Chlorozotocin, 2-[3-(2-Chloroethyl)-3-nitrosoureido]-D-glucopyranose, an Antitumor Agent with Modified Bone Marrow Toxicity

Tom Anderson, Mary G. McMenamin, and Philip S. Schein

Clinical Pharmacology Section, Medicine Branch, National Cancer Institute, Bethesda, Maryland 20014

SUMMARY

Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose, is a newly synthesized, water-soluble nitrosourea antitumor agent that is active against L1210 leukemia in mice. A 701% and a 401% increase in life-span were attained with a dose that was lethal to 10% of the animals (15 to 20 mg/kg, i.p.) in mice treated on Day 2 or Day 6 of L1210 tumor growth, respectively. Sixty % of Day 2-treated mice and 30% of Day 6-treated mice survived for 90 days. At the maximally effective dose against L1210, chlorozotocin produced no significant depression in normal bone marrow DNA synthesis nor in peripheral neutrophil count, in contrast to a sustained >90% inhibition in L1210 ascites cell DNA synthesis.

If the antitumor activity and reduced bone marrow toxicity of chlorozotocin are confirmed in man, the use of this compound would facilitate treatment of patients with neoplastic disease who have preexisting abnormal bone marrow function or would allow for the more effective use of a nitrosourea agent in combination with anticancer agents possessing more potent myelosuppressive properties.

INTRODUCTION

The nitrosourea class of antitumor agents has demonstrated significant activity against specific histological forms of human cancer. The principal clinical toxicity has been depression of bone marrow function, usually delayed in onset, which, after cumulative damage, necessitates discontinuation of treatment (2, 3). In previous structure activity studies it has been demonstrated that nitrosourea myelosuppression in animals can be reduced by attachment of the cytotoxic group to the C-2 position of glucose (5, 6). The addition of a glucose carrier to l-methyl-l-nitrosourea, a bone marrow toxin in animals, resulted in the formation of the antibiotic streptozotocin, an antitumor agent with minimal myelosuppressive activity demonstrated in both animal and human studies (5, 7).

To evaluate the influence of the glucose carrier on chloroethyl nitrosourea bone marrow toxicity, 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose tetraacetate, or chlorozotocin tetraacetate, was synthesized by Dr. John Montgomery. Chlorozotocin tetraacetate was demonstrated to have antitumor activity against the L1210 mouse leukemia system at doses that did not produce leukopenia or inhibit normal bone marrow DNA synthesis (6). The present report describes the biological and biochemical properties of the water-soluble form of this compound, 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose (chlorozotocin). Its antitumor and myelosuppressive activities are compared with those of a representative of this class of antitumor agents in actual clinical use, BCNU.

MATERIALS AND METHODS

Male C57BL/6 × DBA/2 F1 (hereafter called BD2F1) mice, weighing 17 to 25 g and maintained on Purina laboratory chow pellets and water ad libitum, were used throughout. Chlorozotocin (NSC 178248) was dissolved in 0.005 M citrate buffer, pH 4.5; BCNU (NSC 409962) was dissolved in a 10% ethanol solution (Chart I). All drugs were administered i.p. at a volume of 0.1 ml/10 g body weight.

The mouse L1210 leukemia system was utilized to assess antitumor activity. Treatment was administered on the 2nd or 6th day after i.p. injection of 1 × 10^6 L1210 cells. The percentage of increased life-span was calculated from the survival of control tumor-bearing animals that received appropriate volumes of the respective diluents (4).

An investigation of the effects of the individual drugs upon DNA synthesis was carried out using mice with 4 days of L1210 tumor growth. Chlorozotocin, 15 mg/kg, and BCNU, 30 mg/kg, were administered i.p. in their respective diluents. One hr prior to sacrifice the mice were given i.p. injections of 100 μCi [3H]thymidine (specific activity, 1.9 μCi/mole; New England Nuclear, Boston, Mass.). The mice were killed by cervical traction at 0, 8, 24, 48, or 72 hr posttreatment. The ascitic tumor was aspirated from the abdominal cavity into 0.0067 M phosphate-buffered 0.85% NaCl, pH 7.4, at 4°C. Both tibias were removed and the bone marrows were expressed with 0.0067 M phosphate-buffered 0.85% NaCl solution. We have previously determined (9) that the bone marrows of mice examined after 6 days of i.p.

The abbreviation used is: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.
L1210 growth are not replaced by tumor. Ascites and bone marrows from 3 mice were pooled at each point, and the DNA content of each pooled specimen was extracted by a modification of the method of Schneider (8). A 0.5-ml aliquot of the final supernatant was added to 15 ml of Aquasol (New England Nuclear) and counted in a Packard Tri-Carb Model 4322 liquid scintillation spectrometer with a counting efficiency of 26% for tritium. Quench corrections were performed using the automatic external standard. An additional 0.5-ml aliquot of the supernatant was used for the measurement of DNA by the method of Burton (1). Results are expressed as dpm/μg DNA.

Serial WBC determinations were made by counting a suspension of 20 μl of tail vein blood in 10 ml Isoton (Scientific Products, Washington, D.C.) in a Model F Coulter counter after lysis of RBC with Zap-Isoton (Coulter Diagnostics, Inc., Hialeah, Fla.).

WBC differential counts were performed on Wright-stained smears of pretreatment blood and Day 3 posttreatment blood, which was found to be the nadir of WBC depression. Bone marrow histologies on both control and treatment mice, which was found to be the nadir of WBC depression. Bone marrow histologies on both control and treatment mice were compared with WBC counts from mice that had received single i.p. doses of BCNU, 20 or 30 mg/kg. Serial peripheral WBC counts were made by counting a suspension of 20 μl of tail vein blood and Day 3 posttreatment blood, which was found to be the nadir of WBC depression. Bone marrow histologies on both control and treatment mice were compared with WBC counts from mice that had received single i.p. doses of BCNU, 20 or 30 mg/kg. The nadir of WBC depression occurred 3 days after administration for both drugs (Chart 2).

Chlorozotocin, administered at a maximum nonlethal dose, 15 mg/kg, produced an 18% reduction in mean WBC count (Chart 1). This degree of WBC depression was not significantly different from the control mean. A 10% lethal dose, 20 mg/kg, produced a 28% decrease in WBC count with no specific alteration in neutrophil/lymphocyte ratio (N/L) of 0.83 compared to control (0.98) (Table 1).

RESULTS

Bone Marrow Toxicity. Groups of 10 normal BD2F1 mice received a single i.p. dose of chlorozotocin ranging from 10 mg/kg to 50 mg/kg. Serial peripheral WBC counts were performed over a 30-day period of observation. The results were compared with WBC counts from mice that had received single i.p. doses of BCNU, 20 or 30 mg/kg. The nadir of WBC depression occurred 3 days after administration for both drugs (Chart 2).

Chlorozotocin, administered at a maximum nonlethal dose, 15 mg/kg, produced an 18% reduction in mean WBC count (Chart 1). This degree of WBC depression was not significantly different from the control mean. A 10% lethal dose, 20 mg/kg, produced a 28% decrease in WBC count with no specific alteration in neutrophil/lymphocyte ratio (N/L) of 0.83 compared to control (0.98) (Table 1).

Administration of a 100% lethal dose, 50 mg/kg i.p., decreased the WBC count by 76%, with a preferential reduction in circulating lymphocytes (N/L = 3.60). Histological examination of the bone marrows of these animals demonstrated only a moderate reduction in the granulocytic series, whereas there was a major reduction in size of splenic lymphoid follicles. With the 50 mg/kg dose all animals died between the 4th and 6th day following injection.

BCNU, administered at a maximum nonlethal i.p. dose of 20 mg/kg, produced a 40% reduction in WBC count, which was significantly different from control (Chart 2) (p < 0.05). In addition, WBC differential counts demonstrated a preferential reduction in circulating neutrophils (N/L = 0.15) (Table 1). A 20% lethal i.p. dose of BCNU, 30 mg/kg, caused a reduction in WBC count comparable to a 100% lethal dose of chlorozotocin (72%). The N/L ratio at this dose of BCNU was only 0.25, and the bone marrows of mice sacrificed at Day 3 postinjection demonstrated a generalized hypoplastic state, whereas splenic lymph follicles were only moderately reduced in size compared to controls. Animals died between 21 and 30 days after treatment at this dose of BCNU.

Activity against L1210 Leukemia. Mice bearing L1210 leukemia were treated with graded single doses of chlorozotocin on Day 2 of tumor growth. The maximally effective dose, 15 mg/kg i.p., produced a 70% increase in life span compared to untreated controls, and 60% of the mice survived for 90 days (Table 2). In animals treated on Day 6 of tumor growth, chlorozotocin, 20 mg/kg i.p., resulted in a 401% increase in life span, with 30% of the animals surviving for 90 days (Table 3).

The maximally effective dose for BCNU against the Day 2 tumor was 30 mg/kg i.p. This dose resulted in a 632% increase in life span compared to untreated controls, with 7 of 9 animals alive after 90 days of observation (Table 2). When mice were treated after 6 days of tumor growth, a 493% increase in life span was achieved, with 50% of the mice surviving for 90 days (Table 3).

Effects on L1210 and Bone Marrow DNA Synthesis. Chlorozotocin, 15 mg/kg, produced a 96% inhibition of L1210 DNA synthesis within 24 hr of administration, as measured by [3H]thymidine incorporation into DNA. BCNU, 30 mg/kg, resulted in an 85% inhibition at the same
Dose Drug \( \text{mg/kg} \) Lethality in normal mice WBC count \( \% \) of control \( \text{Neutrophils} \) Lymphocytes \( \% \) Monocytes \( \% \) Eosinophils \( \% \) Control 100 44 45 10 1
Chlorozotocin 20 \( \text{LD}_{10} \) 72 43 52 5 0 50 \( \text{LD}_{100} \) 24 72 20 8 0
BCNU 20 \( \text{LD}_{9} \) 60 12 78 8 2 30 \( \text{LD}_{90} \) 28 19 73 9 0

* Median WBC count for controls, 14,000/cu mm.
* \( \text{LD}_{10} \), 10% lethal dose; \( \text{LD}_{100} \), 100% lethal dose; \( \text{LD}_{0} \), 0% lethal dose.

Table 2
Survival of BD2F1 mice treated on day 2 of L1210 tumor growth

<table>
<thead>
<tr>
<th>Drug</th>
<th>( \text{mg/kg} )</th>
<th>Lethality in normal mice</th>
<th>Av. life-span (days)</th>
<th>Increase in life-span ( % )</th>
<th>90-day survivors</th>
<th>Av. life-span of nonsurvivors (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorozotocin</td>
<td>5</td>
<td>19.1</td>
<td>140</td>
<td>1/10</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15 ( \text{LD}_5 )</td>
<td>66.5</td>
<td>701</td>
<td>6/10</td>
<td>27.3</td>
<td></td>
</tr>
<tr>
<td>20 ( \text{LD}_{10} )</td>
<td>48.7</td>
<td>487</td>
<td>4/10</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 ( \text{LD}_{30} )</td>
<td>38.7</td>
<td>366</td>
<td>3/10</td>
<td>16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ( \text{LD}_{50} )</td>
<td>7.3</td>
<td>0/10</td>
<td>0/10</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
<tr>
<td>BCNU</td>
<td>10</td>
<td>23.0</td>
<td>125</td>
<td>1/9</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>20 ( \text{LD}_9 )</td>
<td>55.5</td>
<td>444</td>
<td>5/10</td>
<td>21.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 ( \text{LD}_{10} )</td>
<td>74.7</td>
<td>632</td>
<td>7/9</td>
<td>21.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 ( \text{LD}_{90} )</td>
<td>74.7</td>
<td>632</td>
<td>8/10</td>
<td>13.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ( \text{LD}_{100} )</td>
<td>49.4</td>
<td>384</td>
<td>4/10</td>
<td>22.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.2</td>
<td>0/10</td>
<td>0/10</td>
<td>10.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice inoculated with \( 10^9 \) L1210 cells.
* Compared to untreated controls.
* \( \text{LD}_0 \), 0% lethal dose; \( \text{LD}_{10} \), 10% lethal dose; \( \text{LD}_{30} \), 90% lethal dose; \( \text{LD}_{90} \), 100% lethal dose; \( \text{LD}_{90} \), 20% lethal dose.

Chlorozotocin did not significantly reduce DNA synthesis of bone marrow (Chart 4). BCNU, however, did reduce the incorporation of thymidine into DNA to 37% of control by 24 hr posttreatment. This degree of inhibition was significant \( (p < 0.01) \). With both drugs, a rebound in DNA synthesis above the control level was observed by 48 hr after administration.

DISCUSSION

With the single exception of streptozotocin, the principal treatment-limiting toxicity of nitrosourea antitumor agents in clinical use has been myelosuppression. Because of the relative bone marrow-sparing property of streptozotocin, structure activity studies of other glucose-containing nitrosoureas were undertaken. Chlorozotocin tetraacetate was demonstrated to have relatively little bone marrow toxicity at doses that produced maximum antitumor activity in the L1210 system. Subsequently, 2-[3-(2-chloroethyl)-3-ureido]-2-deoxy-D-glucopyranose itself was nitrosated to give a stable compound (T. P. Johnston, G. S. McCaleb, and J. A. Montgomery, unpublished data). This water-soluble agent, chlorozotocin, is significantly more active against the L1210 leukemia system than is the tetraacetate analog and produces only a minor degree of peripheral WBC count depression and inhibition of bone marrow DNA synthesis when compared to equally effective antitumor doses of BCNU. However, it is important to emphasize that the bone marrow-sparing feature of chlorozotocin is relative and, when the drug is administered at lethal dose levels, myelosuppression is observed. Nevertheless, when the degree of bone marrow toxicity and lethality for maximally
Table 3
Survival of BD2F1 mice treated on day 6 of L1210 tumor growth

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Lethality in normal mice</th>
<th>Av. life-span (days)</th>
<th>Increase in life-span (%)</th>
<th>90-day survivors</th>
<th>Av. life-span of nonsurvivors (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorozotocin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>19.2</td>
<td>137</td>
<td>1/10</td>
<td>11.3</td>
</tr>
<tr>
<td>20</td>
<td>LD₅₀</td>
<td></td>
<td>13.4</td>
<td>65</td>
<td>0/10</td>
<td>13.4</td>
</tr>
<tr>
<td>30</td>
<td>LD₉₀</td>
<td></td>
<td>40.6</td>
<td>401</td>
<td>3/10</td>
<td>19.4</td>
</tr>
<tr>
<td>Control</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCNU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td>17.3</td>
<td>97</td>
<td>0/10</td>
<td>17.3</td>
</tr>
<tr>
<td>20</td>
<td>LD₅₀</td>
<td></td>
<td>25.0</td>
<td>184</td>
<td>1/10</td>
<td>19.0</td>
</tr>
<tr>
<td>30</td>
<td>LD₉₀</td>
<td></td>
<td>52.5</td>
<td>493</td>
<td>5/10</td>
<td>14.4</td>
</tr>
<tr>
<td>40</td>
<td>LD₂₀₀</td>
<td></td>
<td>14.9</td>
<td>69</td>
<td>0/10</td>
<td>14.9</td>
</tr>
<tr>
<td>50</td>
<td>LD₁₀₀₀</td>
<td></td>
<td>23.7</td>
<td>169</td>
<td>1/10</td>
<td>16.3</td>
</tr>
<tr>
<td>Control</td>
<td>8.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice inoculated with 10⁶ L1210 cells.
* Compared to untreated controls.
* LD₅₀, 50% lethal dose; LD₉₀, 90% lethal dose; LD₂₀₀, 20% lethal dose; LD₁₀₀₀, 100% lethal dose.

Chart 3. In vivo uptake of [³H]thymidine into the ascitic L1210 after treatment with chlorozotocin or BCNU. Mean ± S.E.

Chart 4. In vivo uptake of [³H]thymidine into mouse bone marrow after treatment with chlorozotocin or BCNU. Mean ± S.E.

effective antitumor activity against the L1210 system are correlated, chlorozotocin has a greater therapeutic index than does BCNU or the tetraacetate of chlorozotocin.

The potential importance of this study is the identification of a new active nitrosourea antitumor agent with modified bone marrow toxicity. If glucose modification of nitrosourea bone marrow toxicity is confirmed in man, the use of such a compound would facilitate treatment of patients with neoplastic disease who have preexisting abnormal bone marrow function or would allow for the more effective use of a nitrosourea agent in combination with anticancer agents possessing more potent myelosuppressive properties.

REFERENCES


Chlorozotocin, 2-[3-(2-Chloroethyl)-3-nitrosoureido]-d-glucopyranose, an Antitumor Agent with Modified Bone Marrow Toxicity

Tom Anderson, Mary G. McMenamin and Philip S. Schein


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/35/3/761

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.