Ability of the $\alpha$ and $\beta$ Anomers of Chlorozotocin to Kill Rat 9L Tumor Cells *in Vitro*  

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**ABSTRACT**

Chlorozotocin (CLZ), a nitrosourea synthesized in the hope that it would have little bone marrow toxicity, has been shown to be effective against animal tumors and tumor cells in culture. However, the clinical results with CLZ have been disappointing. The original report on the synthesis of CLZ indicated that $\alpha$ and $\beta$ anomers at the $\beta$-glucoside moiety should be expected, particularly when CLZ is placed in aqueous solution. In this study, the $\alpha$ and $\beta$ anomers have been separated by high-performance liquid chromatography and characterized by UV spectrophotometry, mass spectroscopy, and nuclear magnetic resonance. The equilibration and decomposition of the anomers in various physiological solutions were determined as a function of temperature, pH, and serum concentration. In Eagle's basal medium (pH 7.2) held at 37°C, CLZ decomposed with a $t_{1/2}$ of $\approx 82$ min; at 37°C with serum, CLZ decomposed with a $t_{1/2}$ of $< 10$ min. In these two cases, the $\beta\alpha$ ratio reached 1 in $\approx 48$ min and $< 5$ min, respectively. The maximum $\beta\alpha$ ratio obtained in all cases ranged from 1.25 to 1.5. After holding CLZ in tissue culture medium and compensating for its decomposition, 9L rat brain tumor cells were treated in *vitro* with CLZ having different ratios of the $\alpha$ and $\beta$ anomers. These experiments demonstrated that the $\beta$ anomer has little, if any, ability to kill 9L cells. Thus, this anomerization phenomenon may have been responsible for the disappointing clinical results with CLZ. Our data suggest that appropriate preparation, handling, and drug delivery procedures might be devised to minimize this problem in both experimental and clinical situations.

**INTRODUCTION**

Nitrosoureas have been used in the clinic to treat a variety of cancers such as acute lymphocytic leukemia, lymphoma, melanoma, multiple myeloma, glioma, and some types of gastrointestinal tumors (1, 2). Myelosuppression was the major side effect which limited the therapeutic efficacy of these drugs (3). Nitrosoureas with a glucose moiety, such as streptozotocin and chlorozotocin, were found to spare this myelosuppressive effect (4–6). The exact mechanisms for this bone marrow sparing are unknown, but it has been suggested that the glucose chlorehynitrosoureas alkylate transcriptionally inactive regions of the chromatin in bone marrow cells, whereas the other nitrosoureas, such as CCNU, preferentially alkylate transcriptionally active regions of the chromatin (7).

Chlorozotocin has about twice the alkylating activity of BCNU. In the L1210 leukemia model, it took twice the molar dose of BCNU to achieve the same curative level as chlorozotocin (8). Phase I clinical trials suggested that a single dose of 120 mg/m$^2$ of chlorozotocin should be equivalent to approximately 150 mg/m$^2$ of BCNU or 110 mg/m$^2$ of CCNU without the corresponding myelosuppressive toxicity (9). Although some complete and partial responses were observed when leukemias (10) and metastatic malignant melanoma (11) were treated with this dose of chlorozotocin in Phase II trials, it was not effective against several other solid tumors including small cell lung carcinoma (12) and pancreatic carcinoma (13). Unlike the animal studies, the clinical studies indicated that chlorozotocin had no significant advantage over BCNU or CCNU.

Nitrosoureas with a glucose moiety can exist as $\alpha$ and $\beta$ anomers (14, 15). A 1:10 anomic ratio ($\beta\alpha$) is obtained during synthesis of chlorozotocin (14). In contrast, streptozotocin exists predominantly as the $\beta$ anomer (15). In aqueous solutions, chlorozotocin undergoes decomposition (a nonenzymatic hydrolysis to an alkylating species and a carbamoylating species) and anomerization (a mutarotation around C-1). Although the anomers of chlorozotocin might equilibrate rapidly in aqueous solution, there was evidence to suggest that alterations of the stereochemistry at the glucose moiety would not change the antimutator activity of chlorozotocin. For example, Johnston et al. (16) reported that $\alpha$- and $\delta$-chlorozotocin had similar anti-leukemic activity in mice. However there are many cases in the literature where stereoisomers have been shown to produce substantially different biological effects (e.g., Refs. 17 and 18). In this paper, we have separated and characterized the $\alpha$ and $\beta$ anomers of chlorozotocin and have investigated their relative ability to kill 9L rat brain tumor cells in culture.

**MATERIALS AND METHODS**

**Materials.** Two separate lots of chlorozotocin were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. The drug was stored at $-79^\circ$C until used. All HPLC solvents were chromatography grade.

**Separation of Anomers and Chemical Analysis.** The $\alpha$ and $\beta$ anomers of chlorozotocin were separated by HPLC for analysis by MS and NMR. The HPLC system consisted of a Varian Model 500 solvent delivery system (Palo Alto, CA) and a Hewlett Packard 1040 A UV/Vis diode array detector (Palo Alto, CA). Chlorozotocin was dissolved in water and eluted from a C$_18$ reverse-phase column in a Waters radial compression Z-module (Milford, MA) with 10% acetonitrile and 90% water at a flow rate of 1 ml/min. Two distinct peaks with retention times of 5 and 7 min were found, and their spectra were scanned from 200 to 302 nm. The two peaks were collected in test tubes submerged in a methanol-dry ice mixture. The samples were then lyophilized and stored at $-79^\circ$C. Aliquots were dissolved in methanol for MS and methanol-d$_4$ for NMR analysis. The MS was performed on a Ribermag R-10-10C from Nermag (Houston, TX). Positive ion mass spectra of the samples were obtained by desorptive chemical ionization over a scan range of 100 to 400 m/z. Proton NMR spectra were recorded with a Bruker Aspect 3000 spectrometer at 300 MHz. Chemical shifts in the Fourier transformed spectra were made relative to trimethylsilane ($\delta = 0$).

**Decomposition and Anomerization in Aqueous Solutions.** The decomposition and anomerization of chlorozotocin were measured using a Beckman 332 HPLC system (Fullerton, CA) consisting of two Model 110B pumps and a Model 165 variable wavelength detector controlled by the System Gold Software. Chlorozotocin was dissolved in various solvents (0.3 to 1.6 mM) after adjusting the solvents to the specific temperatures and pHs desired. All buffers were 0.05 M, and the specific

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: CCNU, 1-(2-chloroethyl)-3-(cyclohexyl)-1-nitrosourea; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; HPLC, high-performance liquid chromatography; MS, mass spectroscopy; NMR, nuclear magnetic resonance; NBS, newborn bovine serum; BME, Eagle's basal medium.

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pHs were obtained by adding the appropriate salt for each buffer. In those experiments that required serum, the appropriate amount of heat-inactivated NBS (Gibco Laboratories, Grand Island, NY) was added. After various incubation times, a 25-μl aliquot was withdrawn and injected directly into the HPLC system. The sample was eluted from a reverse-phase Ultrasphere-ODS column with 10% acetonitrile and 90% water at a flow rate of 1 ml/min. Two distinct peaks with retention times around 6 and 8 min were eluted, and the amount of each anomer was quantitated by integrating the respective peak areas determined at 230 nm. The total chlorozotocin concentration was determined by summing the peak areas of the two anomers, and the β:α ratio was calculated from the respective peak areas.

Cytotoxicity Assessment. 9L brain tumor cells were maintained in BME containing 10% NBS as described elsewhere (19). Cells were set at 1 x 10⁴ per 75-cm² Falcon tissue culture flask (Becton Dickinson, Oxnard, CA) 72 h prior to each experiment. The cells were grown in an incubator maintained at 37°C with a humidified 5% CO₂ atmosphere.

 Chlorozotocin (5 mg/ml) was dissolved in ice-cold distilled water by sonication (35% maximum output; Fisher Model 300 sonic dismembrator; Fisher Scientific Co., Fairlawn, NJ) for <3 min. The solution was filtered through a 0.22-μm Millipore filter and held on ice until used. An amount of chlorozotocin was added to BME which resulted in the desired total chlorozotocin concentration if left standing at room temperature for 0, 60, or 120 min. Using this procedure, 9L cells were treated with a total (α + β) chlorozotocin concentration of 32, 64, or 96 μM (10, 20 or 30 μg/ml); treatments at each concentration had 1:10, 1:1, and 1:3:1 β:α ratios. At the appropriate time, flasks containing exponentially growing 9L cells were removed from the incubator, the chlorozotocin was added, and the flasks were returned to the incubator in <30 s. After incubating for 30 min, the medium was removed, the cells were washed twice with 3 ml of ice-cold trypsin (0.25% + 20 mg of EDTA per 100 ml), and the cells were detached by incubation for 10 min at 37°C with 2 ml of trypsin. Trypsinization was stopped by adding complete medium, and the cells were counted, diluted, and plated into Petri dishes for colony formation. After incubating for 12 days, the colonies were fixed, stained with crystal violet, and dissolved in methanol, and colonies containing more than 50 cells were counted. The surviving fraction was determined as the ratio of the colony formation efficiency of treated cells to the colony formation efficiency of untreated cells.

RESULTS

Separation and Identification of Anomers. The HPLC chromatograms of both lots of chlorozotocin in either water or methanol at room temperature revealed two separate peaks, and the UV scans of the peaks indicated that they had identical spectra. Initially, the ratio of the integrated area of the peak with the shorter retention time (6 min) to the one with the longer retention time (8 min) was 1:10 in both methanol and water. The 1:10 ratio in water changed to about 1.3:1 when the sample was allowed to stand for a period of time at room temperature.

The component with the shorter retention time was collected. The decay of this component in the HPLC eluting solution (10% acetonitrile:90% water) was studied at room temperature. An aliquot of the sample was analyzed by HPLC after various incubation times. The concentration of the component with the shorter retention time decreased, while the concentration of the component with the longer retention time increased until they reached equilibrium (Fig. 1). The same procedure was repeated starting with the component having the longer retention time and the converse results were obtained (Fig. 1). These results indicated that both components in an aqueous solution would reach the same equilibrium regardless of which component was used as the starting material.

Further identification of the two peaks was performed using MS and NMR spectroscopy. Although the ionization intensities were different, the MS results indicated that the fragmentation patterns of the parent compound (M, 313.69) and of the components in the two peaks were identical. For example, identical mass fragments at 206, 223, 249, 267, 296, and 313 representing the loss of the alkylating species, splitting of the glucose ring, loss of the chloroethyl group, loss of the methoxyl group from the glucose ring, loss of a water molecule, and loss of a proton, respectively, were obtained in all 3 spectra. These mass spectra are identical to the mass spectrum previously reported for chlorozotocin by Johnston et al. (14). From this, we concluded that the two components had the same molecular formula as the parent compound, indicating that they probably represented two isomers of chlorozotocin.

A possible site of isomerization is at the d-glucose moiety, where the d-glucose can exist in either the α or β position. The chemical shifts obtained by NMR were: δ3.3 due to the upfield half of N(CH₂CH₂)Cl; δ3.4 to 4.1 due to CH₂OH, H-5, H-4, H-3, and H-2; δ4.15 due to the downfield half of N(CH₂CH₂)Cl; and δ4.8 due to the OH at C-1, C-3, C-4, and C-6. The α anomer was identified by the H-1 resonance at δ5.2, and the β anomer was identified by the H-1 resonance at δ4.7. These values were similar to those described by Johnston et al. (14). Consequently, the HPLC component with the shorter retention time was designated the β anomer, and the component with the longer retention time was designated the α anomer.

Decomposition and anomerization in Aqueous Solutions. The half-times for decomposition of the total (α + β) chlorozotocin and the anomerization kinetics of chlorozotocin from the α to the β form were determined for chlorozotocin dissolved in aqueous solutions and maintained under a variety of temperatures and pH conditions. The initial β:α ratios were about 0.1 to 0.4, depending on the solution and conditions. The results of a typical anomerization experiment are shown in Fig. 2A. The equilibration curve for the α and β anomers showed an initial rapid rise and finally reached a plateau (Fig. 2A). In this case, it took ≈ 48 min to reach a β:α ratio of 1 and ≈ 150 min to reach the maximum β:α ratio. The decomposition kinetics of total chlorozotocin were exponential for all cases studied.
For the decomposition experiment with BME in Fig. 2B, chlorozotocin was mixed and sonicated in water at 4°C before it was added to BME. No attempt was made to identify or quantitate the previously published decomposition products (20). The same procedure was later used for the cytotoxicity experiments. The effect of temperature, type of aqueous solution, pH, and serum on the breakdown and anomerization of chlorozotocin are summarized as follows.

The decomposition of total chlorozotocin and the equilibration of the α and β anomers depended strongly on the incubation temperature (Table 1). In general, the rate of decomposition of chlorozotocin increased with increasing temperature, particularly between room temperature and 37°C. The maximum β:α ratio remained relatively constant (~ 1.3), but the time to reach both a β:α ratio of 1:1 and the maximum β:α ratio decreased with increasing temperature.

The decomposition rate of the total chlorozotocin was relatively independent of the type of buffer (in Table 2, compare the buffers to water at room temperature). The times to reach a β:α ratio of 1:1 and the maximum β:α ratio in the buffers were slightly shorter than in water. Therefore, the equilibration of the α and β anomers was independent of the type of buffer, but slightly dependent on the osmolarity.

The decomposition rate of the total chlorozotocin in phosphate buffer depended on pH (4 to 7.8) at room temperature (Table 3). The half-time for the decomposition of chlorozotocin decreased as the pH increased. The time to reach the maximum β:α ratio was essentially independent of pH at room temperature in phosphate buffer (Table 3). However, at 4°C, the time to reach the maximum β:α ratio increased as the pH decreased (Table 3).

At 37°C, the decomposition rate of the total chlorozotocin was similar in phosphate buffer (pH 7.4) and BME (pH 7.2). However, the time to reach both a β:α ratio of 1:1 and the maximum β:α ratio was faster in phosphate buffer (Table 4). Addition of serum did not change the decomposition rate of the total chlorozotocin in phosphate buffer, but it increased the decomposition rate in BME by a factor of about 2. Similarly, serum did not greatly alter the time to reach the maximum β:α ratio in phosphate buffer, but it did change the time to reach a β:α ratio of 1:1 by more than a factor of 5 in BME. Serum only produced a small decrease in the time required for the β:α ratio to reach its maximum in BME. It is likely that the change in the rate of decomposition when serum was added to BME resulted predominantly from a slight change in the pH, because the buffering capacity of BME is substantially less than that of the phosphate solutions. Identical results were obtained with serum that was not heat inactivated (data not shown). Thus,

Table 1 Summary of the decomposition and anomerization parameters as a function of temperature

<table>
<thead>
<tr>
<th>Decay</th>
<th>$t_{0.5}^{\beta:\alpha}$ (min)</th>
<th>Max $\beta:\alpha$</th>
<th>$t_{\text{max}}^\beta:\alpha$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dd H$_2$O, RT</td>
<td>1668.1 ± 4.5</td>
<td>1.33</td>
<td>150</td>
</tr>
<tr>
<td>dd H$_2$O, 37°C</td>
<td>533.6 ± 47.3</td>
<td>1.25</td>
<td>80</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 7.4, 4°C</td>
<td>651.8 ± 169.1</td>
<td>1.50</td>
<td>150</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 7.4, RT</td>
<td>123.6 ± 17.6</td>
<td>1.35</td>
<td>120</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 7.4, 37°C</td>
<td>15.2 ± 0.8</td>
<td>&lt;5</td>
<td>30</td>
</tr>
<tr>
<td>BME, pH 7.2, RT</td>
<td>81.7 ± 7.2</td>
<td>1.30</td>
<td>150</td>
</tr>
<tr>
<td>BME, pH 7.2, 37°C</td>
<td>18.6 ± 0.9</td>
<td>1.25</td>
<td>110</td>
</tr>
</tbody>
</table>

* The half-time for decomposition of total (α + β) chlorozotocin obtained from a regression analysis of the data.

Column notations are the same as those in Table 1.

Table 2 Summary of the decomposition and anomerization parameters as a function of buffer type

<table>
<thead>
<tr>
<th>Decay</th>
<th>$t_{0.5}^{\beta:\alpha}$ (min)</th>
<th>Max $\beta:\alpha$</th>
<th>$t_{\text{max}}^\beta:\alpha$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO$_4$ buffer, pH 4, RT</td>
<td>1487.2 ± 178.9</td>
<td>1.35</td>
<td>120</td>
</tr>
<tr>
<td>Citrate buffer, pH 4, RT</td>
<td>1645.9 ± 292.6</td>
<td>1.30</td>
<td>110</td>
</tr>
<tr>
<td>dd H$_2$O, RT</td>
<td>1668.1 ± 447.0</td>
<td>1.33</td>
<td>150</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

Table 3 Summary of the decomposition and anomerization parameters as a function of pH

<table>
<thead>
<tr>
<th>Decay</th>
<th>$t_{0.5}^{\beta:\alpha}$ (min)</th>
<th>Max $\beta:\alpha$</th>
<th>$t_{\text{max}}^\beta:\alpha$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO$_4$ buffer, pH 4, RT</td>
<td>1487.2 ± 178.9</td>
<td>1.35</td>
<td>120</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 6, RT</td>
<td>163.9 ± 22.7</td>
<td>1.40</td>
<td>80</td>
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<tr>
<td>PO$_4$ buffer, pH 7.4, RT</td>
<td>123.6 ± 17.6</td>
<td>1.35</td>
<td>120</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 7.8, RT</td>
<td>33.7 ± 1.4</td>
<td>1.35</td>
<td>120</td>
</tr>
<tr>
<td>PO$_4$, pH 6, 4°C</td>
<td>583.5 ± 203.6</td>
<td>(238)</td>
<td>(1.5)</td>
</tr>
<tr>
<td>PO$_4$, pH 7.4, 4°C</td>
<td>651.8 ± 169.1</td>
<td>1.5</td>
<td>110</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

Table 4 Summary of the decomposition and anomerization parameters with and without serum

<table>
<thead>
<tr>
<th>Decay</th>
<th>$t_{0.5}^{\beta:\alpha}$ (min)</th>
<th>Max $\beta:\alpha$</th>
<th>$t_{\text{max}}^\beta:\alpha$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO$_4$ buffer, pH 7.4, 37°C</td>
<td>15.2 ± 0.8</td>
<td>&lt;5</td>
<td>30</td>
</tr>
<tr>
<td>PO$_4$ buffer, pH 7.4, 37°C, serum</td>
<td>17.7 ± 1.4</td>
<td>&lt;5</td>
<td>10</td>
</tr>
<tr>
<td>BME, pH 7.2, 37°C</td>
<td>18.6 ± 0.9</td>
<td>26</td>
<td>125</td>
</tr>
<tr>
<td>BME, pH 7.2, 37°C, serum</td>
<td>8.8 ± 0.8</td>
<td>&lt;5</td>
<td>90</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
there is little evidence to suggest that serum contains enzymes that will drastically alter either the decomposition or anomerization kinetics of chlorozotocin.

Cytotoxicity of the α and β Anomers. 9L cells were exposed to chlorozotocin concentrations of 32, 64, or 96 μM (10, 20, and 30 μg/ml) containing different ratios of the α and β anomers by holding the drug in BME for 0, 60, and 120 min at room temperature before treating the cells. These time intervals resulted in treatments where the β:α ratios were 1:10, 1:1, and 1:3.1. The cytotoxicity of a 30-min chlorozotocin exposure decreased as the β:α ratio increased (Fig. 3). The decrease in cytotoxicity as a function of holding time (Fig. 3) was virtually identical to the decrease in the concentration of the α anomer that resulted from its conversion to the β anomer during the holding period (Fig. 2A). Consequently, the β anomer appears to have little, if any, ability to kill 9L rat brain tumor cells.

DISCUSSION

The possibility that chlorozotocin existed in two anomeric forms was apparent when we attempted to quantitate the drug using an HPLC method. Two peaks were eluted when chlorozotocin was prepared in aqueous solution. The identical mass fragments, identical UV spectra, and temperature-dependent changes in the peak area ratio in aqueous medium were all consistent with the notion that the two peaks contained anomers of chlorozotocin. Identical results obtained with different batches of chlorozotocin and using different HPLC systems eliminated the possibility that contaminants and measurement artifacts were responsible. The identification of the α and β anomers came from the NMR analysis. The initial β:α ratio of 1:10 obtained for each lot of chlorozotocin was also in agreement with the literature (14).

In aqueous solutions, the disappearance of chlorozotocin as measured by a loss of absorbance at 230 nm reflected the spontaneous decomposition of the nitrosourea moiety into an alkylating and a carbamoylating species similar to that observed for BCNU and CCNU (14), except that an intramolecular carbamoylation bicyclic urethane derivative was formed with chlorozotocin. Unlike BCNU or CCNU (24), serum did not increase the decomposition of chlorozotocin in phosphate buffer, if the pH was held constant (Table 4). Thus, chlorozotocin appears to have decomposition properties similar to those observed for other water-soluble nitrosoureas, such as 1-(2-chloroethyl)-2-(2,6-dioxo-3-piperidyl)-1-nitrosourea, which have low protein binding affinities (24).

Anomerization of chlorozotocin results from the mutarotation of the glucose moiety around C-1 in solution. As expected from a base-catalyzed hemiacetal reaction, the time to reach equilibrium increased as the pH and temperature increased (25). A maximum β:α ratio of 1:25 to 1.5 was found, consistent with the β-glucose being the preferable form in aqueous solutions (25). Serum did not significantly affect the kinetics of anomerization, if the pH was held constant. This is expected if the reaction is not a protein-catalyzed reaction.

Using the data on the decomposition and anomerization of chlorozotocin, the relative cytotoxicity of the α and β anomers could be investigated. Chlorozotocin was held in BME at room temperature because decomposition and anomerization were relatively slow under these conditions. An aliquot of chlorozotocin in BME could be added to flasks of 9L cells without significantly altering the medium composition or temperature. As the β:α ratio increased, the ability of a 30-min treatment to kill 9L cells decreased. Changing the β:α ratio from 1:10 to 1:3:1 resulted in more than a 3-log decrease in the cell kill after a 30-min treatment with 96 μM (30 μg/ml) chlorozotocin (Fig. 3). This result indicates that the β anomer has little, if any, ability to kill the 9L tumor cells.

Although the mechanism for this differential cytotoxicity of the α and β anomers is unknown, Lam et al. (21) and Lazarus et al. (26) have shown that uptake of chlorozotocin in lymphoblasts and bone marrow cells occurs by passive diffusion rather than by a glucose transport mechanism. This makes it unlikely that differential uptake of the α and β anomers is responsible for the differential cytotoxicity in 9L cells. Enzymatic metabolism of chlorozotocin has been reported to occur in lymphoblasts (21), probably by a denitrosation reaction similar to that which occurs for BCNU and CCNU (27). Thus, a difference in the metabolic breakdown of the α and β anomers might account for the differential cytotoxicity observed in our 9L experiments. However, in our study, the time-dependent loss of cytotoxicity (Fig. 3) was virtually identical to the time-dependent loss of a component due to anomerization to the β anomer (Fig. 2A), making it unlikely that this is the mechanism responsible for the differential cytotoxicity observed. Finally, differential alkylation of chromatin sites has been suggested as a possible reason for the differential toxicity of this drug against leukemia and bone marrow cells (7). Although differential alkylation in leukemia and bone marrow cells probably involves differences in the accessibility of their chromatin to chlorozotocin that are associated with differences in their states of differentiation (28), differential alkylation of the chromatin by the α and β anomers may occur because of stereochemical considerations. Further study will be required before the exact mechanism for the differential cytotoxicity of the α and β anomers can be determined.

Regardless of the mechanism responsible for the differential
cytotoxicity reported here for the α and β anomers, this study indicated that preparation and handling procedures can greatly influence the outcome of experimental and clinical treatments with chlorozotocin. Using the data presented here, it should be possible to design procedures that maximize the amount of the α anomer reaching the tumor in both experimental and clinical situations.

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