = 0.060$ for AUC_{0-last}). Although these values approach statistical significance, the 15% relative difference between the mean values for both *C*max and AUC are not felt to be clinically significant. The ratio of the mean carboplatin *C*max values [(*C*max without RPI.4610)/(*C*max with RPI.4610)] was 1.07 (90% CI, 0.77–1.37), and the ratio of the mean AUC_{0-last} values was 1.04 (90% CI, 0.73–1.35). For paclitaxel the ratio of the mean *C*max values was 1.17 (90% CI, 1.03–1.31) and the ratio of the mean AUC_{0-last} values was 1.17 (90% CI 1.04–1.30). On the basis of these values the pharmacokinetics of carboplatin and paclitaxel do not appear to be substantially affected by RPI.4610.

Effect of carboplatin and paclitaxel administration on plasma pharmacokinetics of RPI.4610

Concentrations of RPI.4610 in patients receiving RPI.4610 monotherapy or CP plus RPI.4610 combination therapy are presented in Table 3 and depicted in Fig. 5. No statistical difference was observed between

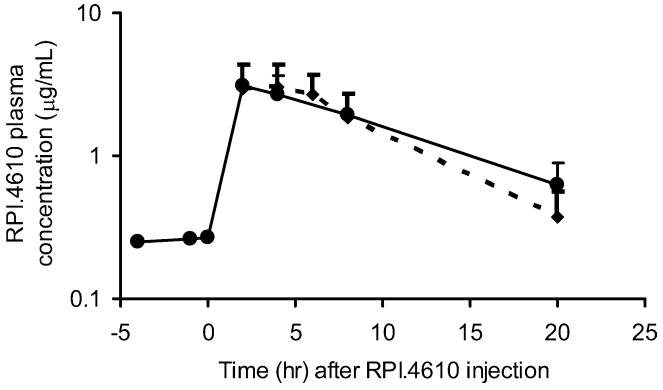

Fig. 5 Concentration of RPI.4610 in patients receiving RPI.4610 monotherapy (*broken line*) or carboplatin/paclitaxel plus RPI.4610 combination therapy (*solid line*). Data presented are mean ± SD of results from six patients

*C*max and AUC_{0-last} of RPI.4610 when administered alone or in combination with CP ($P = 0.679$ for *C*max, $P = 0.581$ for AUC_{0-last}). The ratio of the mean *C*max values was 1.19 (90% CI, 0.65–1.72), and the ratio of the mean AUC_{0-last} values was 0.90 (90% CI, 0.52–1.28). These data are consistent with a lack of pharmacokinetic interaction between CP and RPI.4610 when RPI.4610 was administered as an s.c. bolus injection following a standard regimen of CP.

Antitumor effect

Objective tumor responses were observed in this trial and consisted of a complete response lasting more than 7 months in a patient with chemotherapy naïve metastatic bladder cancer, a partial response lasting more than 3 months in a patient with previously treated (cisplatin, paclitaxel, and fluorouracil) adenocarcinoma of the gastroesophageal junction, and stable disease in three patients with thymoma, lung cancer, and uterine cervical cancer lasting between 2 months and 12+ months.

Discussion

Inhibition of neovascularization driven by VEGF-mediated receptor signaling is a promising approach to cancer treatment. In addition to multiple preclinical models, the benefit of this combined approach has recently been demonstrated for an anti-VEGF monoclonal antibody, bevacizumab, in combination with irinotecan, 5-FU, and leucovorin (IFL) chemotherapy in a phase III clinical trial for patients with metastatic colorectal cancer [8]. It is biologically, pharmacologically and clinically important to evaluate pharmacokinetic interactions and the spectrum and frequency of clinical adverse events when anti-VEGF therapy is combined with conventional chemotherapy.

The present study demonstrated there were no appreciable pharmacokinetic interactions between the anti-VEGFR-1 ribozyme RPI.4610 and carboplatin/paclitaxel. The *C*max and AUC_{0-last} of carboplatin and paclitaxel appeared to be relatively unaltered by coadministration of RPI.4610. The difference between AUC_{0-last} for paclitaxel with or without RPI.4610 was only 2.2 μg mL^{-1} h, a 15% relative difference. Although this approached statistical significance, it is considered not to be clinically significant. The pharmacokinetic parameters of RPI.4610 were also not significantly altered by coadministration of carboplatin/paclitaxel. RPI.4610 is a nucleic acid-based agent and has multiple possible guanine sites for platinum adduct formation by carboplatin; however, this clinical study showed no relevant pharmacokinetic interactions between carboplatin and RPI.4610. The result is comparable with preclinical observations which showed no significant pharmacokinetic interactions between carboplatin and RPI.4610 and no DNA adduct formation in vivo [9].

These results are consistent with studies involving other nucleic acid-based agents. For example, the pharmacokinetics of a bcl-2 antisense oligodeoxynucleotide was not altered by coadministration of carboplatin and etoposide [16]. The pharmacokinetics of an H-ras antisense oligodeoxynucleotide was also not altered by coadministration of gemcitabine [1]. The result is also comparable with data demonstrating there are no pharmacokinetic interactions between bevacizumab and a variety of chemotherapeutic agents [10].

Drug interaction studies can use a randomized crossover, a one-sequence crossover, or a parallel design. A randomized crossover has been considered ideal. We, however, used a one-sequence crossover in the present study because the chances of a carryover effect between treatment cycles were considered minimal. Indeed, for carboplatin and RPI.4610, concentrations were measured just before the next administration and they were found to be negligible. In a previous study of paclitaxel, no significant change in pharmacokinetics was observed in weekly administrations of paclitaxel [2].

In human single agent clinical phase I and I/II studies, RPI.4610 was well tolerated at doses up to 300 mg m^{-2} administered i.v. or s.c. Once-daily s.c. administration resulted in prolonged plasma levels with ($t_{1/2} = 209$ min) [17]. The principal toxicity of RPI.4610 was a mild injection-site reaction. In the present study in which RPI.4610 was administered with carboplatin/paclitaxel, the most common grade 3–4 toxicities were neutropenia (three patients, 25%), thrombocytopenia (three patients, 25%), pain (three patients, 25%), anemia (two patients, 16.7%) and fatigue (two patients, 16.7%). The spectrum, frequency, and severity of these toxicities appear to be similar to those observed as a result of administration of carboplatin/paclitaxel alone. In one such study that utilized doses of carboplatin/paclitaxel similar to those used in this study, the most common grade 3–4 toxicities were neutropenia (62.9%), thrombocytopenia (14.8%), anemia (11.4%), alopecia (44.4%), neuropathy (7.4%), nausea/vomiting (7.4%), and mucositis (7.4%) [14]. These findings suggest that RPI.4610 100 mg m^{-2} day^{-1} did not add appreciably to the rate or severity of toxicities observed with paclitaxel 175 mg m^{-2} and carboplatin AUC 6. Although one patient developed grade 3 rash and another developed grade 4 pain during single agent RPI.4610, these episodes were transient and did not recur upon reintroduction of RPI.4610. Although these events are regarded as possibly related to RPI.4610, this suggests the events may, at least in part, have been attributable to the underlying tumor or other, unrecognized, intercurrent events. Other less common and/or less serious adverse events, such as alopecia, peripheral neuropathy, nausea/vomiting, or fatigue, occurred with a frequency that did not seem to be greater than what might be expected from carboplatin/paclitaxel alone.

No thromboembolic events were observed in this study. This is in contrast to a phase II trial in which RPI.4610 was administered in combination with IFL chemotherapy to patients with metastatic colorectal cancer. In that study, deep vein thrombosis/pulmonary embolism was reported in 19 of 83 patients (23%) [20]. Although 16% incidence of thromboembolic events was reported in patients receiving IFL alone for advanced colorectal cancer [8], it cannot be ruled out that there may be a biological, if not pharmacological interaction between RPI.4610 and specific drugs, and/or clinical situations. Also, we cannot exclude the possibility that adverse pharmacokinetic or pharmacodynamic effects may occur at higher dose levels of RPI.4610 or carboplatin or paclitaxel. Further studies to explore the interaction of selected chemotherapeutic agents and RPI.4610 on the coagulation cascade and/or endothelial cells are warranted.

Although antitumor response was not a primary objective of the present study, tumor responses were observed and consisted of one complete response, one partial response and three patients with disease stabilization.

Because long-term therapy with angiogenesis inhibitors may be necessary to achieve optimum tumor control, it is important to determine if an angiogenesis inhibitor can be administered without cumulative toxicity. In this study no apparent cumulative toxicity occurred during RPI.4610 monotherapy and four patients continued on RPI.4610 for more than 200 days. Furthermore, nucleic acid-based agents seem to be suitable for long-term therapy in terms of lower cost and immunogenicity than antibody-based agents. The lack of pharmacological interaction with chemotherapeutic drugs, the unlikelihood of cumulative toxicity, and encouraging tumor responses observed in this study make RPI.4610 an attractive candidate for further clinical evaluation in combination with carboplatin/paclitaxel.

Acknowledgements This trial was supported by Sirna Therapeutics, Chiron, and NIH Grant RR00095. We thank Drs Jordan D. Berlin

and Bruce J. Roth (Vanderbilt-Ingram Cancer Center), Dr Andrew S. Pierson and Martha S. Persky, R.N. (University of Colorado Cancer Center) for managing all clinical aspects of some study patients. We are also grateful to Drs Gary S. Gordon and Vann P. Parker for their help in performing pharmacokinetic analyses.

References

1. Adjei AA, Dy GK, Erlichman C et al (2003) A phase I trial of ISIS 2503, an antisense inhibitor of H-ras, in combination with gemcitabine in patients with advanced cancer. Clin Cancer Res 9:115–123
2. Akerley W, Herndon JE, Egorin MJ et al (2003) Weekly, high-dose paclitaxel in advanced lung carcinoma: a phase II study with pharmacokinetics by the Cancer and Leukemia Group B. Cancer 97:2480–2486
3. Allain P, Berre S, Mauras Y, Le Bouil A (1992) Evaluation of inductively coupled mass spectrometry for the determination of platinum in plasma. Biol Mass Spectrom 21:141–143
4. Bamberger ES, Perrett CW (2002) Angiogenesis in epithelian ovarian cancer. Mol Pathol 55:348–359
5. Bauer J, Morrison B, Oates R et al (2003) ANGIOZYME and interferon-α2b synergistically inhibit tumor angiogenesis. Proc Am Assoc Cancer Res 44 (Abstr 1159)
6. Buoen C, Holm S, Thomsen MS (2003) Evaluation of the cohort size in phase I dose escalation trials based on laboratory data. J Clin Pharmacol 43:470–476
7. Herbst RS, Takeuchi H, Teicher BA (1998) Paclitaxel/carboplatin administration along with antiangiogenic therapy in non-small-cell lung and breast carcinoma models. Cancer Chemother Pharmacol 41:497–504
8. Hurwitz H, Fehrenbacher L, Novotny W et al (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335–2342
9. Jackson E, Sweedler D, Hartsough K et al (2002) Assessment of pharmacokinetic drug interactions between carboplatin and ANGIOZYME in vivo. Proc Am Assoc Cancer Res 43 (Abstr 1927)
10. Margolin K, Gordon MS, Holmgren E et al (2001) Phase Ib trial of intravenous recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. J Clin Oncol 19:851–856
11. Meert AP, Paesmans M, Martin B et al (2002) The role of microvessel density on the survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 87:694–701
12. Parry TJ, Cushman C, Gallegos AM et al (1999) Bioactivity of anti-angiogenic ribozymes targeting Flt-1 and KDR mRNA. Nucleic Acids Res 27:2569–2577
13. Pavco PA, Bouhana KS, Gallegos AM et al (2000) Antitumor and antimetastatic activity of ribozymes targeting the messenger RNA of vascular endothelial growth factor receptors. Clin Cancer Res 6:2094–2103
14. Pivot X, Cals L, Cupissol D et al (2001) Phase II trial of a paclitaxel-carboplatin combination in recurrent squamous cell carcinoma of the head and neck. Oncology 60:66–71
15. Royer I, Alvinerie P, Armand JP et al (1995) Paclitaxel metabolites in human plasma and urine: identification of 6 alpha-hydroxytaxol, 7-epitaxol and taxol hydrolysis products using liquid chromatography/atmospheric-pressure chemical ionization mass spectrometry. Rapid Commun Mass Spectrom 9:495–502
16. Rudin CM, Kozloff M, Hoffman PC et al (2004) Phase I study of G3139, a bcl-2 antisense oligonucleotide, combined with carboplatin and etoposide in patients with small-cell lung cancer. J Clin Oncol 22:1110–1117
17. Sandberg JA, Parker VP, Blanchard KS et al (2000) Pharmacokinetics and tolerability of an antiangiogenic ribozyme (ANGIOZYME) in healthy volunteers. J Clin Pharmacol 40:1462–1469
18. Sandberg JA, Sproul CD, Blanchard KS et al (2000) Acute toxicology and pharmacokinetic assessment of a ribozyme (ANGIOZYME) targeting vascular endothelial growth factor receptor mRNA in the cynomolgus monkey. Antisense Nucleic Acid Drug Dev 10:153–162
19. U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research (1999) In vivo drug metabolism/drug interaction studies—study design, data analysis, and recommendations for dosing and labeling
20. Venook A, Hurwitz H, Cunningham C et al (2003) Relationship of clinical outcome in metastatic colorectal carcinoma to levels of soluble VEGFR-1: results of a phase II trial of a ribozyme targeting the pre-mRNA of VEGFR-1 (angiozyme) in combination with chemotherapy. Proc Am Soc Clin Oncol 22 (Abstr 1025)
21. Weng DE, Weiss P, Kellackey C et al (2001) Angiozyme pharmacokinetic and safety results: a phase I/II study in patients with refractory solid tumors. Proc Am Soc Clin Oncol 20 (Abstr 393)