A phase I study of rebeccamycin analog in combination with oxaliplatin in patients with refractory solid tumors

Charles J. Nock · Joanna M. Brell · Joseph A. Bokar · Matthew M. Cooney · Brenda Cooper · Joseph Gibbons · Smitha Krishnamurthi · Sudhir Manda · Panayiotis Savvides · Scot C. Remick · Percy Ivy · Afshin Dowlati

Received: 30 June 2009 / Accepted: 2 September 2009 / Published online: 23 September 2009
© Springer Science + Business Media, LLC 2009

Summary Rebeccamycin analog (RA) is an antitumor antibiotic with both topoisomerase I and II inhibiting activity. Topoisomerase inhibitors have demonstrated synergy with platinum agents. We performed a phase I trial of combination RA with oxaliplatin in patients with refractory solid tumors. RA was administered as a 1-hour infusion daily on days 1–5 with oxaliplatin administered on day 5. Cycles were repeated every 21 days. A total of 17 patients were enrolled. The MTD for RA was 80 mg/m²/d for five days along with oxaliplatin 130 mg/m² on day 5. Myelosuppression was a common occurrence but was mild except in one instance. Dose limiting toxicities included atrial fibrillation and hypophosphatemia. There was evidence of antitumor activity including 3 partial responses in patients with esophageal, gallbladder and hepato-cellular carcinoma; 5 additional patients had stable disease. Thus, the combination of RA and oxaliplatin is both tolerable and has evidence of clinical activity, but given the lack of significant activity for

single agent RA across a variety of disease sites, it is unlikely to proceed to phase II development.

Keywords Phase I · Rebeccamycin · Oxaliplatin · Solid tumors · Rebeccamycin analog · Becatecarin

Introduction

Rebeccamycin analog (RA, Bectecarin; (1,11-Dichloro-6 [2-(diethylamino)ethyl]-12,13-dihydro-12-(4-O-methyl-beta-D-glucopyranosyly)-5H-indolo[2,3-a]pyrolo[3,4-c]-carbazole-5,7(6H)-dione), or NSC 655649, is an antibiotic with antitumor properties originally isolated from the actinomycete strain *Saccharothrix aerocolonigenes* [1, 2]. Preclinical studies indicate RA has activity against P388 leukemia, L1210 leukemia, B16 melanoma, M5076 reticulum cell sarcoma and M109 lung carcinoma [3, 4] cell lines. The exact mechanism of RA is unknown. However, its parent compound, rebeccamycin, acts as an intercalating agent leading to inhibition of topoisomerase II, and inducing breaks in eukaryotic DNA. Recent reports also suggest rebeccamycin can inhibit topoisomerase I [4–6].

Two phase I trials of a single infusion of RA in patients with solid tumors have been completed [7, 8]. In the study done by the San Antonio group [7], the MTD was 500 mg/m² given q 21 days in patients who were previously treated. The dose limiting toxicity (DLT) was grade 4 neutropenia. However, above a dose of 400 mg/m², all patients developed grade 2 phlebitis, which improved by increasing the duration and volume of infusion. In the other phase I study performed at the University of Wisconsin [8], the MTD was established at 572 mg/m² with the dose limiting toxicity being local irritation and phlebitis. A phase I study of RA performed at Case Western Reserve University was done using the

Supported in part by grants U01 CA62502, MO1 RR00080 and K23 CA109348.

C. J. Nock · J. M. Brell · J. A. Bokar · M. M. Cooney · B. Cooper · J. Gibbons · S. Krishnamurthi · S. Manda · P. Savvides · S. C. Remick · P. Ivy · A. Dowlati
Case Western Reserve University,
Cleveland, OH, USA

C. J. Nock · J. M. Brell · J. A. Bokar · M. M. Cooney · B. Cooper · J. Gibbons · S. Krishnamurthi · S. Manda · P. Savvides · S. C. Remick · P. Ivy · A. Dowlati (✉)
University Hospitals Case Medical Center,
11100 Euclid Avenue,
Cleveland, OH 44106, USA
e-mail: afshin.dowlati@case.edu

P. Ivy
National Cancer Institute,
Bethesda, MD, USA

daily for 5 consecutive day schedule [9]. The rationale for exploring this schedule was both the occurrence of significant superficial thrombophlebitis at higher doses on the once every 3 week schedule and preclinical experiments showing schedule dependency favoring repetitive dosing [5]. In our study, the MTD for heavily pretreated patients was higher than the 2 prior studies at 705 mg/m² (141 mg/m²/d×5 days) with DLT being grade 4 neutropenia [9]. This trial also defined the MTD in chemotherapy naive patients at 825 mg/m² divided over 5 days [9]. Several responses have been seen including three partial responses in gastric, unknown primary and gallbladder cancers as well as a minor response in pancreatic cancer. In terms of antitumor activity, our trial (CWRU) demonstrated a significantly greater number of responses compared to the other 2 phase I trials in which RA was given on day 1 only. Furthermore, plasma concentrations achieved were comparable to those required for *in vitro* inhibition of topoisomerase II levels. In the day 1 only studies, the level remained in this therapeutic range for 2 days, but in our study of the 5 day schedule, the plasma concentration remained within this range for >6 days [10].

Oxaliplatin (*cis*-[(1*R*,2*R*)-1,2-cyclohexanediamine-*N,N'*] oxalato (2-)-*O,O'*] platinum is a novel platinum coordination complex approved for the treatment of metastatic colorectal carcinoma in combination with fluoropyrimidines. Oxaliplatin is more potent than cisplatin *in vitro*, requiring fewer DNA adducts to achieve equivalent cytotoxicity [11]. Oxaliplatin has demonstrated efficacy in preclinical studies against a broad spectrum of experimental tumors, including some cisplatin- and carboplatin-resistant cell lines. The impetus for combining oxaliplatin and RA stems from: (1) phase I data suggesting possible synergy between cisplatin and RA [12]; (2) non-overlapping toxicities of these 2 agents; and (3) early evidence of activity for both RA and oxaliplatin in biliary cancers [10, 13].

Patients and methods

Eligibility

Enrolled patients were required to have a histologically confirmed solid tumor that was not amenable to conventional surgical, radiation, or chemotherapy treatment programs. Prior chemotherapy and/or radiation were allowed, however, at least 4 weeks must have elapsed since prior large-field radiation therapy, and at least 3 weeks for any previous anti-cancer therapy. Eligibility criteria included (1) age≥18; (2) Eastern Cooperative Oncology Group performance status≤2; (3) life expectancy≥12 weeks; (4) adequate hematopoietic

function (ANC≥1500/μL, platelet count≥100,000/μL, hemoglobin≥9.0 g/dL), hepatic function (total bilirubin<1.5 mg/dL, AST<2.5 times the upper limit of normal), and renal function (creatinine<1.5 mg/dL or calculated creatinine clearance≥60 mL/min/1.73 m²). A central line was required for drug administration. All patients gave informed written consent prior to treatment according to federal and institutional guidelines.

Study design, dosage, and drug administration

RA was supplied by the Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute (NCI), (Bethesda, MD) as a sterile vial containing 20 mL of a solution of 10 mg/mL rebaccamycin analogue and 2.24 mg/mL (1 meq/mL) of L-tartaric acid in sterile water for injection. RA was completely soluble and was mixed with 250–500 mL normal saline. RA was given through a central line over 1 hour daily for 5 days, with oxaliplatin administered over 2 h immediately after RA on day 5 of each cycle. Cycles were repeated every 3 weeks. Patients were treated until disease progression or unacceptable toxicity occurred. Oxaliplatin was supplied in two distinct dosage forms: lyophilized sterile powder or sterile solution. Both formulations were further diluted in D5W prior to administration.

The dose escalation schema is depicted in Table 1. Dosing was based on the documented safety shown in prior phase I trials [7–9]. At least three patients were treated at each dose level. Dose escalation occurred if no DLT was experienced by a minimum of three patients. If one out of the three patients experienced a DLT, at least three additional patients were treated at the same dose level. The DLT was reached when any grade 3 nonhematologic toxicity or grade 4 hematologic toxicity was observed, during cycle one only, in two or more patients at the set dose level. The MTD was defined as the highest dose tested in which none or only one patient experienced a DLT attributable to the study drug combination, when at least six patients were treated at that dose, or one dose level below the highest dose tested in which 2 or more patients experienced a DLT attributable to the study drug. At least six patients were required to be treated at the MTD.

Table 1 Dose escalation schema

Dose level	Rebeccamycin analog (mg/m ² /d)	Oxaliplatin (mg/m ²)
Level 1	80	90
Level 2	80	130
Level 3	110	130

Toxicities were evaluated according to the NCI common toxicity criteria, version 2.0.

Pretreatment and follow-up studies

Baseline evaluations including patient history, physical examination, and routine laboratory studies, were conducted within 2 weeks prior to start of protocol therapy. Baseline radiographic tests were done within 4 weeks prior to start of therapy. Routine laboratory studies included a complete blood cell count, chemistries and liver function tests, and were performed weekly. Physical exams were performed prior to each cycle. Disease burden was formally measured before treatment and every two cycles using standard radiographic procedures. A complete response was defined as disappearance of all disease on two measurements separated by a minimum of 4 weeks. RECIST criteria were used for response definition. Pharmacokinetic sampling was done on a limited basis but not reported herein.

Results

Between June 2004 and September 2006 eighteen patients were enrolled. One patient signed consent but was never treated. Patient characteristics of the 17 patients are listed in

Table 2 Patient characteristics

Characteristics	No. of patients
Total patients assessable:	17
No. of courses per patient:	
Median	3.9
Range	1–8
Age, years:	
Median	56
Range	31–81
Sex:	
Male	9
Female	8
Performance Status:	
0	10
1	6
2	1
Tumor Types:	
Esophagus	3
Pancreas	4
Gallbladder/Cholangiocarcinoma	5
Renal	1
Small Cell Lung	2
Unknown Primary	1
Prostate	1

Table 3 Non-hematological toxicities (all cycles)

Dose Level	RA Dose mg/m ² /d	Oxaliplatin Dose mg/m ²	No pts	AST/ALT				Fatigue				Diarrhea				Vomiting				Atrial Fibrillation				Low phosphate				Low sodium			
				1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	80	90	8	2				4	1			1					2	1													
2	80	130	6	5				2	2			2	1	1			1	1													
3	110	130	3	1				1				2	1								1				1						

Table 4 Hematological toxicities (cycle 1 only)

Toxicity (no. of patients)															
Dose Level	Rebeccamycin dose mg/m2/d	Oxaliplatin dose mg/m2	No. of patients	ANC Grade				Platelet Grade				Anemia Grade			
				1	2	3	4	1	2	3	4	1	2	3	4
1	80	90	8	4	1		1				1	2	1		1
2	80	130	6	4				3				1		1	
3	110	130	3		2	1								2	

Table 2. Fifteen patients completed 1 cycle of therapy and were followed for at least 4 weeks. Of those not assessable for response but assessable for toxicity, one patient suffered grade 4 myelosuppression during the first cycle and subsequently died. This patient was on dose level 1 and this dose level was subsequently expanded with no further significant toxicities. A second patient developed grade 4 atrial fibrillation during cycle 1 one on dose level 3 (DLT) and was taken off study. Fifteen patients received a total of 56 cycles of therapy. The median number of cycles given was 3.9 (range, 1–8). All but one patient had a performance status of 0 or 1.

Nonhematologic toxicity

Grade 4 atrial fibrillation occurred in one of three patients at dose level 3 (RA 110 mg/m²/d, oxaliplatin 130 mg/m²) (cycle 1 – DLT). Over 70% of patients experienced grade 1 nausea, and nearly 50% had grade 1 fatigue (see Table 3). Electrolyte imbalances were seen including one grade 3 hyponatremia and one grade 3 hypophosphatemia (cycle 1 – DLT) requiring dose-reduction. Grade 3 diarrhea occurred twice, with one patient experiencing subsequent grade 3 hypokalemia.

Hematologic toxicity

Hematologic toxicities were common (see Table 4), including one patient with grade 4 and three with grade 3 neutropenia

(1 occurred during cycle 1 and two others in subsequent cycles). The patient with grade 4 neutropenia died of sepsis during cycle one. In addition, one patient experienced grade 4 thrombocytopenia requiring dose-reduction, and one patient developed grade 3 anemia.

Efficacy

All responses were reviewed by the Cancer Center Data Safety and Toxicity Committee. Three patients had partial responses with mean time to progression of 3.7 months. One PR was seen at each dose level. Five patients had stable disease. Of those five, the average number of cycles was six. No one tumor type showed a trend toward response (see Table 5).

Discussion

Based on preclinical synergy between platinum agents and topoisomerase inhibitors we embarked on phase I trial of RA and oxaliplatin. Another rationale was that both agents had demonstrable single agent activity in biliary cancers. In our study 3 DLTs were seen including 1 grade 4 myelosuppression and death from infection. This event was definitely felt to be related to study procedure. Since this occurred during dose level 1, the level was expanded with no further DLTs and dose escalation continued. The

Table 5 Clinical efficacy

Response	Tumor type	Dose level	Time to progression
Partial response	Cholangiocarcinoma	1	4.5
	Gallbladder	2	4
	Esophagus	3	3
Stable disease	Pancreas	1	6
	Renal Cell	1	3
	Pancreas	2 ^a	3
	Cholangiocarcinoma	2	4.5
	Prostate	3 ^b	5.5

^a RA dose reduced with cycle 3

^b Oxaliplatin dose reduced with cycles 2 and 3

attribution of the other 2 DLT's, i.e. hypophosphatemia and atrial fibrillation were considered to be possibly related to study procedure and both occurred at dose level 3. Although it is possible that the occurrence of these two toxicities may not have been drug-related, given the fact that the dose of oxaliplatin used was at its standard dose on a q 3 week basis, dose escalation was halted. Thus, the MTD was dose level two, with RA at 80 mg/m²/d and oxaliplatin at 130 mg/m². In all, six patients were treated at this dose level, and no grade 3 or 4 toxicities were observed in this group. As seen in previous phase I trials, transient benign ALT/AST elevations were seen, as well as mild nausea. Fatigue was common, but not dose-limiting. Electrolyte imbalances were more frequent in this combination regimen compared to the single agent studies, especially after dose level one, which likely is more attributable to oxaliplatin than RA. Superficial phlebitis did not occur as seen in previous trials due to the decision to place central venous access prior to trial initiation.

Concerning anti-tumor activity, three partial responses were achieved. Five patients had stable disease. No one tumor type or dose level showed a trend toward response. However, over half the patients received at least 4 cycles of therapy, with three patients receiving greater than six. The combination of oxaliplatin and other topoisomerase I or II inhibitors has been reported. In three separate phase I studies, oxaliplatin has been combined with topotecan [14–16]. In all three studies oxaliplatin was infused prior to administration of topotecan and the recommended phase I dose of oxaliplatin in these combination studies was similar to our trial. In addition the doses of topotecan were less than its single agent dose. This is concordance with our trial where the doses of RA were reduced compared to its single agent dose. Clinical activity was again seen across all studies. A single phase I pediatric solid tumor trial has looked at the combination of oxaliplatin and a topoisomerase II inhibitor, etoposide [17]. Given the activity seen in this pediatric study further studies were recommended.

Our previous phase I work with RA as a single agent achieved dose levels of 141 mg/m²/d × 5 days (705 mg/m² total dose) in patients with prior therapy, and 165 mg/m²/d × 5 days (825 mg/m² total dose) for chemotherapy-naïve patients [9]. In the current study, the recommended phase II dose of RA, when combined with oxaliplatin, is notably lower at 80 mg/m²/d × 5 days (400 mg/m² total dose). Despite the proposed synergistic effect of oxaliplatin and RA and evidence of antitumor activity, phase II trials of RA across a number of disease sites have failed to disprove the null hypotheses (although responses were seen in non-small cell lung cancer, breast cancers, leukemia, small cell lung and biliary cancers) as set forward in those trials. Thus, this

regimen is unlikely to proceed to further phase II studies. Nevertheless, promising responses were seen in cholangiocarcinoma, gallbladder cancer and esophageal cancer. In these 3 diseases oxaliplatin is an active agent and thus it is difficult to attribute the efficacy to RA.

References

1. Kaneko T, Wong H, Okamoto KT et al (1985) Two synthetic approaches to rebeccamycin. *Tetrahedron Lett* 26:4015–4018
2. Nettledone DE, Doyle W, Krishnan B et al (1985) Isolation and structure of rebeccamycin — a new antitumor antibiotic from *Nocardia aerocolonigenes*. *Tetrahedron Lett* 26:4011–4014
3. Kaneko T, Wong H, Utzig J et al (1990) Water soluble derivatives of rebeccamycin. *J Antibiotics* 43:125–127
4. Bush JA, Long BH, Catino JJ et al (1987) Production and biological activity of rebeccamycin, a novel antitumor agent. *J Antibiot* 40:668–678
5. National Cancer Institute, Division of Cancer Treatment, Investigators Brochure Rebeccamycin Analog NSC 655649 (July 1999) Bethesda, Maryland
6. Bailly C, Riou JF, Colson P et al (1997) DNA cleavage by topoisomerase I in the presence of indolocarbazole derivatives of rebeccamycin. *Biochem* 36:3917–3929
7. Tolcher AW, Eckhardt SG, Kuhn J et al (2001) Phase I and pharmacokinetic study of NSC 655649, a rebeccamycin analog with topoisomerase inhibitory properties. *J Clin Oncol* 19:2937–4710
8. Cleary JF, Berlin JD, Tutsch KD et al (1997) Phase I clinical and pharmacokinetic study of rebeccamycin analog (NSC 655649). *Proc Am Soc Clin Oncol* 16:217a
9. Dowlati A, Hoppel CL, Ingalls ST et al (2001) Phase I clinical and pharmacokinetic trial of rebeccamycin analog given daily for 5 consecutive days. *J Clin Oncol* 19:2309–2318
10. Dowlati A, Posey J, Ramanathan RK, et al (2009) Phase II and pharmacokinetic trial of rebeccamycin analog in advanced biliary cancers. *Cancer Chemoth and Pharmacol*. Apr 28. [Epub ahead of print]
11. Culy CR, Clemett D, Wiseman LR (2000) Oxaliplatin A review its pharmacological properties and clinical efficacy in metastatic colorectal cancer and its potential in other malignancies. *Drugs* 60:895–924
12. Ricart AD, Hammond LA, Kuhn JG et al (2005) Phase I and Pharmacokinetic Study of Sequences of the Rebeccamycin Analogue NSC 655649 and Cisplatin in Patients with Advanced Solid Tumors. *Clin Cancer Res* 11:8728–8736
13. Becouarn Y, Agostini C, Truffandier N et al (2001) Oxaliplatin: available data in non-colorectal gastrointestinal malignancies. *Crit Rev Oncol Hematol* 40:265–272
14. Hochster H, Chen TT, Lu JM et al (2008) Tolerance and activity of oxaliplatin with protracted topotecan infusion in patients with previously treated ovarian cancer. *Gynecol Oncol* 108:500–504
15. Elkas JC, Winter WE, Chernofsky MR et al (2007) A phase I trial of oxaliplatin and topotecan in recurrent ovarian cancer. *Gynecol Oncol* 104:422–427
16. Gross-Goupil M, Lokiec F, Lopez G et al (2002) Topotecan preceded by oxaliplatin using a 3 week schedule: a phase I study in advanced cancer patients. *Eur J Cancer* 38:1888–1898
17. McGregor LM, Spunt SL, Santana VM et al (2009) Phase I study of an oxaliplatin and etoposide regimen in pediatric patients with recurrent solid tumors. *Cancer* 115:655–664