Pharmacokinetics of O⁶-benzylguanine in Pediatric Patients with Central Nervous System Tumors: A Pediatric Oncology Group Study

Kathleen Neville,1 Susan Blaney,1
Mark Bernstein,2 Patrick Thompson,1
Denise Adams,3 Alexander Aleksic,1 and
Stacey Berg1
1Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 2Sainte-Justine Hospital, University of Montreal, Montreal, Quebec, Canada; and 3University of Vermont College of Medicine, Burlington, Vermont

ABSTRACT

Purpose: To report the results of the first pharmacokinetic study in pediatric patients of O⁶-benzylguanine (O⁶BG), which irreversibly inactivates the DNA repair protein alkylguanine-alkyltransferase, thus enhancing the cytotoxicity of nitrosoureas.

Experimental Design: As part of a Pediatric Oncology Group Phase I study, 120 mg/m² of O⁶BG was administered i.v. over 1 h, before 1,3-bis(2-chloroethyl)-1-nitrosourea administration in children with recurrent or refractory brain tumors. Serial blood samples for plasma pharmacokinetic studies were obtained. Concentrations of O⁶BG and its active metabolite O⁶-benzyl-8-oxoguanine (8-oxo-O⁶BG) were measured by high-performance liquid chromatography. A pharmacokinetic model and additional first-order elimination rate constants for each compound were developed.

Results: O⁶BG concentration versus time data were evaluated for 25 patients. The peak concentration of O⁶BG (mean ± SD) was 11 ± 4 μM, and the peak concentration of its active metabolite, 8-oxo-O⁶BG, was 35 ± 10 μM. O⁶BG was rapidly eliminated with a half-life of 85 ± 140 min, area under the curve of 795 ± 320 μM · min and clearance of 760 ± 400 ml/min/m². The area under the curve of 8-oxo-O⁶BG when extrapolated to infinity was 22,700 ± 11,800 μM · min. The clearance and terminal half-life of 8-oxo-O⁶BG were 30 ± 15 ml/min/m² and 360 ± 220 min, respectively.

Conclusions: There is rapid elimination of O⁶BG after i.v. administration over 1 h. In contrast, the terminal half-life for the active metabolite, 8-oxo-O⁶BG, is 4-fold longer. The pharmacokinetic parameters for O⁶BG and 8-oxo-O⁶BG are similar to those reported previously in adults.

INTRODUCTION

Chemotherapeutic nitrosoureas and methylating agents are used to treat a variety of neoplasms including brain tumors. These alkylating agents form a covalent bond at the O⁶ position of guanine in DNA, leading to the formation of alkylguanine adducts and intra- and interstrand cross-links, which disrupt DNA synthesis. The subsequent alterations, which include chromosomal aberrations, rearrangements, and strand breaks (1, 2), all correlate with cytotoxicity and as few as 10 cross-links cause tumor cell death (3, 4). Because DNA cross-linkage is the primary mechanism of action of alkylating agents, DNA repair after cross-linkage is an important mechanism of drug resistance.

O⁶-alkylguanine-DNA alkyltransferase (AGAT) is a DNA repair enzyme that repairs adducts at the O⁶ position of guanine by transferring the alkyl group to a cysteine residue within the enzyme molecule (5, 6). Because the repair enzyme is a single turnover enzyme, the number of DNA lesions that can be repaired is stoichiometrically proportional to the amount of AGAT in the cell (7). Consequently, once the enzyme is depleted, further DNA repair is dependent on de novo enzyme synthesis (8). Thus, depletion of AGAT has been postulated to increase the sensitivity of cells to nitrosourea-mediated DNA damage.

O⁶-benzylguanine (O⁶BG) was developed to be a potent and selective AGAT inhibitor. O⁶BG and its metabolite O⁶-benzyl-8-oxoguanine (8-oxo-O⁶BG) bind to the same cysteine residue on the AGAT molecule that is used for alkyl group transfer, thereby permanently inactivating that enzyme molecule (6). Multiple studies have been performed using O⁶BG before nitrosourea treatment in adult phase 1 and 2 clinical trials. Pharmacokinetic studies of O⁶BG in adults show that elimination of O⁶BG from the plasma is rapid. Essentially, all of the drug was oxidized to the active metabolite 8-oxo-O⁶BG by cytochrome P450 enzymes CYP1A2 and CYP3A4. The metabolite has nearly identical AGAT inhibition activity as the parent drug (9, 10).

We report the results of the first pharmacokinetic study of O⁶BG in pediatric patients. These studies were a component of Pediatric Oncology Group study (POG) 9870 of O⁶BG administered before 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).
PATIENTS AND METHODS

Patients. Patients younger than 22 years of age who met eligibility requirements were enrolled after informed consent was obtained. Eligibility requirements included the following: a histologically or cytologically proven central nervous system tumor refractory to conventional therapy or for which there was no known effective therapy; a Karnofsky performance score $\geq 50$; a life expectancy $>8$ weeks; recovery from previous chemotherapy; and adequate bone marrow, hepatic, renal, and pulmonary function. Patients with brain stem glioma were exempted from the requirement for biopsy.

Drug. O6-BG (NSC 637037, IND 45789) was supplied by the National Cancer Institute, Division of Cancer Treatment and Diagnosis, in dual packs with diluent. The drug was provided in 100-mg vials that contained white lyophilized powder with 670 mg of mannitol, USP, and sodium hydroxide to adjust pH to 7–8.5. Diluent consisted of a 30-ml vial that contained sterile solution of 40% polyethylene glycol 400 in phosphate buffer (pH 8; 106 mg of dibasic sodium phosphate, 102 mg of monobasic potassium phosphate in sterile water for injection USP). Once diluted, each milliliter of the resulting solution contained 3.3 mg of O6-BG, 22 mg of mannitol, USP, 0.4 ml of polyethylene glycol 400, and approximately 0.6 ml (pH 7) of phosphate buffer.

Study Design. Patients received both O6-BG and BCNU. The dose of O6-BG was 120 mg/m$^2$. O6-BG was administered i.v. over 1 h. One h after completion of the O6-BG infusion, BCNU was administered i.v. over 1 h. Courses of O6-BG and BCNU were repeated every 6 weeks provided patients had stable or responsive disease and evidence of recovery from all prior course toxicity. Patients who had progressive disease after any treatment course were removed from protocol therapy. At least three patients within a cohort had to be assessable for toxicity before escalating to the next higher dose level. If one of the first three patients entered at a dose level had dose-limiting toxicity, up to three additional patients were entered at that dose level. There was no intrapatient dose escalation. Standard criteria were used for response and toxicity analysis.

Pharmacokinetic studies were performed during the first course of chemotherapy only. In the first 16 patients, blood samples were collected before O6-BG infusion; at the end of the infusion; at 5, 15, 30 min; at 1, 2, 4, 6, and 24 h; and when possible at 48 h after infusion. Nine patients also had samples collected at 8, 10, and 12 h after initial data analysis revealed the need for additional sampling between 6 and 24 h. Blood samples were immediately centrifuged, and the plasma fraction was stored at $-20$°C or colder before analysis.

Sample Analysis. Plasma samples underwent solid-phase extraction using 3 ml Bond Elut C$_{18}$ prep columns (Analytichem International, Harbor City, CA) rinsed previously with 3 ml of methanol and 3 ml of 0.25 M ammonium acetate (pH 7.0). After loading 500 $\mu$l of plasma, the columns were washed with 3 ml of 0.25 M ammonium acetate (pH 7.0) and eluted with 2 ml of acetonitrile. Eluates were evaporated to dryness under nitrogen at 37°C. Before injection onto the high-performance liquid chromatography system, samples were reconstituted in 500-$\mu$l mobile phase and filtered through a 0.45-$\mu$m filter (Ultrafree-MC; Millipore Corp., Bedford, MA).

RESULTS

Samples for pharmacokinetic studies were collected from 26 patients; however, samples from one patient could not be...
Pharmacokinetics of O6-BG in Pediatric Patients

area under the curve values for O6-BG and 8-oxo-O6-BG were similar to published adult data (Table 1).

Fig. 2 Representative concentration versus time curve for O6-benzyl-8-oxoguanine (O6-BG) and its metabolite, O6-benzyl-8-oxoguanine (8-oxo-O6-BG). Circles represent O6-BG concentration, triangles represent 8-oxo-O6-BG concentration, and lines represent the model-fitted values.

analyzed because of insufficient specimen volume. A representative concentration versus time curve for O6-BG and metabolite 8-oxo-O6-BG is shown in Fig. 2. The peak concentration of O6-BG (mean ± SD) was 11 ± 4 µM. O6-BG was rapidly eliminated with a half-life of 85 ± 140 min, area under the curve of 795 ± 320 µM·min/m², and clearance of 760 ± 40 ml/min/m². The peak concentration of 8-oxo-O6-BG was 35 ± 10 µM. The area under the curve of 8-oxo-O6-BG when calculated to the last time point was 12,100 ± 5,790 µM·min/m². Initial sampling times did not permit extrapolation to infinity or estimation of the terminal half-life for 8-oxo-O6-BG, because drug concentrations were quantifiable at 6 h but below the limits of quantification at 24 h. Additional sampling time points were added, allowing extrapolation to infinity with a resulting area under the curve of 22,700 ± 11,800 µM·min/m², clearance of 30 ± 15 ml/min/m² and a terminal half-life of 360 ± 220 min. Clearance, half-life, and area under the curve values for O6-BG and 8-oxo-O6-BG were similar to published adult data (Table 1).

DISCUSSION

High levels of AGAT activity are common in adult brain tumors (12–14) and may be correlated with poor responses to alkylator-based chemotherapy and decreased time to treatment failure and death (12, 15, 16). High levels of AGAT have also been observed in pediatric brain tumors, including gliomas, medulloblastomas, primitive neuroectodermal tumors, and ependymomas (13). These observations may explain why nitrosourea-based chemotherapy has had little impact on survival in common pediatric brain tumors (17, 18). Therefore, a strategy to inactivate AGAT before administration of an alkylating agent is a logical pursuit for adult and pediatric central nervous system tumors.

We found that O6-BG is rapidly eliminated with a half-life of 85 ± 140 min after i.v. administration. In contrast 8-oxo-O6-BG, an active metabolite, appears rapidly in plasma after O6-BG administration and has a prolonged half-life of approximately 6 h. In most patients, the combined concentrations of O6-BG and 8-oxo-O6-BG exceed 1 µM for 16 h. In extracts from the HT29 cell line, a concentration of 0.3 µM 8-oxo-O6-BG for 30 min resulted in inactivation of 50% of the methyl-guanine-methyl-transferase activity, compared with 0.2 µM O6-BG (10).

In adult studies (19–21), doses of 100–120 mg/m² O6-BG resulted in concentrations of O6-BG and 8-oxo-O6-BG in the micromolar range for up to 24 h (8-oxo-O6-BG) and completely eliminated tumor AGAT activity during that time. Therefore, our results suggest that clinically relevant AGAT-inactivating concentrations are obtained in children after administration of 120 mg/m² O6-BG.

This study also shows that the pharmacokinetic parameters for both O6-BG and 8-oxo-O6-BG in children were similar to those obtained in adults (Table 1). After comparable doses of O6-BG, the terminal half-life of O6-BG was 40 min, and the terminal half-life of 8-oxo-O6-BG was approximately 220 min (19). One interesting difference between adults and children was that 8-oxo-O6-BG concentrations measured 24 h after the O6-BG infusion were substantially lower in children than adults. In adult studies, the 24 h concentration of 8-oxo-O6-BG ranged from 0.5 to 1.6 µM after O6-BG doses of 80–120 mg/m² (19, 22). In contrast, in the present study, the 24-h concentration of 8-oxo-O6-BG after a dose of 120 mg/m² was below the limits of assay quantitation, 150 nm. The reason for this difference is unclear. O6-BG is oxidized to an active metabolite, 8-oxo-O6-BG, by cytochrome P450 enzymes CYPIA2 and CYP3A4. 8-oxo-O6-BG is subsequently metabolized by debenzylation, although the mechanism of this is unclear (10). Thus, it is possible that developmental differences in enzyme activity could account for this observed difference between children and adults.

Future studies of O6-BG combined with BCNU in children are not planned because this combination did not result in tumor regression in adult glioma patients at doses of BCNU that could be delivered with acceptable clinical toxicity (23). Nevertheless,

Table 1 Comparison of pediatric and adult pharmacokinetic parameters for O6-BG and metabolite O6-benzyl-8-oxoguanine

<table>
<thead>
<tr>
<th>Agent</th>
<th>Patient population</th>
<th>Dose of O6-BG (mg/m²)</th>
<th>C_max (µM)</th>
<th>AUC (µmol/l · min)</th>
<th>Half-life (min)</th>
<th>Clearance (ml/min/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O6-BG</td>
<td>Children</td>
<td>120</td>
<td>11 ± 4</td>
<td>795 ± 320</td>
<td>85 ± 140</td>
<td>760 ± 400</td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>80</td>
<td>8 ± 2</td>
<td>570 ± 171</td>
<td>43 ± 18</td>
<td>628 ± 197</td>
</tr>
<tr>
<td>O6-BG</td>
<td>Adults</td>
<td>80</td>
<td>n.r.</td>
<td>827 ± 232</td>
<td>32 ± 8</td>
<td>533 ± 150</td>
</tr>
<tr>
<td>8-Oxo-O6-BG</td>
<td>Adults</td>
<td>80</td>
<td>7 ± 2</td>
<td>n.r.</td>
<td>49.1 ± 16.1</td>
<td>638 ± 205</td>
</tr>
<tr>
<td></td>
<td>Children</td>
<td>120</td>
<td>35 ± 10</td>
<td>22,700 ± 11,800</td>
<td>360 ± 215</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>80</td>
<td>17 ± 4</td>
<td>15,300 ± 5210</td>
<td>550 ± 175</td>
<td></td>
</tr>
<tr>
<td>8-Oxo-O6-BG</td>
<td>Adults</td>
<td>80</td>
<td>n.r.</td>
<td>13,600 ± 5560</td>
<td>340 ± 160</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adults</td>
<td>80</td>
<td>13 ± 3</td>
<td>n.r.</td>
<td>324 ± 39</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: C_max, peak concentration; AUC, area under the concentration versus time curve; n.r., not reported.
the pharmacokinetic profile of O6BG in children is important especially because there are other ongoing pediatric studies that use O6BG. These studies, which are primarily for children with recurrent or refractory central nervous system tumors, include several trials of O6BG plus temozolomide as well as a trial of O6BG plus Gliadel. The potential advantage of using O6BG with a form of loco-regional drug delivery such as Gliadel is that systemic toxicities that limit the dose of BCNU that can safely be co-administered with O6BG are theoretically avoided. In summary, the O6BG pharmacokinetic data from this trial coupled with data from adult studies strongly suggest that there is clinically relevant inactivation of AGAT after administration of 120 mg/m2 of O6BG to children with central nervous system tumors.

REFERENCES

Pharmacokinetics of O\textsuperscript{6}-benzylguanine in Pediatric Patients with Central Nervous System Tumors: A Pediatric Oncology Group Study

Kathleen Neville, Susan Blaney, Mark Bernstein, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/10/15/5072

Cited articles  This article cites 22 articles, 14 of which you can access for free at: http://clincancerres.aacrjournals.org/content/10/15/5072.full.html#ref-list-1

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.