A Phase I and Pharmacokinetic Study of Dihydro-5-azacytidine (NSC 264880)

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ABSTRACT

5,6-Dihydro-5-azacytidine (DHAC; NSC 264880) is an analogue of 5-azacytidine that does not possess the hydrolytically unstable 5,6-imino bond of the parent compound. Thus, unlike 5-azacytidine, DHAC is stable in aqueous solution and may be administered by prolonged i.v. infusion, potentially avoiding acute toxicities associated with bolus administration of 5-azacytidine. In this study, patients with advanced cancer were treated with DHAC administered as a 24-h constant i.v. infusion every 28 days. Treatment began at a dose of 1 g/sq m and was escalated to the maximum-tolerated dose of 7 g/sq m, where the limiting toxicity was pleuritic chest pain. Other toxicities included nausea and vomiting, which were not limiting. There was no evidence for myelosuppression, nephrotoxicity, or hepatotoxicity. DHAC was measured in plasma, urine, and ascites by a sensitive and specific reverse-phase high-performance liquid chromatography assay capable of detecting 50 ng of drug per ml. Steady-state plasma levels were achieved with 8 h and ranged from 10.0 to 20.5 µg of DHAC per ml at the maximum-tolerated dose. Total-body clearance of 311 ± 76 ml/min/sq m and postinfusion half-lives between 1 and 2 h were observed. Between 8 and 20% of the administered dose was excreted unchanged in urine. While ascites DHAC levels were comparable to plasma levels, postinfusion elimination was slower from this compartment than from plasma. No correlation was observed between DHAC plasma levels and duration or intensity of dose-limiting pleuritic chest pain. One patient with progressive Hodgkin’s lymphoma demonstrated stabilization of disease for seven treatment cycles, and two patients with aggressive lymphoma demonstrated dramatic, although transient, disease responses. A dose of 7 g/sq m is recommended for Phase II trials of DHAC using this schedule.

INTRODUCTION

The pyrimidine nucleoside analogue 5-AC\(^2\) (NSC 102816) was originally synthesized by Piskala and Sorm (1) as a potential inhibitor of nucleic acid biosynthesis. The drug is a ring analogue of cytidine and has been shown to interfere with both RNA and DNA synthesis (2). In clinical trials, 5-AC has demonstrated antileukemic activity, inducing complete remissions in a significant number of heavily pretreated patients with acute myelogenous leukemia (3, 4). The dose-limiting toxicity of 5-AC is leukopenia, with less prominent thrombocytopenia. In addition, i.v. bolus treatment with 5-AC may induce severe gastrointestinal symptoms, fever, and occasional life-threatening hypotension (3, 5). These acute toxicities can be ameliorated or eliminated by administering the drug slowly via constant i.v. infusion (6, 7). However, 5-AC is unstable in aqueous solution, making strict dosage control in infusional therapy cumbersome (8–10).

To circumvent the problem of aqueous instability, Beisler and coworkers reduced the hydrolytically susceptible 5,6-imino double bond of 5-AC to produce DHAC (11)(Chart 1). This compound demonstrates excellent aqueous solubility and is stable in solution over a broad pH range.

In preclinical antitumor screening studies, DHAC demonstrated reproducible activity against murine L1210 and P388 leukemia. Screening in solid tumor models has confirmed activity against the human MX-1 mammary xenograft, the murine CD8F mammary tumor, and the s.c. implanted colon 38 tumor (12).

The mechanism of action of DHAC is presumed to be similar to 5-AC (13). Both drugs are incorporated into nuclear RNA, inhibit the methylation of ribosomal and transfer RNA, and inhibit the transcription of ribosomal and nuclear RNA (14). The overall effect is a decrease in the synthesis of methylated bases into RNA and impaired protein synthesis. Similar findings using both DHAC and 5-AC have been reported in Friend leukemia and Chinese hamster ovary cells and confirm drug-induced inhibition of RNA and protein synthesis as well as DNA synthesis (15). Both drugs are cell cycle specific, with S-phase cells most sensitive and stationary cells relatively drug resistant.

We report here the results of a Phase I trial of DHAC, including pharmacokinetic data.

MATERIALS AND METHODS

Patient Selection. Patient characteristics are shown in Table 1. Thirteen patients, 5 men and 8 women ranging in age from 27 to 69 years, were entered into study. All patients had pathological confirmation of cancer and had received prior chemotherapy. Prior to beginning treatment with DHAC, each patient underwent a comprehensive evaluation including complete history and physical examination and evaluation of measurable disease by the appropriate modality (physical examination, X-ray, or scan). Pretreatment evaluation also included complete blood count, with WBC differential, serum chemistries, and creatinine clearance. Complete blood counts were followed weekly while the patient was on study, and other parameters were repeated on Day 1 of each cycle. All patients had adequate pretreatment renal function as defined by a creatinine clearance of 60 ml/min or greater and serum creatinine less than 1.8 mg/dl, normal hemogram with WBC >3000/cu mm, platelet count >100,000/cu mm, normal hepatic function with bilirubin <2 mg/dl, and serum glutamicoxaloacetic transaminase <100 units/ml. All patients gave written, informed consent prior to therapy.

Drug Formulation and Dosage. DHAC was supplied as its hydrochloride salt by the Investigational Drug Branch, National Cancer Institute, Bethesda, MD. The drug was provided in 20-mL vials containing 500 mg of lyophilized DHAC powder with 300 mg of mannitol. When reconstituted with 300 mg of mannitol. When reconstituted with...
with 9.6 ml of sterile water for injection, United States Pharmacopeia, each ml contained 50 mg of DHAC and 30 mg of mannitol. The prescribed dose was further diluted in 500 ml of 5% dextrose and water for administration by constant infusion. When reconstituted as directed, the solution of DHAC exhibited little or no decomposition under these conditions for at least 24 h at room temperature (12).

The starting dose of DHAC was 1000 mg/m² administered by constant infusion over 24 h via an external pump (1-Med Model 927 infusion pump; 1-Med Corp., San Diego, CA). Dose escalation was performed using a modified Fibonacci search scheme, with 3 patients entered at each dose level before escalation was performed. No patients were entered at 3.3 mg/m², as this dosage had been safely tested in other ongoing clinical studies. No dose escalation was performed in individual patients. Treatment was continued for at least 2 cycles unless contraindicated by progressive disease or unacceptable toxicity.

In Vitro Stability. Blood was obtained from a normal human volunteer by venipuncture and centrifuged for 15 min at 400 x g in a Dynac tabletop centrifuge (Clay Adams; Becton Dickinson Co., Parsippany, NJ). The plasma was decanted and used immediately. The appropriate volume of 1.24 x 10⁻² M (3.5 mg/ml) DHAC was added to aliquots of plasma so that the final concentration was either 4.5 or 10 µg/ml. The cytidine deaminase inhibitors THU (16) and 1-arabinofuranosyl-1,3,4,7-tetrahydropyrimidine-2-one (17) (NSC 322096; Chart 1) were then added to deaminase inhibitors THU (16) and 1-/J-D-ribofuranosyl-1,3,4,7-tetrahydropyrimidine-2-one (17) (NSC 322096; Chart 1) were then added to deaminase inhibitors THU (16) and 1-/J-D-ribofuranosyl-1,3,4,7-tetrahydropyrimidine-2-one (17) (NSC 322096; Chart 1) were then added to deaminase inhibitors THU (16) and 1-/J-D-ribofuranosyl-1,3,4,7-tetrahydropyrimidine-2-one (17) (NSC 322096; Chart 1). The plasma was then decanted, frozen in dry ice, and stored at -20°C until analyses. Spontaneously voided urine was collected throughout the infusion period and for up to 24 h following the end of infusion. Urine volume was measured, and a 100-ml aliquot (or the entire amount if the volume was less) was frozen in dry ice and likewise stored at -20°C until analysis. Ascites was sampled in one patient via a Tenckhoff catheter immediately before treatment, at the end of the infusion, and 4 h postinfusion. These samples were treated in the same manner as plasma.

DHAC concentrations in plasma, urine, and ascites were measured by a modification of a previously reported HPLC assay (18). Urine was diluted 100X or 10X depending on whether the sample was obtained during treatment or after the end of the infusion. To a 0.5-ml aliquot of each sample were added 20 µl of 6.23 x 10⁻⁴ M (3.3 µg) 5'-chloro-5'-deoxy-5,6-dihydro-5-azacytidine as an internal standard. The sample was then diluted with an equal volume of distilled water and ultrafiltered by centrifugation at 1000 x g for 45 min in a Centrifree micropartition system (Amicon Corp., Danvers, MA). One-half ml of the resulting ultrafiltrate was diluted with 5.0 ml of water, and this solution was added to an 8- x 30-mm glass column of SP-Sephadex C-25 cation exchange resin (Pharmacia Fine Chemicals, Piscataway, NJ) in the NH₄⁺ form. The column was then washed with 20 ml of water and eluted with 15 ml of NH₄OH, pH 9.25. The NH₄OH eluate was filtered through a 0.45-µm Millipore-HA disposable filter unit (Millipore Corp., Bedford, MA), concentrated to approximately 2-ml volume, and transferred to a 10-ml round-bottom flask. This solution was evaporated to dryness, a Teflon-coated 8-mm stirring bar was added to the flask, and the residue was dried in vacuo for 10 min. A hot air blower was used to warm the flask for the last 2 min to ensure complete drying. Chromogenic derivatization and HPLC analysis were carried out as described previously (18).

Standard curves for DHAC in plasma or urine were prepared for each patient's samples by addition of known amounts of DHAC to the corresponding pretreatment plasma or 10X-diluted pretreatment urine. These spiked standards were processed in the same manner as above. Appropriate standard curves were constructed for each day's analysis and typically covered the range 0 to 20 µg/ml for plasma and 0 to 50 µg/ml for urine. These curves were the best straight lines defined by least-squares regression analysis and possessed correlation coefficients greater than 0.998. The limit of quantitation (S/N = 5) for this modified procedure using only one column chromatography step was 0.5 µg/ml, although smaller amounts of DHAC could be detected. The plasma standard curve was also used for the ascites samples. For the few samples that were below the limit of quantitation of the modified method, the previously described 2-column workup procedure was applied (18). The limit of quantitation for this more time-consuming assay was 50 ng/ml.

Kinetic Calculations. Cₘₐ₅ was the average of measured DHAC plasma levels once these concentrations reached a plateau at about 8 h into the infusion. The apparent total-body clearance was then defined as equal to the rate of infusion divided by Cₘ₅. The postinfusion Cₘ₅ versus time data were then fit to the biexponential function representing a 2-compartment open model by an iterative nonlinear least-squares regression through the MLAB computer program (19).

RESULTS

Toxicity. Thirteen patients were treated with DHAC during this study for a total of 26 cycles of therapy. All patients were evaluable for toxicity; there were no drug-related deaths.
Dose-limiting toxicity consisted of severe pleuritic chest pain which first emerged at a dose of 5000 mg/sq m and became dose limiting at 7000 mg/sq m. Severe chest pain was reported by 4 of 5 patients treated at the MTD (Table 2). This toxicity uniformly occurred 16 to 20 h into the infusion and persisted for 12 to 24 h after the end of the infusion. Pain was not alleviated by pretreatment with nonsteroidal antiinflammatories (ibuprofen, indomethacin) and required treatment with narcotic analgesics. By 24 h after the completion and treatment, narcotics were no longer required, and pain abated without specific intervention. No evidence of either cardiac or pulmonary infarction was detected by serial chest X-rays, electrocardiographs, and cardiac enzymes, arterial blood gases, or technetium cardiac scanning. Nausea and vomiting were first reported by patients receiving a dose of 5000 mg/sq m but were never dose limiting. At the MTD, only one of 5 patients developed Grade 3 gastrointestinal toxicity, but in other patients who developed nausea and vomiting at the MTD, symptoms could be controlled by phenothiazine antiemet-
ics.

No other toxicities were observed. Specifically, there was no evidence of myelosuppression, hepatic toxicity, allergic reaction, hypotension, or nephropathy.

Clinical Responses. Dramatic (>50%) reductions in palpable adenopathy were observed in 2 patients with heavily pretreated diffuse histiocytic lymphoma who both received DHAC at the MTD. However, in both of these patients, disease progressed prior to the initiation of Cycle 2, precluding further therapy. In addition, a patient with progressive Hodgkin’s disease refractory to 4 different combination chemotherapeutic regimens stabilized for 7 months on single-agent DHAC.

In Vitro Stability. DHAC was unstable in human plasma when it was incubated at the clinically achievable concentrations of 4.5 or 10 μg/ml (Chart 2). The addition of THU such that the plasma concentration was 1 x 10^-4 M or 1-|8-D-ribofuranosyl-1,3,4,7-
tetrahydro-2H-1,3-diazepin-2-one at 2 x 10^-5 M completely inhib-
ted this decomposition. Accordingly, the more potent diazepinion

PHASE I DHAC (NSC 264880)

Table 2

<table>
<thead>
<tr>
<th>Dose (mg/sq m)</th>
<th>No. of patients/cycles</th>
<th>Nausea (grade)*</th>
<th>Chest pain (grade)*</th>
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</thead>
<tbody>
<tr>
<td>1000</td>
<td>2/6</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>2000</td>
<td>3/4</td>
<td>0 0 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>5000</td>
<td>3/10</td>
<td>0 2 1</td>
<td>0 1 1</td>
</tr>
<tr>
<td>7000</td>
<td>5/6</td>
<td>2 3 1</td>
<td>0 1 5</td>
</tr>
</tbody>
</table>

* Grade 1, nausea only; Grade 2, transient vomiting; Grade 3, vomiting requiring treatment.

6 Grade 1, chest discomfort not requiring treatment; Grade 2, pain relieved by narcotic analgesics; Grade 3, chest pain decreased but not completely relieved by narcotic analgesia.

Chart 2. Stability of DHAC in human plasma at 37°C. Fresh human plasma was spiked with DHAC hydrochloride at either 4.5 μg/ml (1.6 x 10^-4 w) or 10 μg/ml (3.5 x 10^-4 w) and incubated as indicated in “Materials and Methods.” Solid lines (□, △) depict plasma decomposition in the absence of cytidine deaminase inhibitors. Dotted lines represent the corresponding plasma concentrations in the presence of 2 x 10^-4 w diazepinone riboside, NSC 322096 (Δ). Both the diazepinone riboside and THU inhibited in vitro decomposition at both DHAC concentrations; the data obtained for 1 x 10^-4 w THU were superimposable with those shown for NSC 322096.

Between 8.0 and 20.5% of the administered dose of DHAC could be recovered unchanged in the urine. The vast majority of unchanged drug was excreted during the infusion period with only 1 to 4% appearing after treatment had stopped. Although the data for DHAC urinary excretion are limited and skewed toward the highest doses, there does not seem to be any correlation of the percentage of drug excreted with dose.

DHAC levels were also measured in ascites samples obtained from Patient 6, who had ovarian cancer metastatic to the peritoneum and diaphragm. Comparable levels of drug were seen in the plasma and ascites at the end of the infusion, with concentrations of 20.5 and 20.4 μg/ml, respectively, being measured. However, DHAC removal from the ascites was slower, since by 4 h postinfusion, 1.81-μg/ml plasma concentration was attained, while the ascites level was more than twice as high at 4.06 μg/ml.

DISCUSSION

DHAC is the dihydro analogue of 5-AC, a nitrogen isostere of cytidine. Although Phase I studies with 5-AC demonstrated objective responses in patients with breast cancer, carcinoma of the colon, and melanoma (20), tolerance to bolus therapy was limited by acute toxicities (nausea, vomiting, diarrhea, fever, and hypotension), while more prolonged infusional therapy was limited by drug instability. Thus, the clinical use of 5-AC has been restricted to acute myelogenous leukemia where consistent antitumor activity has been documented in patients with heavily pretreated disease (4). DHAC was specifically synthesized to preclude drug decomposition in solution via opening of the triazine ring at the hydrolytically susceptible 5,6-imino bond. As predicted by design, DHAC is highly stable in solution. When
formulated as directed and diluted in 5% dextrose and 0.9% sodium chloride solution, the compound shows 1 to 2% decomposition over 2 days at room temperature. Under similar conditions, 5-AC would exhibit 16 to 21% decomposition in only 6 h (9). Because of this enhanced stability, DHAC could be administered as a constant infusion over 24 h to patients in this Phase I study.

It was originally hypothesized that DHAC functioned as a prodrug, being converted to 5-AC in vivo. This was suggested by studies demonstrating cross-resistance in 5-AC-resistant L1210 (11). However, preclinical pharmacological studies failed to demonstrate any in vivo conversion to 5-AC. DHAC was extensively metabolized in the rat following bolus injection with only 30% being excreted unchanged in urine (21). Neither 5-AC nor its principal hydrolysis products were detected by HPLC, and subsequent gas chromatography-mass spectroscopy analysis identified only the deamination products 5,6-dihydro-5-azauracil, 5,6-dihydro-5-azauridine, ammelide, and cyanuric acid as the principal metabolites. Similarly, our studies demonstrate that DHAC is extensively metabolized in humans during and following constant i.v. infusion over 24 h; 8 to 19% of the administered dose was excreted unchanged in the urine over the course of the 24-h infusion. Postinfusion urine did not account for more than an additional 4% of the total dose. DHAC is an excellent substrate for cytidine deaminase (22), and the fact that the drug is deaminated in humans is confirmed by inhibition of DHAC decomposition by adding diazepinone riboside (NSC 322096) to patient samples. Indeed, DHAC plasma stability appears to be related to levels of this enzyme even though relatively little cytidine deaminase is found in plasma. For BALB/c x DBA/2 F1 mice, which have practically no plasma cytidine deaminase and very low overall levels, an in vitro plasma half-life of about 42 h was observed (23). This contrasts with the 4-h half-life observed in this study for human plasma, which has much higher levels of cytidine deaminase (16).

The dose-limiting toxicity of DHAC, pleuritic chest pain, is an unusual toxic end point (24), and the clinical presentation and pharmacokinetic parameters suggest that it may not be a direct drug effect. No correlation was observed between plasma DHAC blood levels and the intensity of the chest pain. Moreover, while steady-state plasma levels were achieved within 8 h, chest pain was not reported by any patient until 16 to 20 h into the infusion; and while the postinfusion half-life of drug was 1 to 2 h, chest pain persisted for as long as 24 h after the end of treatment. These data suggest that this toxicity might be mediated by a metabolite.
Encouraging clinical activity was observed during this Phase I study with 2 patients with diffuse histiocytic lymphoma demonstrating >50% reductions in palpable adenopathy after treatment at the MTD. However, disease progression in both patients prior to initiation of Cycle 2 suggests that DHAC may need to be administered more frequently than every 28 days. In addition, an extensively pretreated patient with progressive Hodgkin’s disease stabilized for 7 months at a DHAC dose of 5 g/sq m.

In summary, DHAC is an analogue of 5-AC that was synthesized to overcome the aqueous instability of the parent compound and allow precise dosing with infusional therapy. The drug is extensively metabolized in humans and is rapidly deactivated in plasma; less than 20% of the administered dose is excreted unchanged in urine. The recommended dose for Phase II studies with DHAC using a 24-h infusion is 7 g/sq m. Priority should be given to those diseases in which the parent compound has reproducible antitumor activity.

REFERENCES


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