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Triapine and cytarabine is an active combination in patients with acute leukemia or myelodysplastic syndrome☆

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Abstract

Triapine®, an iron chelator and a potent inhibitor of ribonucleotide reductase, has significant anti-leukemia activity. A phase I study of Triapine in combination with ara-C was conducted in 32 patients with refractory acute leukemia and high-risk MDS. Triapine (105 mg/m^2/day 6-h infusion) was followed immediately by ara-C [100 ($n=4$), 200 ($n=6$), 400 ($n=7$), or 800 ($n=8$) mg/m^2/day] as an 18-h infusion for 5 consecutive days. Dose-limiting toxicities (DLTs) were observed at the 800 mg/m^2 ara-C dose level (one patient each with grade 4 mucositis; grade 4 neutropenic colitis, sepsis; grade 4 neuropathy; and grade 4 hyperbilirubinemia). Therefore, the study was amended to include an ara-C dose level of 600 mg/m^2/day, no DLTs occurred in seven patients treated at this dose level. Mean Triapine C_{max} and AUC were 1.13 μg/mL and 251.5 min μg/mL. Of 31 evaluable patients, 4 (13%) (3 AML, 1 Ph + ALL) achieved a CR (1 at a dose of 800 mg/m^2; 2 at 600 mg/m^2; 1 at 200 mg/m^2). The recommended phase II regimen is Triapine 105 mg/m^2/day followed by ara-C 600 mg/m^2/day for 5 consecutive days every 3–6 weeks.

Keywords: Ribonucleotide reductase; Triapine; Cytarabine; Acute myeloid leukemia; Acute lymphocytic leukemia; Myelodysplastic syndrome

1. Introduction

Ribonucleotide reductase (RNR) catalyzes the rate-limiting conversion of ribonucleotides to deoxyribonucleotides, which are required for DNA synthesis, and is therefore indispensable for cell survival, growth, and proliferation [1]. Mammalian class I RNR is an $\alpha_2\beta_2$ heterodimer composed of two subunits: the M1 (R1; α_2 dimer) subunit that contains the active sites and binding sites for allosteric effectors and the inducible M2 (R2; β_2 dimer) subunit that contains the non-heme iron and the tyrosyl radicals essential for enzymatic activity [2]. The activity of RNR is tightly regulated by the binding of nucleoside triphosphates to allosteric sites in the M1 subunit and by the cell cycle-specific availability of the M2 subunit [3,4]. Cytotoxic drugs that cause DNA damage, e.g. chlorambucil, and cytokines, such as transforming growth factor β1, can cause induction of RNR expression [3,5–8]. Overexpression and/or increased activity of RNR are associated with tumorigenesis, metastasis, invasive potential, and drug resistance [9–15].

Most M1-affecting RNR inhibitors are nucleoside analogues, which include several active anti-leukemic agents, e.g. fludarabine, cladribine, and clofarabine [16,17]. Hydroxyurea is the only clinically prescribed RNR inhibitor acting at the iron/free radical site (M2 subunit); however, its

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potency against RNR is low, and inhibition of the enzyme is reversible due to the ease in regenerating the tyrosyl free radical by mammalian cells [10,18,19]. Unlike hydroxyurea, Triapine® (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) is both an iron chelator and a potent, but reversible, inhibitor of the M2 subunit of RNR [20]. As the M2 subunit requires iron to regenerate the tyrosyl free radical, Triapine's enhanced potency against RNR compared to hydroxyurea may be due to its ability to sequester iron [21]. In addition, iron depletion may also inhibit the p53-inducible form of R2 (p53R2) that complexes with R1 subunit to supply dNTPs for DNA repair [19,22], induce hypophosphorylation of the retinoblastoma protein, and inhibit the expression of proteins critical for cell cycle progression [23,24].

Triapine is 100–1000-fold more potent than hydroxyurea and is active in some hydroxyurea-resistant leukemia cell lines, indicating that Triapine can overcome resistance to hydroxyurea attributed to overexpression of the M2 subunit of RNR [20,25–28]. The median Triapine concentration required for 50% growth inhibition (GI_{50}) in a 48 h assay against the National Cancer Institute's tumor cell line panel was 1.6 μM [29]. Two phase I studies in patients with relapsed or refractory hematologic malignancies demonstrated that single agent Triapine, administered as either a 96-h continuous infusion or a 2-h infusion twice a day for 5 consecutive days twice per cycle, reduced circulating blasts and/or bone marrow blasts in the majority of patients without significant non-hematologic toxicities [29,30].

Cytarabine is a nucleoside analogue that requires phosphorylation to its triphosphate form (ara-CTP) to inhibit DNA polymerase activity and elicit cytotoxicity. Clinical response to cytarabine strongly correlates with cellular retention and accumulation of ara-CTP [31,32]. Unlike other nucleoside analogues, such as fludarabine, cladribine, and clofarabine, it does not inhibit RNR [16,17]. Cytarabine is phosphorylated intracellularly, with the rate-limiting step of cytarabine phosphorylation to its monophosphate form by deoxycytidine kinase (dck). The activity of dck is regulated by the availability of the intracellular pool of deoxynucleotide phosphates (dNTPs). The pool of dNTPs is regulated by RNR. High levels of dCTP inhibit the activity of dck. As previously described, Triapine inhibits RNR, leading to the depletion of dNTPs, including dCTP [29], and removing dCTP feedback inhibition of dck. This results in increased dck activity and increased levels of ara-CTP. In addition, depletion of intracellular dCTP pools may decrease competition with ara-CTP for DNA incorporation, leading to enhanced DNA fragmentation and cytotoxicity.

Sequential exposure of tumor cell lines, including the HL-60 human leukemia cell line, to Triapine for 4–12 h followed by cytarabine for 1–72 h has been shown to increase cytarabine cellular uptake and DNA incorporation and to produce synergistic cytotoxicity [25,28]. Triapine-induced depletion of dNTPs prevents not only DNA synthesis, but also repair of drug-induced damage to DNA [28,29]. These data provide the basis for the clinical trial of Triapine as a biochemical modulator of cytarabine.

Preclinical data indicated that the antitumor activity of Triapine was dependent on achieving both a threshold concentration and a minimum duration of exposure [25]. Effective serum concentrations and duration of Triapine exposure required for modulating cytarabine toxicity in vitro can be achieved by administering Triapine as a 2-h infusion daily for 5 consecutive days every 2–4 weeks. At doses of up to 105 mg/m^2/day, peak Triapine serum concentrations (C_{max}) were in the range of 2–7 μM with a terminal half-life of approximately 1 h; the major toxicities were myelosuppression [25,30,33,34]. The current phase I trial was designed to determine the tolerability, safety, and maximum tolerated dose (MTD) of Triapine administered in a sequence-dependent manner with cytarabine in patients with relapsed or refractory acute leukemias or high-risk myelodysplastic syndrome (MDS). Triapine was administered at a dose of 105 mg/m^2/day over 6 h followed immediately by an 18-h infusion of escalating doses of cytarabine for 5 consecutive days.

2. Patients and methods

2.1. Patient eligibility

Patients with histologically confirmed acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), high-risk MDS [refractory anemia with excess blasts (RAEB), RAEB in transformation (RAEB-t), or an International Prognostic Scoring System (IPSS) score of ≥1.5 based on cytogenetics, percentage of bone marrow blasts, and cytopenias [35]], or chronic myelomonocytic leukemia (CMML) by FAB classification were eligible for enrollment. After the maximum tolerated dose of cytarabine was established for the current study, eligibility for study entry was restricted to (i) patients with AML aged <60 years in first relapse who had not received cytotoxic treatment (other than hydroxyurea) for the relapse; (ii) patients with AML aged ≥60 years in first relapse who had not received cytotoxic treatment (other than hydroxyurea) for the relapse, or who did not receive standard induction chemotherapy; (iii) patients with AML of any age with primary refractory disease who had received only one cytotoxic induction chemotherapy regimen; (iv) patients with high-risk MDS who had received ≤1 prior cytotoxic chemotherapy regimen (other than hydroxyurea).

Other inclusion criteria were adequate performance status (Eastern Cooperative Oncology Groups 0–2), life expectancy of >2 months, adequate hepatorenal function [total bilirubin ≤ 2.0 mg/dL, ALT or AST ≤ 3 times the upper limit of normal (ULN), and creatinine ≤ 2.0 mg/dL unless considered due to malignancy]. Women of child-bearing potential must have a negative pregnancy test. Patients could not be pregnant or breast-feeding, have chronic hepatitis, or any serious medical or psychiatric conditions that could inter-