Leukemia Research 35 (2011) 110–118

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

3-Deazauridine enhances the antileukemic action of 5-aza-2′-deoxycytidine and targets drug-resistance due to deficiency in deoxycytidine kinase

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ARTICLE INFO

Article history:
Received 15 February 2010
Received in revised form 17 April 2010
Accepted 21 April 2010
Available online 26 May 2010

Keywords:
5-Aza-2′-deoxycytidine
3-Deazauridine
Epigenetic therapy
Synergy
Drug-resistance
Deoxycytidine kinase
Acute lymphoblastic leukemia

ABSTRACT

New approaches should be sought to treat high-risk acute lymphoblastic leukemia (ALL). Since aberrant DNA methylation plays an important role in leukemogenesis of ALL, it can be targeted by 5-aza-2′-deoxycytidine (5-AZA-CdR), a potent inhibitor of DNA methylation. 5-AZA-CdR is a prodrug that is activated by deoxycytidine kinase (DCK). Leukemic cells lacking DCK are drug-resistant. In a previous phase I study, we reported that 5-AZA-CdR could induce remissions in ALL. However, some patients developed drug-resistance due to deficiency in DCK. These observations aroused our interest in 3-deazauridine (3-DU), a CTP synthetase inhibitor that is effective against leukemic cells deficient in DCK. In this report, we observed that 3-DU enhanced the in vitro antineoplastic action of 5-AZA-CdR on human leukemic cells by increasing its incorporation into DNA. Using an optimized dose-schedule we showed that this combination could cure some mice bearing L1210 leukemia, even in the presence of a subpopulation of drug-resistant (L1210/ARA-C) leukemic cells lacking DCK. 3-DU alone also cured some mice with L1210/ARA-C leukemia. In a pilot study on 3 relapsed patients with advanced ALL, the combination of 5-AZA-CdR and 3-DU produced a marked reduction in leukemic blasts, confirming our preclinical observations. Furthermore, after several treatments with these agents all three patients developed drug-resistance to 5-AZA-CdR as determined by an in vitro drug sensitivity test. In two patients we showed by enzymatic analysis that the drug-resistance was due to deficiency in DCK. Our preclinical and clinical results provide a strong rationale to further investigate the combination of 5-AZA-CdR and 3-DU for the treatment of advanced ALL.

1. Introduction

Remarkable progress has been made for the chemotherapy of acute lymphoblastic leukemia (ALL) as shown by cure rates of higher than 80% for children diagnosed with this hematological malignancy [1]. However, the prognosis is still poor for adults with ALL and a subgroup of children with high-risk ALL. Recent investigations on the molecular mechanisms involved in leukemogenesis can provide new targets for chemotherapy of ALL. Epigenetic alterations in ALL are frequent, especially aberrant DNA methylation, which can lead to silencing of genes that suppress leukemogenesis [2–5]. Promoter hypermethylation was reported to be more frequent in ALL than in acute myeloid leukemia [6]. DNA methylation of multiple genes in ALL is associated with a poor prognosis [7,8]. These reports suggest that hypomethylating agents, such as 5-aza-2′-deoxycytidine (5-AZA-CdR) [9], may have potential for the treatment of ALL.

Historically, the first patient induced into complete remission with 5-AZA-CdR was a child with relapsed ALL [10,11]. This finding was confirmed recently by successful induction with 5-AZA-CdR of refractory childhood ALL [12]. In our initial clinical trial, we observed that some ALL patients after several cycles of 5-AZA-CdR showed signs of drug-resistance using an in vitro drug sensitivity test [10,13]. The molecular mechanism of this resistance was due to a deficiency in deoxycytidine kinase (DCK), the enzyme that activates the prodrug 5-AZA-CdR by phosphorylation (Fig. 1) [14].

An interesting agent to overcome drug-resistance is 3-deazauridine (3-DU). The active phosphorylated form of 3-DU inhibits CTP synthetase [15] and reduces the intracellular level of CTP and dCTP (Fig. 1) [16]. Due to the competition of 5-AZA-CdR with dCTP for DNA polymerase, reduction in dCTP levels results in an increased incorporation of 5-AZA-CdR into DNA and enhancement of its antineoplastic action [17]. Another interesting aspect

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0145-2126/$ – see front matter.
doi:10.1016/j.leukres.2010.04.014

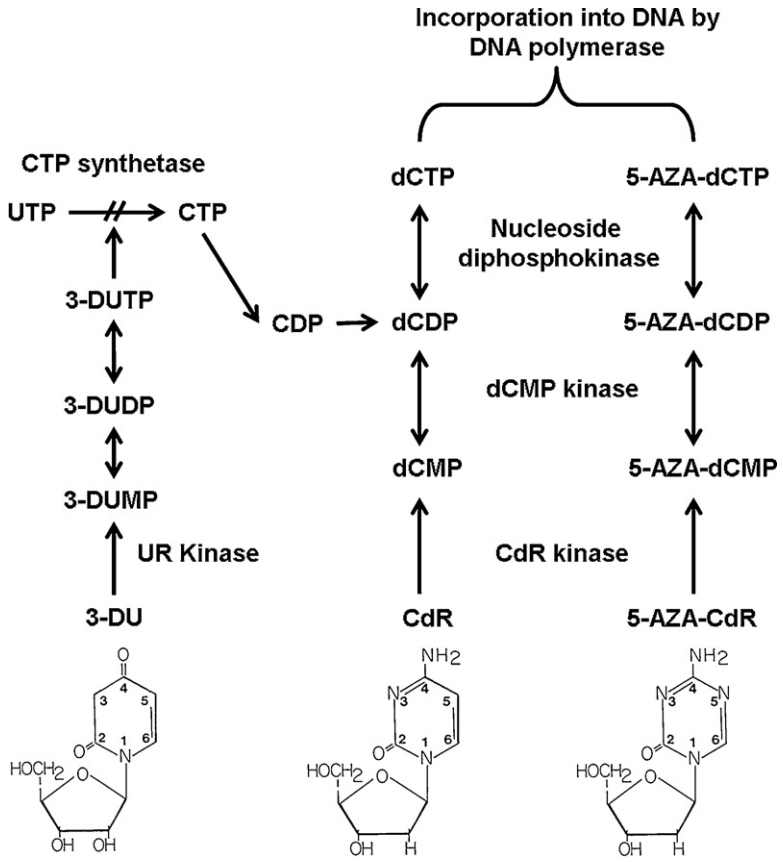

Fig. 1. Molecular mechanism of action of 3-deazauridine (3-DU). After its conversion to the triphosphate 3-DU inhibits CTP synthetase. 3-DU induced reduction in CTP and subsequently in dCTP which results in an enhanced incorporation of 5-AZA-dCTP into DNA due to less competition with dCTP. This interaction results in a marked enhancement of the antineoplastic action of 5-AZA-CdR.

of 3-DU is that leukemic cells lacking DCK are more sensitive to its antineoplastic action than wild type leukemic cells [16,18,19]. It is noteworthy that DCK-deficient cells rely on CTP synthetase for the biosynthesis of CTP and dCTP. Inhibition of CTP synthetase in DCK-deficient cells generates a cytotoxic effect since dCTP is essential for DNA synthesis. In phase I studies, 3-DU was observed to have only minimal activity against leukemia and tumors [20,21]. Consequently, 3-DU generated more interest as a biochemical modulator of deoxycytidine analogues [22]. In preclinical studies, 3-DU was shown to enhance the antileukemic activity of cytosine arabinoside (ARA-C), a deoxycytidine analogue [23,24]. In clinical trials, 3-DU in combination with ARA-C showed significant activity in 5 patients with relapsing acute leukemia resistant to ARA-C [20]. Unfortunately, follow up studies were not performed.

All these reports stimulated our interest to investigate 3-DU as a modulator of 5-AZA-CdR to enhance its antileukemic activity and overcome the problem of drug resistance. In our initial preclinical studies on 5-AZA-CdR and 3-DU, we observed that this combination showed synergy against both leukemic cells and tumor cells [17]. We also demonstrated that this combination was effective against L1210 lymphoid leukemia in mice, even in the presence of drug-resistant cells [19]. However, the dose-schedule used in these studies was sub-optimal and the full chemotherapeutic potential of this combination remains to be determined. In this present study, we optimized the dose-schedule of 5-AZA-CdR in combination with 3-DU in mice bearing L1210 wild type leukemic cells and drug-resistant leukemic cells. In light of these interesting preclinical observations, we report a pilot clinical study on three patients with advanced ALL who were treated with this combination and confirmed some of our preclinical observations.

2. Materials and methods

2.1. Cells and materials

Human myeloid HL-60, lymphoid MOLT-3 and murine leukemic (L1210 and L1210/ARA-C) were cultured as previously described [25,26]. L1210/ARA-C cells are resistant to cytosine arabinoside (cytarabine, ARA-C) and 5-AZA-CdR because of DCK deficiency [18,19,27]. Doubling times of HL-60, MOLT-3, L1210 and L1210/ARA-C cells were about 17, 24, 9 and 12 h, respectively. Stock solutions of 5-aza-2′-deoxycytidine (5-AZA-CdR) (Pharmachemie, Haarlem, Netherlands) and 3-deazauridine (3-DU) (Ash Stevens, Midland, MI) were prepared at 1,000 μg/ml in PBS and stored in aliquots at −70 °C. [6-^{3}H]-5-AZA-CdR was obtained from Moravek Biochemicals (La Brea, CA) and stored at −20 °C.

2.2. Measurement of 5-AZA-CdR incorporation into DNA

Incorporation of [6-^{3}H]-5-AZA-CdR into the DNA of HL-60 and MOLT-3 cells (3×10^5 cells) was performed as described previously [26]. Cells were incubated with 0.2 μM [6-^{3}H]-5-AZA-CdR alone or with 3-DU (0.1–40 μM) for 4 h. Cells were poured onto glass fiber filters and placed into scintillation liquid for measurement of radioactivity. The experiments were performed in triplicate and repeated three times.

2.3. In vitro clonogenic assay

Human leukemic cell lines in log phase at 5×10^4 cells/ml were treated with 5-AZA-CdR at 0.02 and 0.2 μM alone or in simultane-