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CLINICAL TRIAL REPORT

A phase I study of Triapine® in combination with doxorubicin in patients with advanced solid tumors

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Abstract
Purpose To assess the maximum-tolerated dose (MTD), dose-limiting toxicity (DLT), pharmacokinetics and antitumor activity of Triapine® administered in combination with doxorubicin.
Study design Patients were treated with doxorubicin intravenously (IV) on day 1 and Triapine® IV on days 1–4 of a 21-day cycle. The starting dose (level 1) was doxorubicin 60 mg/m^2 and Triapine® 25 mg/m^2. PK analysis was performed at various time-points before and after treatment.
Results Twenty patients received a total of 49 courses of treatment on study. At dose level 2 (doxorubicin 60 mg/m^2, Triapine® 45 mg/m^2), two patients experienced DLTs (febrile neutropenia, grade 4 thrombocytopenia). An additional three patients were enrolled at dose level 1 without initial toxicity. Enrollment then resumed at dose level 2a with a decreased dose of doxorubicin (45 mg/m^2) with Triapine® 45 mg/m^2. The two patients enrolled on this level had two DLTs (diarrhea, CVA). Enrollment was planned to resume at dose level 1; however, the sixth patient enrolled to this cohort developed grade 5 heart failure (ejection fraction 20%, pretreatment EF 62%) after the second course. Thus, doxorubicin and Triapine® were reduced to 45 and 25 mg/m^2, respectively (level 1a), prior to resuming enrollment at dose level 1, the MTD. The main drug-related toxicity was myelosuppression. Non-hematologic toxicities included mild-to-moderate fatigue, grade 3 diarrhea and grade 4 CVA. There was one treatment-related death due to heart failure. While no objective responses were observed, subjective evidence of clinical activity was observed in patients with refractory melanoma and prostate cancer.
Conclusions Pretreated patients with advanced malignancies can tolerate the combination of Triapine® and doxorubicin at doses that achieve subjective clinical benefit with the main treatment-related toxicities being myelosuppression and fatigue. The MTD was determined to be doxorubicin 60 mg/m^2 on day 1 and Triapine® 25 mg/m^2 on days 1–4 of a 21-day cycle.

Keywords Triapine® · 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone · Doxorubicin · Phase I · Ribonucleotide reductase

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Introduction

Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) is a potent small molecule ribonucleotide reductase (RR) inhibitor that exerts its antineoplastic activity by inhibiting DNA synthesis and repair [1]. RR is an enzyme that is important for cell division and tumor growth [2–4], and the human RR has a tetrameric structure, composed of two non-identical homodimers [5]. The RRM1 subunit contains a nucleotide binding site, and the RRM2 subunit contains a metal binding site. The RRM2 subunit is comprised

of a non-heme iron and a tyrosine free radical, which are required for the enzymatic reduction of ribonucleotides [6]. Therapeutic agents that inhibit RR include gemcitabine, fludarabine and hydroxyurea. While gemcitabine and fludarabine are not specific inhibitors of RR, they act, in part, by altering the binding of RRM1. Hydroxyurea, on the other hand, inhibits RRM2 subunit by destroying the free radical [7]. Triapine® belongs to the class of RRM2-affecting RR inhibitors that are derived from α-heterocyclic carboxaldehyde thiosemicarbazone (HCT), the most potent class of RR inhibitors [8, 9]. Triapine® is an iron chelator that acts by inhibiting the enzymatic activity and the tyrosyl radical of the RRM2 and p53R2 subunits of RR [10, 11]. It also causes DNA damage in the presence of H_2O_2 and iron. These two functions can combine synergistically resulting in cell cycle arrest and apoptosis.

Triapine® alone has anti-tumor effects, both in vitro and in vivo. In vitro activity has been observed in leukemia, non-small-cell lung cancer, renal cancer, and melanoma cell lines. In murine models, Triapine® displayed anti-tumor activity against L1210 leukemia and various solid tumors [12], and the activity of Triapine® was schedule dependent, with twice daily administration showing greater effects on tumor growth than once daily administration. Triapine® was also effective against subcutaneously implanted Madison 109 (M109) lung tumors and A2780 ovarian tumors, when given twice daily (approximately 8 h apart) intra-peritoneal for 5–6 days at 8–10 mg/(kg dose) (30 mg/m^2 per dose).

Recent phase 1 clinical trials have evaluated Triapine® in patients with advanced or metastatic solid tumors, using three schedules: a single 2-h IV infusion administered every 4 weeks [13], a 2-h infusion daily × 5 repeated every 4 weeks [14], and a 96-h infusion repeated every 2–3 weeks [15]. For the single IV infusion schedule, no DLTs were observed at doses up to 105 mg/m^2; however, no anti-tumor activity was observed, and emphasis in subsequent development was placed on multiple dose schedules. Maximum tolerated doses were established for the 2-h IV infusion daily × 5 repeated every 2–4 weeks schedule and for the 96-h IV infusion repeated every 2–3 weeks schedule. The major DLT on the 2-h infusion schedule was transient neutropenia, but there was substantially less toxicity when Triapine® was administered for 4 days rather than 5. In addition, the 2 h infusion of ≥140 mg/m^2 was associated with reversible hypotension, hypoxia, dyspnea, cough, and EKG changes including ST-T wave changes and prolongation of the QT interval. The 96-h IV infusion schedule appeared to result in more severe hepatic and renal toxicity (increases in bilirubin and creatinine, and decreases in serum bicarbonate) compared to the 2-h IV infusion schedule. Additional toxicities (across all schedules) included anemia, thrombocytopenia, hyperbilirubinemia, nausea, vomiting, fatigue, hypertension, dyspnea, elevated creatinine, and low serum bicarbonate. All adverse events were rapidly reversible. Clinical activity was observed in breast, esophageal, head and neck, uterine and prostate cancers and in non-Hodgkin's lymphoma. In addition, a patient with an islet cell tumor producing vasoactive intestinal peptide (VIP) had a reduction in the size of a liver lesion and circulating VIP levels.

Doxorubicin has several mechanisms of action [16]. Similar to other anthracyclines, it intercalates between base pairs in the DNA helix, resulting in the inhibition of chain elongation. It also acts as a topoisomerase II inhibitor. These effects ultimately lead to DNA double-strand breaks and cell death. RR inhibitors have been shown to potentiate the activity of DNA damaging agents by inhibiting DNA repair. Synergy between doxorubicin and Triapine® has been demonstrated in a study, where L1210 leukemia cells were injected into mice which were then treated with intraperitoneal Triapine®, doxorubicin or both [12], and animals receiving the combination had prolonged survival times when compared to either agent alone. Their complimentary and synergistic activities make Triapine® and doxorubicin an attractive combination for clinical evaluation.

Here, a phase 1 study was conducted to determine the safety and tolerability of Triapine® in combination with doxorubicin in patients with refractory solid tumors. In addition, pharmacokinetic analyses of Triapine® and doxorubicin were performed in plasma and erythrocytes.

Patients and methods

Patient selection

Eligible patients were over 18 years of age and had a histologically documented, advanced stage, primary or metastatic solid tumor, that was refractory to standard therapy or for which no curative standard therapy was available. Other inclusion criteria included: Eastern Cooperative Oncology Group performance status of 0–2; adequate bone marrow (WBC ≥3,000/μl, absolute neutrophil count ≥1,500/μl, platelet ≥100,000/μl); adequate hepatic function (total bilirubin within institutional normal limit and alanine aminotransferase, ≤2.5 × the institutional upper limit of normal); adequate renal function (creatinine ≤1.5 mg/dl or measured creatinine clearance ≥60 ml/min/1.73 m^2 for patients with creatinine levels about institutional normal); an LVEF >45%; and life expectancy greater than 12 weeks.

Exclusion criteria included the following: untreated brain metastasis; ≤4 weeks since prior chemotherapy or radiation therapy; prior treatment with an anthracycline; G6PD deficiency; major surgery within 4 weeks; active infection; and any serious concomitant conditions that

would place the patient at excessive or unacceptable risk of toxicity. All patients had to practice effective birth control and give written informed consent indicating that they were aware of the investigational nature of the study. Before entering the study, each patient gave written informed consent, according to institutional and federal guidelines. The protocol was approved by the Health Sciences Institutional Review Board at the University of Wisconsin-Madison.

Study design

This was a phase 1, dose-escalating trial designed to determine the safety and tolerability of Triapine® in combination with doxorubicin in advanced solid tumors. Triapine® was administered as a 2 h infusion on days 1–4. Doxorubicin was administered as an IV bolus over 15 min on day 1, immediately following the end of the Triapine® infusion. Treatment was repeated every 21 days. The starting dose (level 1) was doxorubicin 60 mg/m^2 and Triapine® 25 mg/m^2. A maximum of $\leq$360 mg/m^2 total doxorubicin was allowed. Adverse events were evaluated using the National Cancer Institue Common Toxicity Criteria, version 3.0 guidelines.

Drug administration

Triapine® was supplied by Vion Pharmaceuticals, Inc. (50 mg/vial; 5 mg/ml) and distributed by the Cancer Therapy Evaluation Program, the Division of Cancer Treatment and Diagnosis, National Cancer Institute. Triapine® was further diluted in D5W (final concentration between 0.01 and 2 mg/ml) in non-polyvinyl chloride (non-PVC) bags and administered as a 2 h infusion through non-PVC tubing.

Acute reactions to Triapine®, occurring either during the infusion or soon after the infusion was completed, have been observed, primarily at doses $\geq$ 140 mg/m^2 infused IV over 2–4 h. The reactions included hypoxia (with or without dyspnea and with or without associated cough) and hypotension. Patients were observed clinically for 3–4 h after each Triapine® infusion for hypoxia and hypotension during the first week of cycle 1. Vital signs and oxygen saturation by pulse oximetry were recorded prior to Triapine® infusion, every 30 min during infusion and every 60 min post-infusion for several hours. If the patient became symptomatic or developed hypoxia (oxygen saturation $\leq$92%) requiring oxygen, a methemoglobin level was obtained and was repeated prior to the next infusion (or, in the case of the last day's infusion, 24 h later) to determine whether a dose modification was indicated or whether the patient was removed from the study. Since pulse oximetry is not reliable in the presence of significant methemoglobinemia, isolated hypoxia was managed with supplemental oxygen, unless the patient was symptomatic. In cases where there was doubt, arterial blood gases were obtained. If oxygen saturation did not return to >92% with oxygen supplementation, Triapine® was discontinued and the patient was removed from the study. Likewise, Triapine® infusion was stopped if patients developed dyspnea at rest or hypotension (systolic blood pressure <85 mm Hg). If the toxicity did not resolve within 4 h, patients were removed from the study. If the patient experienced symptoms of dyspnea, pulse oximetry was measured at regular intervals. If the patient experienced hypotension or bradycardia, an EKG was obtained. Since EKG changes consisting of ST-T wave changes and mild prolongation of the QT interval have been observed immediately following treatment with Triapine®, changes such as ST-T wave changes and mild prolongation of the QT interval, unaccompanied by hypotension or dyspnea, were not indications to stop treatment.

Patients were expected to show a transient rise in methemoglobin levels (up to 10–15%) with Triapine®. Unless accompanied by hypoxia (oxygen saturation $\leq$92%) or symptoms, or failure of methemoglobin levels to drop under 5% within 24 h, treatment continued unmodified. As described above, if patients became symptomatic or developed hypoxia (oxygen saturation $\leq$92%) requiring oxygen, a methemoglobin level was obtained. If patients remained asymptomatic without hypoxia and had a methemoglobin level <5%, treatment continued without change in dose. If oxygen saturation was $\leq$92%, methemoglobin levels were >15% or moderate to severe symptoms were experienced, patient were monitored hourly and given supportive care. Patients with moderate to severe symptoms that did not resolve within several hours, but did not require hospitalization were allowed to continue on trial with a one level dose reduction; otherwise, they were removed from the study. Patients with methemoglobinemia >15% which did not resolve within a few hours were removed from the study.

If no changes in oxygen saturation were seen in cycle 1 during the treatment and observation periods, and if the patient experienced no clinically significant symptoms, patients were discharged 1–2 h after the Triapine® infusion during subsequent cycles. If patients had prolonged methemoglobinemia or hypoxia requiring dose adjustment during first week of cycle 1, monitoring was performed as described above during subsequent cycles until stable.

Doxorubicin hydrochloride was obtained from commercially available supplies and prepared per institutional standards. Doxorubicin was administered intravenously as a bolus over 15 min into a free flowing IV line using extravasation precautions.

Dose escalation

For dose escalation to occur, three assessable patients had to complete their first cycle without DLT. With each DLT,

three additional assessable patients had to be accrued, and further escalation could occur if no more DLTs were observed. Hematologic DLT was defined, using the Common Toxicity Criteria, version 3.0, as grade 3 or 4 neutropenia lasting ≥7 consecutive days, febrile neutropenia and grade 3 or 4 thrombocytopenia lasting ≥7 consecutive days. Non-hematologic DLT was defined as any ≥grade 3 adverse event thought to be related to Triapine® or doxorubicin, except for the following: injection site reaction, alopecia and fatigue, or clinically insignificant biochemical abnormalities. Patients were also removed from study following a treatment delay of more than 2 weeks, due to delayed recovery from toxicity related to treatment with Triapine® or doxorubicin. Maximum tolerate dose (MTD) was defined as the highest dose level at which 0 or 1 out of six evaluable patients experienced DLTs.

Assessment of methemoglobinemia

In early clinical trials with Triapine®, several instances of unexpected hypoxemia were noted with pulse oximetry. This was confirmed and was probably secondary to Triapine®-induced methemoglobinemia. Triapine® iron complexes are thought to be redox active and thus may secondarily catalyze hemoglobin oxidation. In this study, methemoglobinemia was tested in patients receiving Triapine®. Blood samples were collected prior to study drug administration during day 1 of the first cycle, at the end of infusion and then at 2, 4.5 and 22 h after Triapine® administration. Samples were be assayed for methemoglobin levels by our clinical laboratory.

Pretreatment and follow-up studies

History, physical examination, weight, estimation of ECOG performance status, CBC, total bilirubin, AST, ALT, creatinine, sodium, potassium, chloride, bicarbonate, albumin, calcium, and alkaline phosphatase were obtained from all patients at baseline and at the beginning of subsequent cycles. Tumor assessment was obtained at baseline and every cycle if measured by physical examination, or every other cycle if measured by imaging, utilizing, whether clinically or radiographically, identical methods. Other pre-registration studies included measurement of height, serum pregnancy testing for women of childbearing age, an EKG, glucose-6-phosphate dehydrogenase testing (in patients of African, Asian or Mediterranean origin/ancestry) and baseline pulse oximetry. In addition, a CBC with differential was obtained on days 8 and 15 of each cycle. On day 1 of cycle 1, a methemoglobin level was obtained prior to treatment, at the end of the infusion and then at 2, 4.5 and 22 h after Triapine® administration.

All patients who completed at least one treatment course followed by 2 weeks of observation were considered evaluable. The determination of antitumor efficacy was based on objective tumor assessments made according to the RECIST system of unidimensional evaluation. Baseline imaging-based tumor assessments were performed within 28 days prior to the start of treatment, and all tumor assessments were re-evaluated every 6 weeks thereafter. All patients with responding tumors (CR and PR) were required to have response confirmed 4 weeks after the first documented response.

Sample collection for pharmacokinetics

Triapine®

Blood sample were collected after obtaining written informed consent. Patient blood samples were collected in heparin-containing tubes and centrifuged. Plasma samples were split into two cryovials and frozen at −70°C. Blood samples were collected on the following schedule during cycle 1/day 1: pre infusion, 1 h into the infusion, 1–2 min just before the end of the infusion, and at 10, 20, 30, 45 min and 1, 2, 4.5, 6, 8, 10 and 22 h after the end of the infusion.

Doxorubicin

Blood collection was performed pre infusion (1–2 min prior to the end of infusion for Triapine®) and at 30 min, 1, 2, 4.5, 6, 8, and 22 h after the end of the Triapine® infusion.

Pharmacokinetic analysis

Triapine®

HPLC with UV detection using a Spectra Physics P2000 HPLC system was used to analyze plasma and erythrocyte samples for Triapine® concentration by the method of Murren et al. [14]. Chromatographic separation was achieved using a Supelco Discovery C18 column (5 μM, 250 mm × 4.6 mm; Supelco, St. Louis, MO) with detection at 400 nm. Plasma or erythrocyte samples (0.5 ml) were extracted with 1.0 ml of methanol (containing 4 mM EDTA). After centrifugation, the extract was concentrated to dryness and was reconstituted with 0.25 ml of a solvent consisting of 10% acetonitrile and 90% mobile phase A [20 mM potassium phosphate buffer, 15 mM 1-heptanesulfonic acid, and 1 mM EDTA (pH 3.0)]. The reconstituted solution sample (30 μl) was then injected into the HPLC system. External calibration standards were prepared in pooled control human plasma and were processed identically to test samples. The validated assay was linear over 0.02–10 μg/ml for plasma ($r^2 = 0.99$), with an intraday

variability ranging from a coefficient of variation (CV) of 0.41–3.4% and an interday variability ranging from a CV of 2.60–5.5%. The lower limit of quantitation was 0.078 μg/ml and an absolute recovery from plasma of 92%. Pharmacokinetic parameters in plasma were compared to pharmacokinetic parameters in erythrocytes using a non-parametric Wilcoxon Signed Rank test. All P values were two-sided, and $P < 0.05$ was used to indicate statistical significance.

Doxorubicin

Doxorubicin was analyzed as previously described [17] on a Thermoseparation SpectraSystem P4000 pump with an AS 3000 autosampler, and a Shimadzu RF-551 fluorescent detector, Ex = 550, Em = 470. Data was collected on a CR501 Chromatopac integrator, attenuation 3, with noise level set by integrator. Separations were achieved with an isocratic solvent system, at 1.0 ml/min and an Agilent Rx-C8 4.6 × 250 mm steel column with inline upchurch pre-column filter.

Ten microliter of 1 μg/ml working stock daunorubicin (internal standard) were added to 300 μl of plasma sample. Samples were vortexed briefly, and 600 μl acetonitrile was added. Samples were centrifuged at 14,000 rpm for 10 min, and 700 μl of the resulting supernatant was removed. Supernatants were then concentrated under a stream of N_2 for 1.5–2 h, and the residue was reconstituted with 100 μl HPLC solvent (70% 10 mM sodium acetate/5% acetonitrile, pH 4.5).

The standard curve was linear from 2.5 to 40 ng/ml, $r^2 = 0.996$, with an intraday variability of <3% for high standard (40 ng/ml), $n = 3$ and 13% for low standard (2.5 ng/ml), $n = 3$. Interday variability was 10.2% for the high standard, $n = 5$ and 10.6% for the low standard, $n = 5$ over 3.5 weeks. The limit of detection was 0.25 ng/ml, and the lower limit of quantitation was 1.0 ng/ml. Recovery was based on standard addition: 91.3% for high standard and 103.7% for low standard.

Statistical methods

The primary outcome measure of this study was assessment of toxicity. The number and severity of toxicity incidents determined the level of tolerance for Triapine® and doxorubicin in the treatment of advanced cancer. Hematologic toxicity measures were assessed using continuous variables as the outcome measures (primarily nadir and percent change from baseline values), as well as categorization via CTC standard toxicity grading. Non-hematologic toxicities were evaluated via the ordinal CTC standard toxicity grading only. Frequency distributions and other descriptive measures formed the basis of analysis of these variables. The number of treatment anti-tumor responses served as the secondary outcome measure. Treatment efficacy was summarized by simple descriptive summary statistics delineating complete and partial responses as well as stable and progressive disease.

Pharmacokinetic analysis for both Triapine and doxorubicin was performed by noncompartmental methods using the WinNonlin program, version 5.2 (Pharsight, Cary, NC). The maximum plasma concentration (C_{max}) and the corresponding time of the maximum concentration were identified from the measured samples and recorded. Plasma concentration–time data were plotted on a semi-logarithmic scale, and the terminal log–linear phase was identified by best fit. The elimination rate constant (λ) was determined as the slope of the linear regression for the terminal log–linear portion of the concentration–time curve. A terminal half-life value was calculated as $\ln(2)/\lambda$. AUC was calculated by the trapezoidal method using extrapolation to infinity. Both C_{max} and AUC were dose adjusted and summarized using means and standard deviations.

Results

Patient characteristics

Twenty patients were enrolled onto this study between November 2004 and December 2006 and received a total of 49 courses of therapy. Pretreatment characteristics are outlined in Table 1. All 20 patients who entered the study completed the first cycle of therapy, and all patients were included in the safety analysis. The dose escalation schema together with the number of PK dosing days are listed in Table 2.

Dose escalation and toxicity

All 20 patients were evaluable for toxicity. The dose escalation schedule is outlined in Table 2, and the most common toxicities are shown in Table 3. The starting dose (level 1) was doxorubicin 60 mg/m^2 and Triapine® 25 mg/m^2. No DLTs were observed in the first three patients, and subsequent doses were escalated according to Table 2. At dose level 2 (doxorubicin 60 mg/m^2, Triapine® 45 mg/m^2), two patients experienced DLTs (febrile neutropenia, grade 4 thrombocytopenia). An additional three patients were enrolled at dose level 1 without initial toxicity. Enrollment then resumed at dose level 2a with a decreased dose of doxorubicin (45 mg/m^2) with Triapine® (45 mg/m^2). The two patients enrolled on this level had DLTs (diarrhea, CVA). Enrollment was planned to resume at dose level 1; however, the sixth patient enrolled to this cohort with advanced bladder cancer was hospitalized on course 2, day

Table 1 Patient demographics

	n
Number of patients	20
Median age (range)	61.1 (34–84)
Sex	
Male	12
Female	8
Performance status	
0	3
1	16
2	1
Primary tumor type	
Melanoma	3
Prostate	3
Cholangiocarcinoma	2
Colon	2
Esophageal	2
Unknown primary	2
Breast	1
Transitional cell	1
Gastric	1
Small cell lung	1
Hepatocellular	1
Gallbladder	1
Prior systemic therapy	
0	0
1	4
2	7
≥3	9

10 with dyspnea and neutropenia and died of heart failure (ejection fraction 20%, pretreatment EF 62%) after the second course of therapy. Thus, doxorubicin and Triapine® were reduced to 45 and 25 mg/m^2, respectively (level 1a). No DLTs occurred at this level, and an additional six patients were enrolled at dose level 1, the MTD.

Safety

The main drug-related toxicity was myelosuppression, with 63.3% of courses having grade 3 or 4 neutropenia, 8.2% of courses having grade 3 or 4 anemia and 4.1% of courses having grade 3 or 4 thrombocytopenia (Table 3). The most common non-hematologic toxicities included mild-to-moderate fatigue and nausea and vomiting. Grade 3 diarrhea and grade 4 CVA that met criteria for DLT were observed at dose level 2a and necessitated a dose reduction to dose level 1a.

Efficacy

Although not a primary endpoint of this trial, patients underwent disease assessment prior to every even numbered cycle. While no objective antitumor responses were seen, one patient with metastatic melanoma enrolled at dose level 1 had stable SD through six cycles of treatment. Another patient with previously rapidly progressive metastatic melanoma enrolled at dose level 1 had SD for over six cycles of treatment. One patient with prostate cancer at dose level 1 also had SD, with a stable PSA, until maximal therapy was received at six cycles.

Triapine® pharmacokinetics

Concentrations of Triapine® were measured in plasma and erythrocytes before dosing and at multiple times during cycle 1, day 1, as detail in "Patients and methods", in all patients who received Triapine® dosed at 25 mg/m^2 ($n = 14$) or 45 mg/m^2 ($n = 6$). The pharmacokinetic parameters are described in Table 4. The half-life of Triapine® was 5.3 ± 4.6 h and 4.2 ± 2.1 h in plasma and erythrocytes, respectively. The dose-adjusted AUC was 1.21 ± 0.43 mg × h/ml in plasma and 1.45 ± 0.67 mg × h/ml in erythrocytes, and the C_{max} was 0.65 ± 0.18 and 0.71 ± 0.18 mg/ml, respectively. Both the dose-adjusted AUC and C_{max} values were significantly lower in plasma, when compared to those levels in erythrocytes [AUC: $P = 0.0121$; C_{max}: $P = 0.0181$, non-parametric Wilcoxon Signed Rank test (two-sided)]. Additionally, the T_{max} occurred in the erythrocytes at 0.04 ± 0.11 h compared to 0.22 ± 0.11 h in plasma.

Doxorubicin pharmacokinetics

Plasma concentrations of doxorubicin were measured during cycle1, day 1 as described in "Patients and methods" in all patients who dosed doxorubicin at 45 mg/m^2 ($n = 4$) or

Table 2 Dose escalation schema and frequency of DLTs

Dose level	*n*	Triapine (mg/m^2)[a]	Doxorubicin (mg/m^2)[b]	Courses no.	No. of patients with DLTs (cycle 1)	Description of DLTs (cycle 1)
1[c]	12	25	60	37	0	–
1a	3	25	45	5	0	–
2	3	45	60	5	2	Febrile neutropenia, thrombocytopenia
2a	2	45	45	2	2	Diarrhea, CVA

[a] Administered IV on days 1–4

[b] Administered IV on day 1 of a 21-day cycle

[c] MTD

Table 3 Drug-related adverse events, worst per course (49 total courses)

Selected toxicities	Dose level																Total (%)
	1				1a				2				2a				
	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4	
Hematologic																	
Neutropenia		6	8	13[a]			2	2			1	4[a]				1	37 (75.5)
Anemia		6	1	1						2	2			1			13 (26.5)
Thrombocytopenia		3		1						1	1	1[a]					7 (14.3)
Non-hematologic																	
Nausea/vomiting	1	1					1		4	1							8 (16.3)
Diarrhea			1												1[a]		2 (4.1)
Fatigue	3	7	1			1			1	1	1				1		16 (32.7)
Renal failure											1						1 (2)
Hyponatremia							1				1				1		3 (6.1)
Hypokalemia			1														1 (2)
CVA																1[a]	1 (2)

CVA cerebral vascular accident

[a] Includes one or more events that met criteria for dose-limiting toxicity

Table 4 Pharmacokinetic parameters in plasma and erythrocytes in patients receiving Triapine® dosed at 25 mg/m^2 ($n = 14$) or 45 mg/m^2 ($n = 6$)

	Plasma		Erythrocytes		*P* value
	Mean	SD	Mean	SD	
$T_{1/2}$ (h)	5.3	4.6	4.2	2.1	0.5217
T_{max} (h)	0.2	0.11	0.04	0.11	0.9500
C_{max} (μg/ml)[a]	0.65	0.18	0.71	0.18	0.0181
$AUC_{0-\infty}$ (mg × h/ml)[a]	1.21	0.43	1.45	0.67	0.0121
Cl (ml/min/m^2)	0.38	0.11	0.34	0.17	0.0107
V_{ss} (l/m^2)	107	62	83	28	0.1536

Cl clearance, *SD* standard deviation, V_{ss} distribution volume at steady state, *AUC* area under the plasma concentration time curve from 0 to ∞, $T_{½}$ half-life, T_{max} time of maximum concentration

[a] Dose adjusted

Table 5 Pharmacokinetic parameters in plasma in subjects receiving doxorubicin dosed at 45 mg/m^2 ($n = 4$) or 60 mg/m^2 ($n = 16$)

	Plasma	
	Mean	SD
$T_{1/2}$ (h)	10.14	8.46
T_{max} (h)	0.20	0.07
C_{max} (ng/ml)[a]	143	97
$AUC_{0-\infty}$ (ng × h/ml)[a]	86	51
Cl (ml/min/m^2)	252	12
V_{ss} (l/m^2)	219	223

Cl clearance, *SD* standard deviation, V_{ss} distribution volume at steady state, *AUC* area under the plasma concentration time curve from 0 to ∞, $T_{½,}$ half-life, T_{max}, time of maximum concentration

[a] Dose adjusted

60 mg/m^2 ($n = 16$). The pharmacokinetic parameters are described in Table 5, and demonstrate a half-life of 10.14 ± 8.46 h. The dose adjusted AUC was 86 ± 51 (45 mg/m^2) and the C_{max} was 143 ± 97 ng/ml.

Discussion

Triapine® is a novel RR inhibitor that inhibits the M2 unit and has been shown to act synergistically with DNA damaging agents in vitro. This phase I study was designed to evaluate the safety and tolerability of Triapine® in combination with doxorubicin and to determine the MTD and examine pharmacokinetic and exploratory pharmacodynamic analysis.

The MTD in this study was established at doxorubicin 60 mg/m^2 IV on day 1 and Triapine® 25 mg/m^2 IV over 2 h on day 1 through 4. The dose of Triapine® in this combination was not able to be increased above 25 mg/m^2 due to excessive toxicity. Dose-limiting toxicities (febrile neutropenia and grade 4 thrombocytopenia) were experienced in two of three patients at dose level 2 (doxorubicin 60 mg/m^2 and Triapine® 45 mg/m^2) early in the first treatment course. When Triapine® was increased to 45 mg/m^2 in dose level 2a, with a decreased dose of doxorubicin (45 mg/m^2), the first two patients enrolled also had DLTs (diarrhea, CVA). No DLTs were noted at dose level 1a when doxorubicin was administered at 45 mg/m^2 with Triapine® at 25 mg/m^2. Examination of the 12 evaluable patients enrolled to dose level 1 shows that only one who experienced a treatment-related

DLT after the second course of therapy. Otherwise, treatment was fairly well-tolerated with 83% (10/12) experiencing grade 3 or 4 neutropenia, and one each (8%) having grade 3/4 anemia, fatigue or hypokalemia during the first cycle. These symptoms were transient and tolerable and did not result in significant dose-delays, and there were no episode of febrile neutropenia. The average number of cycles completed per patient at the MTD level was 3.1.

Previous studies have documented the myelosuppressive effects of Triapine®. In the phase 1 study with Triapine® given by 2 h IV infusions for 5 days of every 28-day cycle, the major hematologic toxicities were transient leucopenia (grade 4 in 93% of the patients in at least one course) and anemia (grade 2 in 73% and grade 3 in 22%) [14]. Thrombocytopenia was less common, and was grade 3 or 4 in 22% of patients. In a phase II study of Triapine® in combination with gemcitabine in patients with advanced pancreatic cancer, Triapine® was initially given as a 4 h infusion weekly prior to gemcitabine [20]. Treatment with the 4 h infusion was well tolerated with little additional myelosuppression. However, the protocol was subsequently amended to give Triapine® over a 24 h continuous infusion weekly prior to gemcitabine to enhance the synergistic effect between the two agents. Excessive myelosuppression was seen in the first continuous infusion cohort necessitating a reduction in the Triapine® dose. All subsequent patients also experienced excessive myelosuppression necessitating a dose reduction in gemcitabine. Taken together, these studies suggest that the myelosuppressive effects of Triapine® may be related to dose intensity over a given period of time.

The Triapine® dose in this study was well below the single-agent MTD identified by Murren et al. [14]. In that trial, the MTD was 96 mg/m^2 administered by 2 h IV infusions for 5 days of every 28-day cycle [14]; however, the recommended phase II dose was 96 mg/m^2 daily for 4 days since that schedule had a lower incidence of leucopenia. Our finding that that Triapine® in combination with doxorubicin yielded significant hematologic toxicity is not surprising since severe myelosuppression is one of the DLTs of doxorubicin, and leucopenia occurs in approximately 75% of patients treated with 60 mg/m^2 every 21 days [21, 22]. Anemia and thrombocytopenia are less common with single-agent doxorubicin. The myelosuppressive effects of Triapine® may limit the dose schedules and chemotherapy agents that may be chosen when considering other combination studies.

One 71-year-old male with metastatic bladder cancer with lung metastases, who was enrolled at the MTD level experienced grade 5 CHF, following the second course of chemotherapy (cumulative doses of Triapine® = 385 mg and doxorubicin = 228 mg). The patient was otherwise healthy and had previously undergone transurethral resection of the tumor and was treated with mitomycin-C and gemcitabine in combination with pemetrexed for metastatic disease. While reversible hypotension, hypoxia, dyspnea, cough, and EKG changes, including ST-T wave changes and prolongation of the QT interval, were observed in a prior phase I study with Triapine® [14], CHF has not been observed in single-agent studies. Congestive heart failure related to chronic cardiomyopathy is a well-documented toxicity attributed to doxorubicin; however, the incidence of CHF is typically related to the cumulative dose administered, occurring in 1% in patients treated with up to 300 mg/m^2 and 4% of patients who receive 450 mg/m^2 doxorubicin [23]. Acute cardiac toxicity has also been observed with doxorubicin, but this is usually characterized by transient arrhythmias or other EKG changes. Three cases of severe and fatal (two cases) doxorubicin-induced cardiotoxicity have been reported at cumulative doses below 400 mg/m^2 [24]. In all three cases, the patients were receiving multi-agent chemotherapy with bleomycin, cyclophosphamide, dactinomycin, high-dose methotrexate and cisplatin, and the risk of this toxicity appeared to be enhanced with concurrent administration of other cardiotoxic or hepatotoxic agents. It is unclear whether there may be any synergistic effects between Triapine® and doxorubicin in terms of cardiac toxicity, but this may be a consideration for patient selection if this combination is used in other clinical studies.

The plasma concentrations of Triapine® [14] and doxorubicin [17] in this study were similar to previously reported values, suggesting no interaction between the agents. Our observed half-life of doxorubicin was shorter than previous reports, which is most likely explained by our limited sampling schedule. The dose-adjusted AUC and C_{max} for Triapine® in plasma were significantly lower than the corresponding values in erythrocytes (AUC: $P = 0.01$; C_{max}: $P = 0.02$, non-parametric Wilcoxon Signed Rank test (two-sided)) which suggests an accumulation of Triapine® in the erythrocytes and could be related to the development of methemoglobinemia. Additionally, the T_{max} occurred in the erythrocytes at 0.04 ± 0.11 h compared to 0.22 ± 0.11 h in plasma. This may indicate that Triapine® preferentially enters the erythrocyte and that the accumulation of Triapine® may be the result of decreased diffusion out of the erythrocyte.

Despite the lack of objective tumor responses in this study, two patients with melanoma and another two patients with prostate cancer derived clinical benefit with this combination. While antitumor activity with Triapine® has been shown in melanoma in preclinical models [12] and in a patient with prostate cancer in a single-agent phase I study [13], there are no in vitro data showing activity of this combination in either of these malignancies. Since preclinical data and the findings from this study support a synergistic

interaction between Triapine® and doxorubicin, we believe that further clinical development of this combination in these two tumor types is warranted.

A growing body of evidence suggests that RR expression may influence survival and response in a number of malignancies. Studies examining RRM1 expression in lung tumors have shown that higher levels of this subunit may be associated with growth suppression and a less malignant phenotype, particularly in patients with resectable disease [25]. However, patients with metastatic NSCLC who have increased RRM1 levels are potentially less responsive to cytotoxic therapy with gemcitabine and platinum [26]. Likewise, overexpression of RRM2 resulted in increase in resistance to gemcitabine chemoresistance in lung [27] and pancreatic [28] cancer cells in vitro. In cell lines selected for resistance to hydroxyurea, increased RRM2 protein levels and ribonucleotide reductase activity were detected [29–31]. Taken together, these findings suggest that alterations in RRM1 and RRM2 expression may confer resistance to chemotherapy and may be useful biomarkers for predicting and monitoring responses to various therapies including Triapine® [32, 33]. Pooled analyses from 40 different tumor samples obtained at baseline in three studies evaluating Triapine® at our institution showed that RRM2 protein and gene expression varied by tumor type following treatment with Triapine® (data not shown) [18, 19]. Although the number of samples was too small to determine whether the level of RRM2 protein correlated with response or resistance to Triapine® in specific tumor types, evaluation of RRM1 and RRM2 expression should be considered in future studies with Triapine®.

Based on the clinical activity observed in this study, the combination of Triapine® and doxorubicin may be considered for use in future studies in melanoma and prostate cancer. While this combination was fairly well tolerated at the MTD, it was associated with significant myelosuppression. It is possible that the hematologic effects would be less pronounced in patients with adequate marrow reserve who have not received multiple prior therapies. In addition, liberal use of growth factors could minimize treatment-related neutropenia and associated sequelae. The recommended phase II combination dose based on our data would be Triapine® 25 mg/m^2 administered as a 2 h infusion on days 1–4 and doxorubicin 60 mg/m^2 given by IV bolus on day 1 immediately following Triapine®.

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