Eradication of Human Medulloblastoma Tumor Xenografts with a Combination of $O^6$-Benzy1-2′-deoxyguanosine and 1,3-Bis(2-chloroethyl)-1-nitrosourea

Demetrios M. Kokkinakis,2 Robert C. Moschel, Anthony E. Pegg, and S. Clifford Schold

Department of Neurosurgery, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-8855 [D. M. K.]; National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201 [R. C. M.]; Departments of Cellular and Molecular Physiology and Pharmacology, Pennsylvania State University, College of Medicine, The Milton Hershey Medical Center, Hershey, Pennsylvania 17033 [A. E. P.]; and Division of Neurology, Duke University Medical Center, Durham, North Carolina 27710 [S. C. S.]

ABSTRACT

$O^6$-Benzy1-2′-deoxyguanosine (dBG), a water-soluble inhibitor of $O^6$-methylguanine-DNA methyltransferase (MGMT), potentiates the efficacy of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) against MGMT-positive, BCNU-resistant Daoy human medulloblastoma tumor xenografts in athymic mice (S. C. Schold et al., Cancer Res., 56: 2076–2081, 1996). Such potentiation was comparable to that observed for $O^6$-benzylguanine, the prototype MGMT inhibitor that is currently undergoing clinical trials. In this study, we optimized the therapeutic effect of the dBG and BCNU combination against brain tumor xenografts without inducing substantial toxicity in the host by adjusting the doses of both compounds. dBG was escalated from 133 mg/m² to 200 and 300 mg/m², whereas corresponding doses of BCNU were reduced from 25 mg/m² to 17 and 11 mg/m², respectively. The growth delays of 30.2, 38.4, and 22.3 days, respectively, observed for the above regimens suggest that the optimal drug combination is not achieved with maximum doses of dBG. In fact, the highest doses of dBG (300 mg/m²) contributed to more frequent BCNU-related toxicities, despite the reduced BCNU dosage, and a reduction of the therapeutic effect. Toxicity was related to the depletion of MGMT activity in the gut of host mice and was manifested by edema, inflammation, and hemorrhage in the bowel wall by subsequent BCNU administration. With additional dosage adjustments, we found that tumor suppression of >90 days without toxicity was observed at 200 mg/m² dBG and 23 mg/m² BCNU. At these doses, tumors were eradicated (regressed to an undetectable size for >90 days) in 8 of 12 animals. Thus, dBG is the first of the MGMT inhibitors to show a curative effect in combination with BCNU against a human central nervous system tumor xenograft in athymic mice.

INTRODUCTION

Inhibitors of MGMT1 potentiate the cytotoxic effect of chloroethy1ating and methylating antitumor drugs that produce $O^6$-substituted guanine adducts. Such potentiation has been shown against a variety of tumors both in culture (1–7) and in animal models (8–10) using the prototype MGMT inhibitor BG, which is currently undergoing clinical trials (11). BG is one of the most potent compounds in suppressing MGMT activity in vitro and has an $ED_{50}$ of <0.2 μM (12). However, BG is only marginally soluble in aqueous solvents and has a short half-life in rodents and humans (13, 14). Rapid oxidation to $O^6$-benzyl-8-oxoguanine, an equally potent MGMT inhibitor, accounts for part of its metabolic clearance and also for its persistent inhibitory capacity in vivo (13). Decomposition involving the loss of the benzyl group and inhibitory activity is believed to be a prevailing pathway in the metabolism of BG, accounting for as much as 60% of metabolic clearance in humans (14). These observations have stimulated the development of additional inhibitors with $ED_{50}$s comparable to that of BG but with greater solubilities in aqueous solvents to ensure rapid systemic distribution. Persistence of the parent compound or active metabolites to ensure prolonged suppression of MGMT, lack of toxicity due to the parent compound or its metabolites, and possibly expression of activity against mutant MGMT proteins that are resistant to BG (15–17) are also desirable features of a new generation of MGMT inactivators. In this context, we have tested several 9-substituted derivatives of BG, and we have obtained most promising results with dBG (18). This compound, which has an $ED_{50}$ of 2 μM in cell-free systems and 0.5 μM in HeLa cells (12), was expected to be less effective than BG in suppressing the growth of tumor xenografts in athymic mice. However, dBG was as effective as BG in delaying the growth of medulloblastoma xenografts (Daoy) in mice when used at equimolar doses to BG (8). Because the dBG dose can be escalated more easily than BG, at least in the mouse model, we tested various doses of dBG to optimize the potentiation of BCNU. In previous experi-

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2 To whom requests for reprints should be addressed, at Department of Neurosurgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8855. Phone: (214) 648-6314; Fax: (214) 648-2265.

The abbreviations used are: MGMT, $O^6$-methylguanine-DNA methyltransferase; dBG, $O^6$-benzy1-2′-deoxyguanosine; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; CNS, central nervous system; PEG, polyethylene glycol.
iments, we have shown that dBG is relatively resistant to metabolism as compared with BG (19, 20). dBG persists in the circulation for about 4 h after administration to rodents and reduces the MGMT activity in xenografted tumors to about 1% of its base value for at least 16 h after its administration (18). Conversion of dBG to BG and to O6-benzyl-8-oxoguanine may account for the enhanced and prolonged suppression of MGMT even after the clearance of the parent nucleoside from circulation (8, 18) that is required to effectively cross-link DNA after treatment with bifunctional nitrosoureas (21, 22). In this study, we demonstrate that optimal doses of dBG and BCNU in combination can actually eradicate human CNS tumor xenografts in the athymic mouse model.

MATERIALS AND METHODS

Chemicals. dBG was synthesized and purified according to previously published methods (23, 24). 3H-labeled methylated DNA was prepared as described previously (20, 25) with [3H-CH3]MNU (specific activity, 17.5 Ci/mmol).

Animals. Four-week-old BALB/c nu/nu athymic mice were purchased from Simonson (Gilroy, CA). Mice were maintained under barrier conditions and given sterilized food (Harlan Teklad laboratory diet) and water.

Tumor Lines. Daoy, a hyperdiploid human medulloblastoma line, grows s.c. in athymic mice, with a doubling time of about 1% of its base value for at least 16 h after its administration (18). Conversion of dBG to BG and to O6-benzyl-8-oxoguanine may account for the enhanced and prolonged suppression of MGMT even after the clearance of the parent nucleoside from circulation (8, 18) that is required to effectively cross-link DNA after treatment with bifunctional nitrosoureas (21, 22).

Treatment Efficacy of s.c. Injections and Effect of dBG Dose. The median time from treatment to five times the tumor volume at treatment for the Daoy medulloblastoma was 9 days when tumor-bearing animals were injected with the vehicle (40% PEG and 60% PBS) and 8.8 days when animals were treated with the same vehicle plus BCNU (23 mg/m2). On the other hand, when animals with Daoy tumors were treated with various combinations of dBG and BCNU, the median time to five times the tumor volume at treatment was markedly greater (Table 1), and growth delay (as compared to treatment with BCNU only) was highly dependent on the dBG dose. A dose of 133 mg/m2 dBG in combination with 25 mg/m2 BCNU resulted in a tumor growth delay of 30.2 days, with one death and an average weight loss of 5.3%. A dose of 200 mg/m2 dBG in combination with doses of BCNU at 11 and 23 mg/m2 resulted in tumor growth delays of 38.4 days and more than 81 days, respectively (Fig. 1). A modest average weight loss of 4.2% and 9.0%, respectively. A 300 mg/m2 dose of dBG in combination with BCNU (17 mg/m2) produced a growth delay of only 14.4 days, with treatment-related mortality of 4 out of 12 animals and an average weight loss of 12.9%. Reduction of the BCNU dose to 11 mg/m2 produced similar results. The combination of dBG (200 mg/m2) and BCNU (17 mg/m2) was curative for 3 of 11 animals with no measurable tumor for more than 90 days after treatment. Eight of 12 animals were tumor-free survivors for at least 90 days.
after treatment when the BCNU dose was escalated to 23 mg/m² in combination with 200 mg/m² dBG. There were no 90-day survivors after treatment with any of the other dosage combinations.

### Effect of dBG Dose on MGMT Levels in Tumor and Normal Tissue and Associated BCNU-related Toxicity.

Inhibition of MGMT activity in Daoy tumors implanted in athymic mice to levels as low as 8% of the base value was accomplished within 2 h after administration of 100 mg/m² dBG (Fig. 2). Doses higher than 150 mg/m² dBG reduced the activity to less than 10 fmol/mg protein, a state that is considered comparable to the mer-negative phenotype (nonexpressive). MGMT activity remained suppressed for at least 16 h after dBG administration. At doses of 134, 200, and 300 mg/m² dBG, the levels of MGMT in the tumor were 3.5 ± 2%, 1.6 ± 1%, and less than 1% of the baseline level at 16 h after treatment. However, at 24 h, the MGMT levels were elevated to 20 ± 3%, 15 ± 2%, and 14 ± 4% of the baseline, respectively. The MGMT activity of the intestinal epithelium of the host was more difficult to suppress by dBG and remained nearly unaffected at doses less than 100 mg/m². Suppression of MGMT activity in the intestinal epithelium was observed at dBG doses greater than 150 mg/m², but residual activity (12–18 fmol/mg protein) was still detectable at 300 mg/m². Acute toxicity of BCNU administered 1 h after dBG was usually manifested as early as 24 h by weight

<table>
<thead>
<tr>
<th>Agent*</th>
<th>Doses (mg/m²)</th>
<th>Median time to 5× treat. volume (days)</th>
<th>Tumor regressions</th>
<th>Mortality</th>
<th>Weight loss (%)</th>
<th>T-C*</th>
<th>P versus control</th>
</tr>
</thead>
<tbody>
<tr>
<td>dBG/BCNU 133/25</td>
<td>38</td>
<td>11/11</td>
<td>1/12</td>
<td>5.3</td>
<td>30.2</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>dBG/BCNU 200/17</td>
<td>46</td>
<td>12/12</td>
<td>0/12</td>
<td>10.4</td>
<td>38.4</td>
<td>&lt;0.001</td>
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<tr>
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<td>&gt;90</td>
<td>12/12</td>
<td>0/12</td>
<td>9.0</td>
<td>&gt;81.2</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td>dBG/BCNU 300/17</td>
<td>22</td>
<td>5/8</td>
<td>4/12</td>
<td>12.9</td>
<td>14.4</td>
<td>&lt;0.050</td>
<td></td>
</tr>
<tr>
<td>dBG/BCNU 300/11</td>
<td>33</td>
<td>5/8</td>
<td>4/12</td>
<td>9.1</td>
<td>22.3</td>
<td>&lt;0.036</td>
<td></td>
</tr>
<tr>
<td>PEG/ethanol</td>
<td>–/–</td>
<td>9.0</td>
<td>0/5</td>
<td>0/5</td>
<td>0.0</td>
<td>–</td>
<td>NS</td>
</tr>
<tr>
<td>PEG/BCNU</td>
<td>–/25</td>
<td>7.8</td>
<td>0/12</td>
<td>0/12</td>
<td>0.0</td>
<td>–</td>
<td>1.2</td>
</tr>
<tr>
<td>PEG/BCNU</td>
<td>–/23</td>
<td>8.8</td>
<td>0/5</td>
<td>0/5</td>
<td>0.0</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td>PEG/BCNU</td>
<td>–/17</td>
<td>7.6</td>
<td>0/12</td>
<td>0/12</td>
<td>0.0</td>
<td>–</td>
<td>1.4</td>
</tr>
<tr>
<td>PEG/BCNU</td>
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<td>10.7</td>
<td>0/12</td>
<td>0/12</td>
<td>0.0</td>
<td>1.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

* A drug was administered i.p. at a volume of 20–25 ml/m².

* T-C: tumor growth delay as compared to the group treated with BCNU alone (drug vehicle). Tumor growth delay (T-C) is the difference between median time to 5× treatment volume in treated groups minus the median time to 5× treatment volume in the control groups.

* NS, not significant.

The table above shows the treatments of s.c. Daoy xenografts in athymic mice with dBG/BCNU: effect of dose. The agents and their corresponding doses, median time to 5× treatment volume, tumor regressions, mortality, weight loss, T-C, and P versus control are listed in the table. The figure shows the suppression of MGMT in mouse intestinal epithelium and human medulloblastoma xenografts (Daoy) implanted s.c. in athymic mice. The MGMT activities are shown individually for three animals at each dose.
patients with "high" MGMT activity. This relationship remained
statistically significant when other known prognostic factors
were considered. Jaekle et al. (37) has recently analyzed the
largest subset of patients from the Belanisch series (the South-
west Oncology Group patients), all of whom were treated on the
same protocol. The statistical relationship between MGMT ex-
pression and outcome was even stronger in this subset, despite
the fact that the total number of patients was smaller.

Tumor resistance due to MGMT expression can be over-
come with agents that inactivate the MGMT protein and reduce
the efficiency of repair of 6-chloroethylguanine adducts. The
first compound to be tested in vivo as both an inactivator of
MGMT and a potentiator of BCNU was BG. This compound is
capable of rapidly inactivating high levels of MGMT for pro-
longed time periods at relatively low concentrations (7). When
BG is administered to animals bearing a MGMT-positive,
BCNU-resistant human tumor, MGMT activity in the tumor is
inhibited for several hours, and during that time, the tumor
becomes highly sensitive to BCNU (8, 9). BG is not toxic as a
single agent, and Phase I trials have indicated that 100–200
mg/m² BG results in nearly complete elimination of MGMT
activity in brain tumors as early as 4 h after administration.4 In
glioblastomas, such activity remains low for at least 18 h after
administration (11).

We have previously shown that dBG, a 9-substituted de-
rivative of BG, can be as effective as BG in potentiating
BCNU against human CNS tumor xenografts in athymic mice (8),
despite the difference in the ED₅₀ of the two compounds, which
favors BG as a more effective inhibitor of MGMT and predicts
that BG might be more effective than dBG in potentiating the
antitumor activity of BCNU or other alkylating chemotherapeu-
tic drugs. The reason for the discrepancy between the in vitro
and in vivo results is not fully understood. Examination of the
pharmacokinetics and metabolism of dBG in rodents has shown
that the unexpectedly robust capacity of dBG to potentiate
BCNU against tumors is most likely due to its rapid systemic
distribution to tissues and its metabolic conversion to com-
ounds that are more active as MGMT inhibitors than dBG
itself. In rats, dBG is converted to BG, which is found in the
circulation and in several tissues examined at concentrations
peaking between 2 and 6 h after dBG administration (20).
Because MGMT levels are nearly depleted in both liver and
tumor 1 h after the administration of dBG, it is speculated that
dBG itself reacts with the MGMT, causing its initial decline. A
subsequent decline in activity and prolonged suppression to
about 1% of the baseline activity for at least 16 h after dBG
treatment probably results from the generation of BG and its
metabolite O⁶-benzyl-8-oxoguanine (13, 19). Such potent
and extensive suppression is thought to lead to maximum effective-
ness of cross-linking after BCNU treatment (21, 22). Thus, dBG
in effect acts as a pro-drug for the more active MGMT inhibitors
BG and O⁶-benzyl-8′-oxoguanine, and the greater solubility and
tissue distribution of dBG enhance its therapeutic effect in
combination with BCNU.

In this study, we have demonstrated that dBG in combina-
tion with BCNU not only delays the growth of s.c. MGMT-

Table 2  Acute toxicity of dBG/BCNU treatment on the intestinal
epithelium of athymic mice bearing Daoy xenografts

<table>
<thead>
<tr>
<th>Dosesa</th>
<th>Inflammationb</th>
<th>Apoptoticsc</th>
<th>Mitotic</th>
<th>Hemorrhagee</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG/ethanol</td>
<td>1</td>
<td>1.8</td>
<td>3.4</td>
<td>0/6</td>
</tr>
<tr>
<td>133/ethanol</td>
<td>1</td>
<td>1.9</td>
<td>3.9</td>
<td>0/6</td>
</tr>
<tr>
<td>133/17</td>
<td>3</td>
<td>—</td>
<td>0.5</td>
<td>0/6</td>
</tr>
<tr>
<td>133/25</td>
<td>19</td>
<td>2.9</td>
<td>0.4</td>
<td>0/6</td>
</tr>
<tr>
<td>200/17</td>
<td>4</td>
<td>1.6</td>
<td>0.3</td>
<td>0/6</td>
</tr>
<tr>
<td>200/23</td>
<td>9</td>
<td>2.8</td>
<td>0.7</td>
<td>0/6</td>
</tr>
<tr>
<td>300/11</td>
<td>28</td>
<td>5.9</td>
<td>3.1</td>
<td>1/6</td>
</tr>
<tr>
<td>300/23</td>
<td>56</td>
<td>15.3</td>
<td>6.9</td>
<td>6/12</td>
</tr>
</tbody>
</table>

a Animals were treated with dBG and BCNU 1 h apart and sacri-
ficed 48 h later.

b Dose: dBG/BCNU (mg/m²). In controls, dBG and BCNU were
replaced with PEG and ethanol, respectively.

c Inflammation with expansion of lamina propria of the villi (per-
tcentage of total villi).

d Apoptotic and mitotic indices (apoptotic bodies or mitosis per
1000 cells).

e Number of animals developing hemorrhage per total number of
animals treated.

4 Unpublished observations.
positive tumors but can also cause their eradication in a large percentage of animals receiving treatment at optimal doses. A dose of 200 mg/m^2 dBG appears to reduce MGMT activity in the tumor to a degree and duration that allow maximum efficacy of BCNU without excessive toxicity. An equivalent therapeutic effect has not been found with BG and BCNU at any of the doses tested thus far in animals (8, 9). One of the recognized problems with MGMT inhibitors, including dBG, is the potentiation of BCNU toxicity in the host. BCNU is tolerated well as doses tested thus far in animals (8, 9). One of the recognized effects on both efficacy and toxicity. Both the toxicity and tumor suppression data presented here also demonstrate that although the ED50 dose is a good prognostic factor for predicting the effectiveness of MGMT inactivators in potentiating BCNU against tumors, it is only a rough guide for predicting the outcome of in vivo experiments. Metabolism and clearance of the inhibitor are of paramount importance in determining the outcome of animal and human trials. Additional effort must be devoted to understanding the metabolism of other MGMT inhibitors and preparing additional drugs that are resistant to rapid metabolic degradation and are more specific for MGMT inactivation in tumors than in normal tissues.

REFERENCES


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