Fetal Hemoglobin Gene Activation in a Phase II Study of 5,6-Dihydro-5-azacytidine for Bronchogenic Carcinoma

Brian I. Carr, Samuel Rahbar, James H. Doroshaw, Douglas Blayney, David Goldberg, Lucille Leong, and Yayesh Asmeron

MATERIALS AND METHODS

Adult patients with histologically proven unresectable non-small cell bronchogenic carcinoma who had bidimensionally measurable lesions and no prior chemotherapy were treated. Patients had normal hepatic, renal, and bone marrow function and were excluded from the study if they had cardiac or pleural effusions or a pleural or pericardial friction rub prior to DHAC therapy, in order to assess toxicities. DHAC at a dose of 1650 mg/m²/day was administered as a continuous i.v. infusion for 5 days in 5% dextrose:0.9% NaCl solution. Treatment cycles were repeated every 21 days. Base-line investigations included measurement of tumor size and fetal hemoglobin levels, and pre- and posttreatment values were obtained from arterial blood gases on room air, pulmonary function tests, echocardiogram, ventilation/perfusion lung scan, EKG, cardiac enzymes, MUGA cardiac heart scan, antinuclear antibody, and chest X-rays as well as routine values for complete WBC count and liver and renal function studies. HbF levels (normal range in adults, 0.2 to 1.0%) were determined before and after each cycle of treatment by the alkali denaturation method (22), exactly as described (23). Briefly, a hemolysate of peripheral venous blood was converted to cyanferrihemoglobin and then treated for exactly 2 min with NaOH solution, and after neutralization with ammonium sulfate, the precipitated adult hemoglobin was removed and the fetal hemoglobin which remained in solution was quantitated by spectrophotometry at 540 nm. Day-to-day variation of controls in the assay was 0.75 ± 0.45% (mean ± SD) and was measured using fresh hemolysate from one of the investigators (S. R.). All reagents were freshly made before each assay. In addition, the presence of fetal hemoglobin in the hemolysates was checked by cellulose acetate electrophoresis on Titan III plates at pH 8.6 (24). In selected patients, the percentage of F-cells (normal adult values, 0.4 ± 0.4%; Ref. 25) was determined in the peripheral blood smears using a monoclonal antibody against fetal hemoglobin (26). Criteria for tumor response were exactly as published (27).

RESULTS

The patient characteristics, response data, major toxicities, and laboratory abnormalities are shown in Table 1. Fourteen patients with advanced, unresectable non-small cell bronchogenic carcinoma were treated with DHAC. Four patients were not evaluable for disease response due to rapid clinical deterioration within the first cycle of therapy (1 patient) or refusal of further chemotherapy (3 patients). No objective partial or complete responses were observed. Five patients had stable disease lasting 3 or more mo. Twelve of the 14 patients had chest pain requiring analgesia. Because of the pleuritic nature of the chest pain, an additional 3 patients with mesothelioma were also treated with DHAC. Of the 2 mesothelioma patients who were evaluable for response, both had stable disease for 3 mo and 6 mo, respectively. All 33 mesothelioma patients had DHAC-induced chest pain.

For all patients on the study, the chest pain was central and nonradiating in character and usually required narcotic analgesia for its control. After the first 4 patients received their initial cycles of DHAC chemotherapy, all patients were treated with morphine sulfate (1 to 2 mg/h) by continuous i.v. infusion throughout the 5 days of their DHAC chemotherapy. No patient required more than 2 mg/h of morphine sulfate for adequate control of the chest pain induced by DHAC. EKGs were obtained in all the patients at the beginning and end of each cycle.
PHASE II DHAC

Table 1 5,6-Dihydro-5-azacytidine study: patient characteristics, disease response, and main toxicities

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Histology*</th>
<th>No. of treatment cycles</th>
<th>Disease response</th>
<th>Chest pain</th>
<th>EKG changes</th>
<th>Elevated cardiac</th>
<th>LDH*</th>
<th>ANA*+</th>
<th>Increased HbF</th>
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*Bronchogenic carcinoma, unless otherwise specified.

† Levels observed at peak of HbF synthesis.

‡ ANA, antinuclear antibody; NE, nonevaluable.

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course of therapy. EKG changes were not associated with the onset of the chest pain, but nonspecific ST-T wave elevation or inversion occurred in 12 of the 17 patients treated, from 1 to 3 wk after the onset of chest pain. Elevations in cardiac lactate dehydrogenase isoenzyme levels were found in 5 of the patients, occurring from 1 to 2 wk after the initiation of DHAC infusion. One patient (Table 1, No. 10) developed severe chest pain and abdominal pain. The chest pain cleared spontaneously at the end of the first course of chemotherapy, but the abdominal pain did not. Because of clinical signs consistent with appendicitis, despite a normal complete blood count and blood biochemistry profile (Sequential Multiple Analyzer 18), the patient underwent an exploratory laparotomy. The appendix was removed, and no evidence of abdominal adenopathy or peritoneal fluid was found. Microscopic examination revealed a normal appendix; only focal fibrosis and mesothelial cell proliferation were found in a biopsy taken from the peritoneum. The patient's abdominal tenderness resolved completely postoperatively. The patient received a second course of DHAC at a 50% dose reduction with only mild chest pain and without any recurrence of the abdominal pain. He then received an additional three courses of therapy with full doses of DHAC without any recurrence of the abdominal pain and with only mild pleuritic-type chest pain. The pleuritic pain was usually observed only on the second or third day of each treatment cycle. It invariably resolved rapidly on cessation of DHAC chemotherapy.

No abnormalities were found in the blood gases, the ventilation perfusion (V/Q) lung scans, or the MUGA cardiac scans of any of the patients on this study. Antinuclear antibodies were measured in all patients before and after each course of treatment. Four patients developed positive antinuclear antibodies (levels greater than 1:40 dilution) during the course of their DHAC chemotherapy, which did not disappear after cessation of DHAC treatment. The patterns were: homogeneous (2 patients); speckled (1 patient); and nucleolar (1 patient). Twelve of the 17 patients experienced nausea, but emesis occurred in only 2 of these. No significant hemopoietic, hepatic, or renal toxicities were found.

Fetal hemoglobin levels were measured in the peripheral blood in all patients at the beginning and end of each course of DHAC therapy (Table 2). Eleven of the 17 patients developed an increase of fetal hemoglobin synthesis and an increase in the proportion of F-cells in the absence of clinically significant anemia. Five of the 6 patients who did not increase their HbF values only had 1 cycle of DHAC treatment. The time course for the increase in fetal hemoglobin levels is shown for 5 patients who developed large increases (Fig. 1). In one patient (No. 13), increased HbF occurred during the first course of treatment. In other patients (Nos. 6, 9, 10, and 12) two or more cycles of therapy were needed to induce increased HbF synthesis (Fig. 1). Twelve control patients with bronchogenic carcinoma had received standard chemotherapeutics (mainly Adriamycin and cis-platinum) had fetal hemoglobins (mean ± SD) of 0.75 ± 0.13 %, and they had no detectable time-dependent change in their fetal hemoglobin measurements.

In summary, 17 patients were treated with DHAC. Of the 12 patients who were evaluable for disease response, stable disease was found in 5 of 10 evaluable with bronchogenic carcinoma and 2 of 2 evaluable with mesothelioma. No objective disease responses were seen. Transient, pleuritic-type chest pain was observed in 15 of the 17 patients, positive antinuclear antibody tests were found in 4, and 13 patients developed an increased synthesis of fetal hemoglobin.

DISCUSSION

This study reports the therapeutic results of 5-6-dihydro-5-azacytidine treatment in 14 patients with advanced unresectable bronchogenic carcinoma and 3 patients with mesothelioma. No tumor responses were observed, although 5 patients with bronchogenic carcinoma and 2 patients with mesothelioma had
disease stability for 2 or more mo. The 3 patients with mesothelioma were chosen for evaluation of disease response because of the pleuritic chest pains that were seen in the patients treated with bronchogenic carcinoma. It was reasoned that a chemical pleuritis-type reaction might result in antitumor action in patients with mesothelioma. However, no clear tumor responses were seen in any of the mesothelioma patients.

The chest pain which occurred in 15 of the 17 treated patients had several peculiarities. It usually occurred only on the second or third day of treatment, and it disappeared within hours of cessation of therapy. It was pleuritic in nature and could be made tolerable by concomitant continuous infusion of morphine sulfate. There was no radiation of the pain, and the transient elevations of the LDH level and the electrocardiographic changes did not occur until several days after the onset of DHAC chemotherapy. No accompanying abnormalities were found in the lung or heart scans or arterial blood gases. The seroconversion of 4 patients to positive antinuclear antibody production was interesting. However, this unexplained result was not associated with any skin reaction, vasculitis, or arthralgias and did not correlate well with the extent of the chest pains.

Fetal hemoglobin levels were measured in all of our patients because of the known ability of DHAC and other analogues of 5-azacytidine to inhibit DNA methylation and to increase gene expression in a variety of experimental conditions (3, 16, 28), including an increased fetal hemoglobin production in anemic baboons (17) and humans (18). It was therefore of interest that, despite the absence of clinically significant anemia, 11 of the patients increased their fetal hemoglobin production, although not to the extent seen in anemic patients treated with 5-azacytidine (18). This study shows that continuous infusion of DHAC at 1650 mg/m²/day for 5 days is clinically toxic, but is capable of inducing increased levels of fetal hemoglobin. It is thus possible that lower and less toxic doses might be capable of inducing fetal hemoglobin production which could be useful in the treatment of patients with hemoglobinopathies. DHAC does not appear to be converted to 5-azacytidine in vivo (29), and unlike 5-azacytidine (30–32), DHAC does not appear to be a carcinogen. Since several other analogues of 5-azacytidine have also been shown to inhibit DNA methylation and increase gene expression (3), they might usefully be tested for their clinical gene-activating properties.

REFERENCES


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