Clinical and Pharmacologic Effects of High Dose Single Agent Busulfan with Autologous Bone Marrow Support in the Treatment of Solid Tumors


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ABSTRACT

A Phase I-II clinical trial of high dose single agent busulfan (16–20 mg/kg) administered over a 4-day period was undertaken. Pharmacokinetic measurements reveal that steady state total plasma busulfan levels between 2 and 10 μM were achieved by the second day and maintained through the remaining treatment period. Urinary excretion of mutagenic activity monitored by the Salmonella mutagenesis assay persisted for up to 48 h following the last dose of busulfan. The treatment showed specificity for myelocytic precursors as evidenced by selective depression of granulocytes with relative sparing of lymphocytic elements, and by differences in DNA damage as measured by a nucleoid sedimentation assay. Dose limiting toxicity was mucositis, anorexia, and hepatic toxicity. Transient autoimmune disorders were observed in three of the six patients. Partial responses were seen in two of five patients with melanoma, but these lasted for only 2 and 3 months. High dose busulfan represents an alkylating agent with marked myelocytic selectivity and may be useful for inclusion in intensive combination regimens.

INTRODUCTION

The administered dose is a major prognostic factor in effective therapeutic regimens for both solid tumors and leukemias (1). Because the major dose-limiting toxicity of many anticancer agents is myelosuppression, attempts have been made to ameliorate this toxicity by bone marrow infusions from autologous, syngeneic, or allogeneic sources (2, 3). This technique has permitted modest dose escalations (2–15 μM) before dose-limiting nonmyelosuppressive toxicities occurred (4), as well as improvements in objective response rates in resistant solid tumors (5–7). However, these responses have, in general, been partial and not durable, emphasizing the need for more effective agents.

Busulfan is an alkylating agent of the alkyl alkane sulfonate class which is widely used in the treatment of chronic myelogenous leukemia. However, clinical trials in solid tumors at standard doses have been limited (8). Trials of high dose busulfan and cyclophosphamide with allogeneic bone marrow transplantation in the treatment of acute leukemia have demonstrated that substantial dose escalation in the administered dose of busulfan can be achieved before dose-limiting mucositis is encountered (9), and that the therapeutic efficacy is equivalent, if not superior to cyclophosphamide and total body irradiation as a preparative regimen (10). The maximum tolerated dose of busulfan as a single agent with bone marrow support has not been established, nor its efficacy at high doses in solid tumors.

The present trial was undertaken to establish the maximum tolerated dose, toxicity, and pharmacology of busulfan as a single agent and to evaluate the hematological, antitumor, and immunosuppressive effects of high dose busulfan with autologous bone marrow support in patients with resistant solid tumor malignancies. In addition, the urinary excretion rate of mutagenic metabolites and effects on DNA structure were studied.

MATERIALS AND METHODS

Patient Population

Six patients with measurable metastatic melanoma or hepatocellular carcinoma were treated with two-dose escalations of busulfan (Table 1). No patient had evidence of bone or bone marrow involvement with tumor at the time of marrow harvest or evidence of central nervous system metastatic disease. Two patients each had received prior chemotherapy or local radiotherapy; all except one had visceral disease. Pretherapy evaluation included physical examination, liver chemistries, pulmonary function testing including DLCO, and lymphocyte subset analysis.

Treatment Protocol

Bone marrow was aspirated from bilateral posterior iliac crests under general anesthesia using 12-gauge modified Rosenthal needles and anticoagulated with heparin and TC-199 (GIBCO, Grand Island, NY). Buffy coat concentration was performed using a Cobe 2991 cell washer and cryopreserved in 10% dimethyl sulfoxide and 20% autologous plasma. A mean of 1.58 × 10^8 cells/kg were cryopreserved. All patients had double-lumen silastic catheters inserted. Patients were treated in private rooms with access via reverse isolation and provided a low bacterial, low fungal content diet.

Busulfan was administered over 4 days (day –6 to day –3) in 16 doses given orally every 6 h. The first two patients received each 1 mg/kg dose as commercially available pills, either whole or crushed. Subsequent patients received doses as gelatin capsules prepared from bulk busulfan (Burroughs-Wellcome, Ltd., Research Triangle Park, NC). Perphenazine was used as the sole antiemetic; steroids or barbiturates were not administered during chemotherapy to any patient because of possible effects on alkylating agent pharmacokinetics. Two days following completion of chemotherapy, autologous bone marrow was rapidly thawed and i.v. infused. Toxicity and response are reported from day of marrow infusion. Red blood cells were transfused for a hematocrit less than 42% (11), and platelets transfused to maintain a platelet count above 20,000/mm^3.

Salmonella Mutagenesis Assay

Tester strain TA100 was obtained from Dr. Bruce Ames. Urine specimens from initiation of treatment until the time of bone marrow infusion were filter sterilized (0.22 μm; Millipore, Waltham, MA) and stored frozen at –70°C. The urine was diluted 1 to 250, and 0.3 cc of urine mixed with 0.1 cc overnight culture of TA 100 as previously described (12, 13). After 72 h of incubation at 37°C colonies were counted and corrected for spontaneous reversion frequency.

Nucleoid Sedimentation Assay

Sedimentation of the nuclei of lymphocytes anduffy coat cells derived from patients treated with busulfan were performed as previously described (14, 15). Lymphocytes were prepared from whole blood by sedimentation through Ficol-Hypeaque (LSM), and cryopreserved with 20% autologous plasma and 10% dimethyl sulfoxide until use.
Buffy coat cell collections were obtained from 10 cc of peripheral blood centrifuged in a RC-3B HB-4 rotor at 2000 rpm for 10 min and cryopreserved in a similar manner. Lymphocytes obtained in this manner were thawed at 37°C, washed with media containing 20% fetal calf serum. Buffy coat cells were rapidly thawed at 37°C and applied directly to the sucrose gradient described below. Rapid processing and layering of the BC preparations was essential to obtaining reproducibility with granulocyte preparations. Lymphocytes or BC specimens obtained as above were directly applied to a 14-ml 15-30% sucrose gradient containing 30 μg/ml ethidium bromide (Sigma Chemical Co., St. Louis, MO) overlayed with buffer containing 0.1% Triton-X-100, 2.0 m sodium chloride and Tris-HCl, pH 8.0. After lysis for 15 min at room temperature, the cells were sedimented through the sucrose gradient and AH-627 (Sorvall) rotor, 25,000 rpm for 120 min (lymphocytes) or 45 min (BC). The distance sedimented by nucleoids was directly measured via short-wave UV fluorescence, and compared to sedimentation of a pretreatment sample.

Pharmacokinetics

Plasma samples were obtained from three patients via indwelling venous catheters in heparinized tubes (Vacutainer) at 0, 30 m, 60 m, 90 m, 120 m, and 240 m after the first, 5th, 9th, and 13th doses. Samples were centrifuged and 5 ml was frozen and shipped on dry ice to Baltimore (L. B. G., M. C.) and Boston (W. D. H.) for analysis. Plasma concentrations were measured using two independent methods described elsewhere (16). Concentrations were fit to a one-compartment model with first order absorption adjusted for multiple dosing using PCNonlin (Statistical Consultants, Lexington, KY) for the analysis done in Baltimore and using MLAB (National Cancer Institute, Division of Research Resources) for the analysis done via Boston methodology.

Toxicity

Acute Toxicity. There were no complications in the marrow harvesting and reinfusion procedure. Administration of the busulfan was uneventful. Two patients experienced minor episodes of nausea and vomiting which did not require readministration of drug. No seizures occurred during drug administration. Two patients (UTN 7 and 35) developed unexplained acute pain at the sites of tumor shortly after drug administration which resolved after several days.

Hematological Effects. Severe myelosuppression (Polymorphonuclear cell < 100/mm³) followed administration of busulfan at both dose escalations. The median polymorphonuclear cell count following administration of high dose busulfan and autologous bone marrow reinfusion is shown in Fig. 1A. Severe granulocytopenia resolved by a median 16 days from marrow reinfusion. No dose effect on leucopenia was seen between the two dose escalations examined.

Fever was seen during neutropenia in all patients; however, no blood cultures were positive. Three patients developed oral candidiasis and one patient developed a fatal systemic infection with Candida albicans. Viral infections were not documented.

Immunological Toxicity. The median absolute lymphocyte count remained unchanged throughout therapy (Fig. 1B). Further, measurement of T-cell subsets by fluorescence-activated cell sorting revealed no significant change in the number of T4, T8, Ia, Bl, T3, or T11 positive lymphocytes or the T4/T8 ratio at any time following therapy. This finding is in marked contrast to our experience with high dose combination alkylating agent regimens where there is a decrease in T4 and B1 positive cells, an increase in T8 and Ia positive cells and an inversion of T4/T8 ratio which develops soon after therapy and persists for 6 to 12 months (17).

Three patients developed unusual "immunological syndromes" following therapy. One patient (UTN 6) developed a heliotrope rash and a severe sero-negative arthritis requiring transient steroid therapy 20 days following bone marrow infusion. This patient also developed antigranulocyte and antiplatelet antibodies and required steroid treatment for 2 weeks to reverse neutropenia. A third patient (UTN 30) developed chronic active hepatitis beginning 18 days from marrow infusion which resolved over 2 months without specific therapy. In this case, antimitochondrial and anti-smooth muscle serologies were negative.

B-Cell function appeared intact with patients retaining the ability to

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Unique treatment number</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Sites</th>
<th>Prior therapy for metastases</th>
<th>Busulfan dose (mg/kg)</th>
<th>Response</th>
<th>Duration (days)</th>
<th>Tumor volume (cm³)</th>
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<tr>
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<td>26</td>
<td>M</td>
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<td>Nodes</td>
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<td>16</td>
<td>NR</td>
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<td>BOLD, DBD, XRT</td>
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<td>NE</td>
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<tr>
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<td>PR</td>
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<td>21.4</td>
<td>3.2 (20)</td>
</tr>
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</table>

Table 1. Patient characteristics

* XRT, radiation therapy; BOLD, bleomycin, vincristine, lomustine, dacarbazine; DBD, dibromodulcitol.
* NR, no response; NE, not evaluable; PR, partial response.
* Day from bone marrow reinfusion of maximum response is given in parentheses.

Fig. 1. A, median absolute polymorphonuclear cell (PMN) count following high dose busulfan from the day of bone marrow reinfusion. B, median absolute lymphocyte count (ALC) following high dose busulfan from the day of bone marrow reinfusion.

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mount antibody response against Herpes and cytomegalovirus antigens following therapy. Sensitivities to specific challenge antigens were not tested.

Nonhematological Toxicity. Dose-limiting toxicity in the trial was a diffuse mucositis with accompanying diarrhea (Table 2). The mucositis was more severe at 20 mg/kg, requiring narcotic administration for pain and precluding oral food intake for more than 2 weeks. Frequent diarrhea also accompanied the mucositis and all patients required i.v. fluid supplementation. While nausea and vomiting was not a feature of the acute drug administration, five of six patients subsequently developed nausea, vomiting, and accompanying profound anorexia of more than 2 weeks.

All patients developed hyperbilirubinemia greater than 2.0 mg/dl with half of the patients developing jaundice with bilirubin in excess of 5.0 mg/dl. Hepatic biopsy in one patient showed chronic active hepatitis. At autopsy 6 months later, there was tumor present but resolution of the liver active hepatitis. Autopsy in a second patient demonstrated hepatic candidiasis but no evidence of drug toxicity.

Pulmonary symptoms or toxicity were not encountered. Pulmonary function testing including diffusing capacity for carbon monoxide and arterial blood gases before and after therapy were not significantly different in four evaluable patients. Response. Two patients with melanoma achieved a partial response of 2 and 3 months' duration. Five patients demonstrated evidence of tumor regression by measurement of tumor volumes (Table 1); however, these were frequently transient and therefore not qualifying as partial responses. All patients had evidence of regrowth of tumor by 4 months following treatment.

Pharmacokinetics. Pharmacokinetic measurement by two independent methods indicated that the oral absorption of busulfan was consistent both in an individual patient and among several patients evaluated. Fig. 2 shows that steady state plasma levels of between 2 and 8 μM were achieved by the second day of treatment and maintained throughout the remainder of the treatment course. The daily peak plasma level of busulfan achieved was similar among several evaluated patients (Table 3) with good correlation between samples analyzed by the two different analytic methods.

Salmonella Mutagenesis Assay. Urinary excretion of mutagenic metabolites was monitored using the Salmonella mutagenesis assay (13, 14). Excretion of mutagenic activity was seen from days -5 to -2 (Fig. 3). However, by the time of bone marrow infusion urinary levels of mutagenic activity had returned to baseline. In one patient (UTN 40) treated at 20 mg/kg urinary mutagenic activity was noted until day -1, suggesting a limited margin of safety in the timing of bone marrow infusion.

Nucleoid Body Assay. DNA strand breaks of lymphocytes from patients before and after treatment (Fig. 4) were unchanged as monitored by sedimentation of the nucleoid in neutral sucrose gradients whereas strand breaks and damage to theuffy coat DNA increased progressively following each day of busulfan treatment. The differential effect on lymphocytes and granulocytes was not seen in nucleoids obtained from patients treated with cyclophosphamide, cisplatin, and
membranes and proteins, and that because immune function is otherwise unaltered, cell surface alkylated products might be immunogenic, leading to the development of the transient immunological syndromes. An alternative explanation for the noted immunological syndromes would be modification of specific subpopulations of lymphocytes which were not detected by the reagents used. That such immune syndromes have not been observed when busulfan is administered in combination with the immunosuppressive agent cyclophosphamide (10) is consistent with the possibility that these effects are immunologically mediated.

Busulfan has been widely used at conventional dosage for the treatment of chronic myelogenous leukemia (18). At doses similar to those employed in this study, busulfan has been used successfully as a component of preparative regimens in allogeneic bone marrow transplantation for acute and chronic leukemia (10). Animal experimentation with busulfan and dimethylmyleran, a dimethylderivative of busulfan, suggests that these agents produce a selective granulocytopenia with little effect on cellular or humoral immune function (19). Further, the marrow suppressive and therapeutic effects of busulfan and dimethylmyleran were shown to be relatively independent of the proliferative state of the marrow (20, 21). This property is attractive for the selection of agents for autologous marrow transplantation efforts as most solid tumors have a low growth fraction. Further, many of the complications of high dose therapy are related to the immunosuppressive properties of the therapeutic regimens. Since immunosuppression is not required for high dose chemotherapy using autologous or syngeneic transplants, agents lacking immunosuppressive effects may provide a therapeutic advantage.

Analysis of sedimentation of the nucleoid from lymphocytes and granulocytes in high salt, neutral sucrose gradients shows that less damage occurs to lymphocyte DNA after in vivo busulfan exposure. Whether this difference is due to more effective repair or to differences in transport require further analysis.

Busulfan demonstrates efficacy in several solid tumor animal screens (22, 23). Recent experimental data suggests that busulfan at transplant doses is effective in the treatment of B16 melanoma.* However, few trials of the activity of busulfan in human solid tumors have been reported (reviewed in Ref. 8).

Even though administered at doses approximately 100 times standard doses as in this study, busulfan produced limited therapeutic results as a single agent in melanoma. Two patients developed temporary partial responses and evidence of transient tumor volume regression (50%) was seen in five of six (83%). Kaiser has demonstrated that the therapeutic efficacy of busulfan in P388 leukemia is highly dependent upon its temporal administration with other agents. Alteration of the schedule of administration may also affect toxicity and antitumor effect.

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* H. Kaiser, personal communication.


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