Original Study

Preclinical and Clinical Evaluation of Forodesine in Pediatric and Adult B-Cell Acute Lymphoblastic Leukemia

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Abstract

Forodesine was originally developed for T-cell leukemias and was effective in T-cell acute lymphoblastic leukemia (T-ALL). The current study was done to test its utility in B-cell ALL (B-ALL). Our preclinical investigations (lymphoblasts from pediatric patients with B-ALL [n = 12]) demonstrate activity in vitro. Minimal activity in the clinic suggests that this agent should be used in combination with other established or novel ALL agents.

Background: The discovery that purine nucleoside phosphorylase (PNP) deficiency leads to T-cell lymphopenia was the basis for introducing PNP inhibitors for T-cell leukemias. Forodesine is an orally bioavailable PNP inhibitor with picomolar potency. Because T lymphoblasts and indolent chronic lymphocytic leukemia (CLL) B cells inherently elicit favorable pharmacokinetics to accumulate deoxyguanosine triphosphate (dGTP), forodesine demonstrated promising activity in preclinical and clinical settings for patients with T-cell acute lymphoblastic leukemia (T-ALL) and B-cell CLL (B-CLL). However, the use of forodesine in B-cell ALL (B-ALL) is unknown. **Patients and Methods:** Leukemic blasts obtained from pediatric patients with de novo B-ALL (n = 10) were incubated with forodesine and deoxyguanosine (dGuo), and the biological end points of apoptosis, intracellular dGTP accumulation, and inhibition of RNA and DNA synthesis were measured. Additionally, adult patients with B-ALL (n = 2) were intravenously infused with 80 mg/m^2/d daily for 5 days. After therapy, clinical response, toxicity, laboratory biomarkers including PNP enzyme inhibition, and plasma forodesine, dGuo, and intracellular dGTP levels were analyzed. **Results:** Our in vitro investigations demonstrated that forodesine treatment inhibited proliferation and induced modest apoptosis in de novo B-ALL lymphoblasts. There was time-dependent accumulation of dGTP and inhibition of RNA and DNA synthesis. During therapy, neither patient achieved a complete response (CR), but there was disease stabilization for several weeks in both patients. There was significant maintained inhibition of PNP enzyme in red blood cells, accumulation of forodesine and dGuo in plasma, and intracellular dGTP accumulation in both patients. **Conclusion:** Our preclinical and clinical investigations suggest that forodesine has activity in B-ALL. However, it needs to be either infused with dGuo or combined with established chemotherapeutic agents based on mechanistic rationale.

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Introduction

The discovery of purine nucleoside phosphorylase (PNP) deficiency, a metabolic disorder that results in congenital immunodeficiency in association with severe T-cell immune depletion, and the elucidation of its pathophysiologic characteristics provided the rationale for the development of deoxyguanosine (dGuo) analogs for leukemias.[1,2] Because dGuo is readily catabolized by PNP, pharmacologic inhibition of this enzyme manifests a deoxyguanosine

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triphosphate (dGTP)-mediated T-cell lymphopenia. Although T-cell–specific depletion was observed with dGuo, the efficient catabolism of dGuo by PNP limited its use clinically. Taken together, these data provided a rationale for the development of PNP-resistant dGuo analogs or PNP inhibitors for the treatment of leukemias.

The PNP-resistant dGuo derivative arabinosylguanine (ara-G) is toxic to T lymphoblasts and resistant to degradation by endogenous PNP.[3] Once within cells, ara-G is phosphorylated by deoxycytidine (dCyd) kinase and deoxyguanosine kinase[4,5] into ara-GTP. The resultant accumulation of intracellular ara-GTP inhibits DNA synthesis.[6] Similar to dGuo, ara-G has shown antileukemic activity in T-lymphoblastic cell lines and in the clinic[3,7,8] through a T-lymphoid lineage-specific accumulation of ara-GTP during therapy.[9,10] A clinical trial conducted by the Childrens Cancer Group and the Pediatric Oncology Group of nelarabine, a prodrug of ara-G, in refractory T-cell leukemias and lymphomas[11] demonstrated higher responses in T-ALL. Additionally, a phase II trial in patients with refractory T-cell malignancies demonstrated substantial single-agent activity, with an objective response rate of more than 50% in the subset of patients with T-cell leukemia.[8] Of note, some activity was also seen in patients with B-lineage disease.

Forodesine (also known as BCX-1777/immucillin H) was developed as an orally bioavailable novel PNP transition-state inhibitor with a low picomolar inhibitory constant for human enzymes.[12] The principal requisite for this agent to demonstrate its action relies on complete inhibition (> 95%) of the PNP enzyme and subsequent accumulation of dGTP, the intracellular metabolite of substrate dGuo. Forodesine exhibited cytotoxicity in T-cell leukemia cell lines through accumulation of large amounts of intracellular dGTP perturbing the cellular milieu.[13] This in vitro activity in cell lines provided the impetus to initiate a clinical study in patients with T-cell leukemia to investigate the pharmacokinetic and pharmacodynamic profile of this inhibitor. In agreement with its preclinical activity, forodesine demonstrated promising activity in patients with T-cell leukemia through accumulation of dGuo in plasma and dGTP in leukemia cells.[14] Forodesine is also effective against indolent B- and T-cell diseases (both in vitro[15,16] and in clinical trials),[17,18] as well as in peripheral T-cell lymphoma.[19,20]

With this background, we proposed to test forodesine in B-ALL based on the following rationale. First, akin to T cells, inhibition of PNP promotes accumulation of dGTP, subsequent imbalance in the deoxyribonucleotide triphosphates (dNTPs), and eventually cell death in B cells.[21] Second, T-cell therapies such as nelarabine have demonstrated clinical activity in B-cell leukemias.[22] Third, B cells have high levels of deoxycytidine kinase (dCK), the enzyme that facilitates the accumulation of dGTP.[21] Fourth, the rate-limiting enzyme dCK that converts dGuo to dGTP is present in high levels in pediatric lymphoblasts.[23] Finally, forodesine has demonstrated some activity in adult patients with relapsed or refractory B-ALL.[18]

The goal of the present study was to evaluate the efficacy of forodesine in B-ALL. Given that B cells contain high levels of dCK, we hypothesized that this agent could sensitize B-ALL lymphoblasts to apoptosis. Our preclinical studies with 12 pediatric patients with de novo acute leukemia (most had B-ALL [10 patients] with 1 each T-ALL and acute myelogenous leukemia [AML]) and extended studies on clinical evaluation of adult patients with B-ALL (n = 2) provide the rationale for utility of this agent in B-cell leukemias.

Patients and Methods

Drug and Other Chemicals

Forodesine for clinical use was provided by BioCryst Pharmaceuticals (Birmingham, AL). For quantitation of deoxynucleotides, dNTPs were obtained from Amersham/GE Healthcare Life Sciences (Pittsburgh, PA) and were used as standards. [^{3}H]deoxyadenosine triphosphate (dATP) and [^{3}H]deoxythymidine triphosphate were purchased from PerkinElmer Life Sciences (Waltham, MA) and MP Biomedicals (Santa Ana, CA), respectively.

Patients

This project consists of reports from 2 studies: in vitro investigations in blasts from de novo pediatric acute leukemia with forodesine (n = 12) and pharmacodynamic and pharmacokinetic evaluations of forodesine in adult patients with B-ALL (n = 2) during therapy. Among pediatric patients, the majority were patients with active B-ALL (B-ALL [n = 10]; acute myelogenous leukemia [AML] [n = 1]; acute T-cell lymphoblastic leukemia [T-ALL] [n = 1]).

Collection and Isolation of Lymphoblasts from Peripheral Blood Obtained from Pediatric Patients With B-ALL

Whole blood or bone marrow was collected in heparinized tubes from pediatric patients with leukemia at initial diagnosis. The specimen was diluted 1:3 with cold phosphate-buffered saline (PBS) (0.135 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4 [pH 7.4]) and layered onto Ficoll-Hypaque (specific gravity, 1.086; Life Technologies, Grand Island, NY). The blood was then centrifuged at 433*g* for 20 minutes, and mononuclear cells were removed from the interphase.[18] Cells were washed twice with cold PBS and resuspended in 10 mL RPMI 1640 (Life Technologies), supplemented with 10% fetal bovine serum. The isolated lymphoblasts were incubated with or without 2 μM forodesine and 20 μM dGuo in a culture incubator at 37°C. These concentrations were selected based on plasma pharmacologic data obtained during a phase I study of forodesine.[8] Cultures were maintained and aliquots (1×10^7cells/mL) were removed at the end of incubation times. A Coulter channelyzer (Coulter Electronics) was used to determine cell number and mean cell volume. These cells were measured for intracellular dNTP, macromolecule syntheses, and apoptosis levels.

Assays

Measurement of Cell Apoptosis in Response to Forodesine and dGuo. The lymphoblasts were incubated with or without 2 μM forodesine and 20 μM dGuo. Cultures were maintained and aliquots (1×10^7cells/mL) were removed at the end of incubation times (24 or 48 hours). Percent apoptosis was measured by annexin V binding assay (PharMingen, San Diego, CA) according to the manufacturer's instructions. Briefly, fresh cells were washed with PBS and resuspended in 200 μL of 1× annexin binding buffer obtained from BD Biosciences (San Jose, CA), at a concentration of