Increased Survival and Multilineage Hematopoietic Protection from Delayed and Severe Myelosuppressive Effects of a Nitrosourea with Recombinant Interleukin-11

Rodney Maze, Thomas Moritz, and David A. Williams

ABSTRACT

The chloroethylnitrosoureas, such as 1,3-bis(2-chloroethyl)-1-nitrosourea, are alkylating agents which are thought to exert antitumor activity by initiating lethal DNA interstrand cross-links. Although nitrosoureas are among the most active agents against childhood and adult gliomas, the utility of this class of agents has been limited by severe and cumulative myelosuppression, which can be fatal. Nitrosourea-induced myelosuppression in humans is delayed and may continue after withdrawal of the agent. We have developed a murine model which mimics the delayed and cumulative myelosuppression seen in humans receiving nitrosoureas. In this model, we demonstrate that interleukin-11, a stromal-derived hematopoietic growth factor with pleiotropic effects in a number of preclinical ablation models, markedly diminishes nitrosourea-induced pancytopenia and leads to a significant reduction in chemotherapy-related mortality. These data suggest that interleukin-11 could allow significant dose intensification in the treatment of tumors which are nitrosourea sensitive.

INTRODUCTION

Chloroethylnitrosoureas, such as BCNU, are among the most active chemotherapy agents used against both childhood and adult glial tumors (1). The lipophilic nature and low molecular weight of these molecules are associated with rapid transition across the blood-brain barrier and achievement of cerebrospinal fluid levels as high as 15–30% of concurrent plasma levels (2, 3). The chloroethylnitrosoureas are alkylating agents which are thought to exert antitumor activity by an initial alkylation of bases, which is followed by a series of reactions that result in a lethal DNA interstrand cross-link (4). The utility of this class of chemotherapeutic agents has been limited by severe and cumulative myelosuppression, which can be fatal (5).

Nitrosourea-induced myelosuppression is characterized by delayed, administration in humans results in anemia and neutropenia in three to four weeks and thrombocytopenia in four to six weeks (6–8). Prolonged myelosuppression continues after treatment with these agents is withdrawn. The exact nature of the bone marrow damage associated with nitrosourea use has not been completely delineated. However, previous studies have shown that BCNU administered to mice reduces the number of primitive hematopoietic stem and progenitor cells (9). Damage to this compartment may then lead to chronic impairment of the capacity of hematopoietic stem cells to maintain steady-state hematopoiesis. Other tissues damaged by the chloroethylnitrosoureas, particularly lung, also limit the use of these agents, but the delayed myelosuppression limits intensification either by increasing the frequency of administration or by combination with other myelosuppressive chemotherapy agents.

IL-11 is a secreted M, 20,000 growth factor that was isolated from a cloned based on IL-6-like biological activity (11). IL-11 shares no sequence homology with IL-6. In vitro, IL-11 in combination with IL-3 and IL-4 have been shown to stimulate the proliferation of early murine hematopoietic progenitor cells (12, 13). In addition, IL-11 in combination with stem cell factor supports the formation of murine multilineage colony formation and the proliferation of blast colony-forming cells (14). IL-11 along with granulocyte colony-stimulating factor and IL-6 have been proposed to be synergistic factors which stimulate primitive cells into cycle by shortening G0 in combination with early-acting cytokines such as IL-3 and granulocyte-macrophage colony-stimulating factor (12–15). Preclinical studies using IL-11 in a variety of murine myeloablation and bone marrow transplant models have consistently demonstrated accelerated recovery of peripheral blood platelets. Other studies have noted effects on blood neutrophil recovery (16–18). Detailed analysis of the effects of IL-11 on the bone marrow in these models have demonstrated increased bone marrow cellularity and increased numbers of multilineage and myeloid-restricted progenitor cells. More recently, our laboratory has demonstrated that administration of IL-11 results in a significant increase in the survival of mice given combination radiation/chemotherapy, due to the stimulation of small intestinal crypt progenitor cells and the rapid recovery of the small intestinal villi after severe damage (19).

In this report, we demonstrate that the administration of BCNU markedly diminishes the number of primitive HPP-CFC, a more primitive hematopoietic cell which may represent an in vitro equivalent of a reconstituting stem cell. These data further define the stem cell toxicity of this agent in the bone marrow. In addition, we report a murine model which closely mimics the delayed and cumulative severe myelosuppression seen in humans. In this model, concomitant administration of IL-11 when used as a single agent markedly diminishes BCNU-induced mortality and is associated with multilineage protection of peripheral counts. This increase in multilineage peripheral blood counts was associated with a significantly higher number of hematopoietic lineage CPC and HPP-CFC in the bone marrow. These data demonstrate that administration of IL-11 as a single cytokine can protect all hematopoietic lineages in mice from the delayed and severe myelosuppressive effects of BCNU and significantly reduce chemotherapy-related mortality. Such therapy may provide an opportunity for dose-intensification and/or schedule compression for the purpose of improving cure rates of nitrosourea-sensitive tumors.

MATERIALS AND METHODS

Mice and Myeloablative Therapy. Female C57Bl/6 mice, ages 8 to 10 weeks (Jackson Laboratories, Bar Harbor, ME), received 40 mg/kg BCNU (National Cancer Institute, Bethesda, MD) i.p. on a weekly schedule as shown.

Received 4/13/94, accepted 7/14/94.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the National Cancer Institute, Program Project Grant PO1 CA59348. T. M. is supported by Grant 300 402 653/2 from the German Cancer Aid/Mildred Scheel Foundation.

2 To whom requests for reprints should be addressed, at Howard Hughes Medical Institute Research Laboratories, Indiana University School of Medicine, 702 Barnhill Drive, Indianapolis, IN 46202-5225.

3 The abbreviations used are: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; IL, interleukin; HPP-CFC, high proliferative potential-colony forming cells; CPC, committed progenitor cells; BSA, bovine serum albumin; CFU-GM, colony-forming unit-granulocyte/macrophage; CFU-GEMM, colony-forming unit-granulocyte/erythroid/macrophage/megakaryocyte; BFU-E, burst-forming unit-erytroid.
in Fig. 1. BCNU was dissolved in anhydrous ethanol and diluted in 0.9% NaCl so that the final concentration of ethanol never exceeded 0.1%. Test mice received 250 μg/kg/day recombinant human IL-11 (Genetics Institute, Boston, MA) that was diluted in Hanks’ balanced salt solution (GIBCO Laboratories, Grand Island, NY) containing 0.1% BSA albumin (Boehringer Mannheim, Indianapolis, IN) and 0.025 mol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (GIBCO) s.c. in a 0.2-ml volume twice per day. Control mice received 0.2 ml Hanks’ balanced salt solution/0.1% BSA/0.025 mol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (vehicle) by the same schedule. IL-11 treatment started one day before the first weekly dose of BCNU was administered.

Peripheral Blood Analysis. Total peripheral blood leukocyte counts and platelet counts were analyzed on tail vein bleeds with a Coulter model ZM (Coulter Electronics, Hialeah, FL) using a 100-μm aperture for leukocyte determinations and a 50-μm aperture for platelet determinations. RBC were lysed using Zapoglobin (Coulter Electronics) according to the manufacturer’s recommendations. Peripheral blood hematocrits were performed by spinning capillary tubes for 5 min in a model MB Micro-Capillary centrifuge (IEC, Boston, MA).

Hematopoietic Progenitor Cell Assays. Bone marrow and spleen cells were harvested as described previously (16), and cellularity was determined using a Coulter model ZM as described above. Bone marrow and spleen cells were evaluated for committed progenitor colony formation by using a standard progenitor cell assay for CFU-GM, BFU-E, and CFU-GEMM. Bone marrow cells (1 × 10⁵) or 1 × 10⁶ spleen cells were plated in Iscove’s modified Dulbecco’s medium (GIBCO) with 0.9% methylcellulose (Fluka, Hauppauge, NY), 30% fetal calf serum (GIBCO), 100 ng/ml recombinant rat stem cell factor (Amgen), 10,000 units/ml murine IL-3 (specific activity, 1 X 10⁶ units/mg protein based on support of proliferation of FDC-P2 cells; Genzyme, Boston, MA), 0.1 mmol/l hemin (Eastman Kodak, Rochester, NY), 2 × 10⁻³ mol/l L-glutamine (GIBCO), and 1 × 10⁻³ M β-mercaptoethanol (Sigma Chemical Co.). Cultures were incubated at 37°C in a humidified environment at 5% O₂ and 5% CO₂ and were scored after 7 to 10 days of incubation.

HPP-CFC Assay. Double-layer agar cultures were prepared as previously described (20, 21). The bottom agar (1%) layer contained 100 ng/ml recombinant rat stem cell factor (Amgen), 10 units/ml murine IL-3 (specific activity, 1 × 10⁶ units/mg protein based on support of proliferation of FDC-P2 cells; Genzyme, Boston, MA), 0.1% BSA/0.025 mol/l 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (GIBCO), and 1 × 10⁻³ M β-mercaptoethanol (Sigma Chemical Co.). Cultures were incubated at 37°C in a humidified environment at 5% O₂ and 5% CO₂ and were scored after 7 to 10 days of incubation.

Statistical Analysis. Three plates were scored for each CPC and HPP-CFC sample. Each mouse was evaluated separately. Results are expressed as a mean ±1 SEM of all samples derived from the averages of each individual mouse within a group. The probability of significant differences between groups was determined with the use of a Student’s t test (two-tailed).

RESULTS

Effect of BCNU on Stem Cell Compartment. In an effort to determine more precisely the cytotoxicity of BCNU with respect to hematopoietic cells within the bone marrow and spleen, we administered single doses of BCNU at 40 or 80 mg/kg to mice and harvested cells from these animals 24 h later. Although single doses of BCNU at these concentrations had little effect on peripheral blood counts (data not shown), significant damage to the stem cell compartment could be demonstrated. The number of CPC was decreased 4-90-fold within a group. The probability of significant differences between groups was determined with the use of a Student’s t test (two-tailed).
matically reduced, with an 8-fold reduction in the number of HPP-CFC in animals treated with 80 mg/kg of BCNU (Fig. 2C). These changes in primitive cell populations were more dramatic than the decrease in bone marrow and spleen cellularity, which represent mainly differentiated cells. Bone marrow cellularity was decreased by 40% (BSA-control: 22.7 ± 1.2 × 10⁶/femur; BCNU-40 mg/kg: 12.2 ± 0.6 × 10⁶/femur, P < 0.01; BCNU-80 mg/kg: 12.3 ± 1.1 × 10⁶/femur, P < 0.01), while spleen cellularity was not significantly affected in BCNU-treated mice (BSA-control: 62.3 ± 3.5 × 10⁶/spleen; BCNU-40 mg/kg: 59.1 ± 4.5 × 10⁶/spleen, P = 0.52; BCNU-80 mg/kg: 52.0 ± 2.2 × 10⁶/spleen, P = 0.08).

**Effect of IL-11 on Delayed Myelosuppression of BCNU.** Because IL-11 has been shown to affect the proliferative status of primitive hematopoietic stem cells, we analyzed the effect of IL-11 administration on mice undergoing repeated doses of BCNU (Fig. 1). Although a single dose of BCNU had little or no effect on bone marrow and spleen cellularity or peripheral blood counts, mice given repeated weekly doses of BCNU at 40 mg/kg developed profound pancytopenia (Fig. 3A-C; BSA group). The onset of cytopenia was delayed and became extreme at between 6 and 8 weeks. In contrast, mice treated concomitantly with IL-11 (250 μg/kg/day divided into two doses), maintained significantly higher hematocrits, platelet counts and leukocytes compared to vehicle-treated control mice receiving BCNU (Fig. 3). The increased hematocrits and peripheral leukocyte cell counts reached significance at week 5 of treatment. Interestingly, since IL-11 affects platelet counts in normal mice, the peripheral platelet counts were higher in IL-11-treated mice after only 1 week of therapy and remained significantly elevated for all 9 weeks of therapy.

The changes in peripheral blood counts were accompanied by changes in bone marrow and spleen cellularity and hematopoietic stem cell and CPC content. As seen in Table 1, IL-11 administration to BCNU-treated mice resulted in improved bone marrow and spleen cellularity. Bone marrow cellularity after 7 weekly injections of BCNU was ~10% of normal in vehicle-treated mice but remained 50% of normal in IL-11-treated mice. BCNU-treated mice administered IL-11 had >80% of normal spleen cellularity, while vehicle-treated control mice had ~20% of normal spleen cellularity.

**Effect of IL-11 on Hematopoietic Stem and CPC Compartments in BCNU-treated Mice.** Since a single dose of BCNU was shown to severely damage the CPC and stem cell compartments in mice, we anticipated that chronic therapy would lead to depletion of these cells in vivo. As seen in Fig. 4 and Table 2, such depletion took place by the 7th week of BCNU treatment. BCNU-treated vehicle control mice demonstrated nearly complete exhaustion of bone marrow (Fig. 4A) and spleen (Fig. 4B) progenitor cells and a marked reduction in the number of more primitive HPP-CFC in the bone marrow (Table 2). IL-11 administration was associated with a 3–10-fold increase in bone marrow CPC, a 32-1000-fold increase in spleen CPC, and a 10-fold increase in the number of HPP-CFC in the bone marrow. These changes were significant at P < 0.05 in the bone marrow and at P < 0.01 in the spleen.

**IL-11 Administration Markedly Reduced BCNU-associated Mortality.** The attenuating effect of IL-11 on development of delayed myelosuppression from BCNU treatment was associated with decreased therapy-related mortality. Animals treated with BCNU and BSA (vehicle control) died beginning at week 7 with profound thrombocytopenia, anemia, and leukopenia (see above). In three separate experiments, after 10 weeks, 69% (18 of 26) of the BCNU-treated mice receiving IL-11 survived, while 0% (0 of 26) BCNU-treated mice receiving vehicle survived (Fig. 5). Mortality began in the vehicle control group at week 7. While a few animals treated with IL-11 died slightly earlier in one experiment, this difference was not significant compared to the control group. Both control and IL-11-treated mice which succumbed during the experiment demonstrated evidence of bacteremia and focal pneumonia in a perivascular distribution, a pattern consistent with hematogenous spread. No gross or microscopic evidence of fibrosis was found in the lung. No gross or microscopic abnormalities were seen in the liver or kidneys. Animals surviving the entire study and sacrificed at week 10 demonstrated no gross or microscopic evidence of fibrosis in the lungs nor any evidence of liver damage by histological examination.

**DISCUSSION**

Alkylating agents which lead to interstrand DNA cross-linking are among the most frequently used chemotherapeutic agents in intensive treatment protocols. Nitrosourea compounds lead to mispairing of
BCNU adversely affects a primitive, multipotential cell population responsible for long-term hematopoiesis in vivo.

We have developed a mouse model which effectively mimics the delayed myelosuppression of BCNU seen in humans. In this model, weekly administration of BCNU is associated with profound and cumulative myelosuppression, which is fatal. In BCNU-treated mice, IL-11 administration increases primitive HPP-CFC stem cells and committed myeloid committed progenitor cells significantly compared to vehicle-treated mice. IL-11-treated mice demonstrated increased peripheral blood counts in all lineages, as well as reduced therapy-related bone marrow and spleen hypoplasia. These changes are associated with increased survival of chronically treated mice, presumably due to reduced neutropenia-related bacteremia.

Many in vitro and preclinical in vivo models suggest that IL-11 in combination with other cytokines stimulates primitive hematopoietic cells (reviewed in Ref. 28). In vitro, IL-11 alone increases the number of committed myeloid progenitors, including CFU-megakaryocyte, CFU-GM, CFU-GEMM, and CFU-spleen, while in vivo IL-11 has pleiotropic effects, depending on the method of cytoablation (28). The different effects may relate to changes in expression of other cytokines in the myeloablated animal. The dramatic beneficial effects of IL-11 in the BCNU model reported here suggests that IL-11 stimulates the proliferation of an hematopoietic cell population more primitive than the population killed by BCNU. As a result, there is increased recruitment of this expanded multilineage cell population into the diminished compartment. The testing of other early- and late-acting cytokines in this model and secondary transplantation/reconstitution assays may help to clarify this hypothesis and determine whether IL-11 can reverse the stem cell toxicity demonstrated previously in BCNU-treated mice (27).

Our laboratory has also recently reported that IL-11 stimulates the proliferation of small intestine crypt progenitor cells and induces the rapid recovery of small intestine villus structure after combined chemotherapy/irradiation damage (19). In the present study, no evidence of

<table>
<thead>
<tr>
<th>Table 1 Effect of IL-11 on bone marrow and spleen cellularity in BCNU-treated mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone marrow Cellularity</strong> (×10⁸ cells/femur)</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>BSA (n = 8)</td>
</tr>
<tr>
<td>IL-11 (n = 8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> X ± SEM.  
<sup>b</sup> p < 0.01 vs BSA.

---

**Table 2 Effect of IL-11 on bone marrow HPP-CFC in BCNU-treated mice**

<table>
<thead>
<tr>
<th></th>
<th>HPP-CFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA (n = 8)</td>
<td>1.25 ± 0.63a&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-11 (n = 8)</td>
<td>11.8 ± 0.55b</td>
</tr>
</tbody>
</table>

<sup>a</sup> HPP-CFC × 10<sup>7</sup> cells/femur; mean ± SEM.  
<sup>b</sup> P < 0.01 versus BSA.

---

Fig. 5. Effect of IL-11 on survival of BCNU-treated mice. Survival curve of IL-11 versus BSA (vehicle control) with 26 mice from three experiments in each group. IL-11 versus BSA, P < 0.01.
villus damage was seen in BCNU-treated mice, and no differences were observed in the small intestine mucosa of mice treated with IL-11 versus vehicle-treated control mice. From previous studies (reviewed in Ref. 28), it is apparent that IL-11 has effects on nonhematopoietic cells. Careful analysis of the response of tumor cells to IL-11 is required to determine if IL-11 will be useful in treatment of therapy-related cytopenias. However, the data presented here suggests that IL-11 administration may allow significant dose intensification for tumors which are BCNU sensitive. Initial human trials using IL-11 in women with advanced-stage breast cancer undergoing intensive chemotherapy have recently suggested reduced thrombocytopenia compared to historical controls (29, 30).

ACKNOWLEDGMENTS

We would like to thank Dr. Claire Doerschuk for reviewing the histological sections and Lijun Feng and Xiangli Xiao for technical help. We thank R. Jakacki and members of our laboratory for reviewing the manuscript and D. Giarla for manuscript preparation. One author (D.A.W.) receives payments from Children’s Hospital, Boston, MA, based on certain milestones set forth in an IL-11 agreement between Genetics Institute, Cambridge, MA, and Children’s Hospital.

REFERENCES


Increased Survival and Multilineage Hematopoietic Protection from Delayed and Severe Myelosuppressive Effects of a Nitrosourea with Recombinant Interleukin-11

Rodney Maze, Thomas Moritz and David A. Williams


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/18/4947

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.