Experimental Antitumor Activity and Toxicity of a New Chemotherapeutic Agent, BBM 928A

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ABSTRACT

The experimental antitumor activity and toxicity of a new antibiotic, BBM 928A (Luzcopeptin A), was evaluated in mice. When administered as a suspension in 0.9% NaCl solution, the compound demonstrated good activity [median survival time of dying mice in a drug-treated group divided by the median survival time of 0.9% NaCl solution-treated mice in a tumor control group, (T/C) >150%] against i.p. B16 melanoma (B16) and i.p. P388 leukemia (P388) and weak activity (T/C of 125 to 150%) versus i.v. P388, i.p. L1210 leukemia (L1210), Lewis lung (LL), and Madison 109 lung (M109) carcinomas. In terms of tumor cell kill, BBM 928A induced net reductions in the body burdens of L1210 and P388 leukemias following single-drug injections but usually failed to yield net reductions following multiple-injection therapies despite achieving T/C >125%. Similarly, mice receiving i.m. implants of LL or M109 or i.v. implants of P388 did not generally achieve a net reduction in tumor burden following treatment with BBM 928A despite associated median increases in life span in excess of 25%, but multiple-injection therapies with BBM 928A against i.p. B16 did result in consistent net reductions in tumor levels. The 0.9% NaCl solution-suspended drug was inactive against i.p. Colon 26, s.c. M109, and s.c. B16. Compared to echinomycin, which it resembles in structure, BBM 928A performed very similarly against various murine tumors. For example, against i.p. B16, BBM 928A displayed superior (average net cell kill advantage of 3.7 log10), but it was comparable against i.p. L1210, P388, LL, and Colon 26 carcinomas, and slightly inferior in the i.p. M109 model. A soluble formulation of BBM 928A was found to be equivalent in activity to the suspension against i.p. P388 and B16 and was slightly active (T/C of 151% or 1.4 log10 net cell kill) versus s.c. B16, whereas the suspension was inactive. In toxicity studies, the soluble formulation, a 0.1-mg/kg dose of which could kill 50% of the treated animals, did not cause leukopenia, nephrotoxicity, or hepatotoxicity as indicated by the lack of an effect upon WBC, blood urea nitrogen levels, and serum glutamic-pyruvic transaminase levels, respectively. Although there was no leukopenia, there were both a dose-related neutrophilia and lymphopenia. Because of its antitumor activity, novel structure, high potency (effective at μg/kg dose levels), strong binding affinity to DNA, and apparent lack of certain organ toxicities, further development of this compound or its analogs is being considered.

INTRODUCTION

A new antitumor antibiotic, BBM 928A, has been described recently by investigators in Japan (7, 10). It is a naturally occurring antibiotic isolated from among a family of structurally similar compounds produced via fermentation by an aerobic strain of actinomycetes, Actinomadura luzonensis. The compound is related chemically to echinomycin (also known as quinomycin A) but differs, for example, in that BBM 928A contains substituted quinoline rings, whereas echinomycin contains substituted quinoxaline rings (Chart 1).

In a recent investigation of the molecular biological properties of BBM 928A, Huang et al. (4) found the drug to be a very potent bifunctional DNA intercalator (like echinomycin); no double- or single-strand DNA breaks have been observed (5).

Because of the relative novelty of its chemical structure, strong binding affinity for DNA, high potency, and an initial observation of antitumor activity, further evaluation of BBM 928A was made. Herein, we describe the results of our investigations involving the experimental antitumor activity and toxicity of this new compound as well as the comparative antitumor results obtained with echinomycin.

MATERIALS AND METHODS

Animals. BALB/c, DBA/2, C57BL/6, BALB/c × DBA/2 F1 (hereafter called DBA/2F1), and C57BL/6 × DBA/2 F1 (hereafter called BDF1) mice of both sexes, 16 to 20 g, were used for antitumor testing. Male BDF1, mice, 22 to 29 g, were used for toxicity studies. All mice were obtained from either Charles River Breeding Laboratories, Inc. (Wilmington, Mass.), Taconic Farms, Inc. (Germantown, N. Y.), or Lab Supply Co., Inc. (Indianapolis, Ind.).

Drugs. BBM 928A (NSC 308312) and echinomycin (NSC 526417) were usually suspended in 0.9% NaCl solution and finely dispersed by either grinding or sonication; in some specifically mentioned instances, DMSO was used initially to dissolve the drugs followed by dilution with 0.9% NaCl solution (final DMSO concentration, <2%). A soluble salt of BBM 928A, formulated by reaction with NaHPCX» (prepared by M. Kaplan, Bristol-Myers Co.), was dissolved in water. All injections were i.p. except where otherwise indicated. The structures of both BBM 928A and echinomycin are shown in Chart 1.

Tumors. P388 and L1210 leukemias were maintained in ascitic form in DBA/2 mice. LL and B16 were maintained as s.c. growing tumors in C57BL/6 mice. M109 (6, 8) and C26 were maintained as s.c. growing tumors in BALB/c mice.

For certain experiments, lines of B16 and P388 tumors were obtained from the tumor bank of the National Cancer Institute (courtesy of Dr. A. Bogden, Mason Research Institute, Worcester, Mass.), and their sensitivity to BBM 928A was compared concomitantly to that of our own tumor lines.

Antitumor Testing. Experiments involving L1210 were initiated by implanting 106 leukemic cells i.p. into BDF1 or C57BL/6 mice. P388 experiments were begun by implanting 106 leukemic cells i.p. or i.v. into...
CD2F, mice. Each drug-treated group consisted of 6 mice, and leukemia control groups were composed of 10 mice.

LL experiments were begun by implanting $10^6$ viable (those excluding trypan blue) tumor cells i.p. into BD2F, mice. B16 experiments were begun by implanting 0.5 ml of a 10% tumor brei suspension i.p., or 25 mg (approximately) tumor fragments s.c. (via trocar), into BD2F, mice. In both the LL and B16 studies, drug-treated and tumor control groups consisted of 10 mice. M109 and C26 experiments were begun by implanting 0.5 ml of a 2% (M109) or 1% (C26) tumor brei suspension i.p., or 0.1 ml of a 2% tumor brei suspension (M109) s.c., into CD2F, mice. Both drug-treated and tumor control groups consisted of 8 mice.

L1210 and P388 experiments were terminated on Day 30, and B16, LL, M109, and C26 experiments were terminated on Day 60. Mice alive at the end of an experiment were autopsied and judged to be cured if no signs of disease were visible. Each drug in an experiment was evaluated at a minimum of 3 dose levels for each treatment schedule used. The various treatment schedules are described in "Results" and in Tables 1 to 3. Mice were observed daily, and antitumor drug activity was also judged by the relative median time for tumors to reach the percent T/C. For mice receiving s.c. implants of M109, antitumor activity was not determined, since no suggestion of drug activity was apparent. The TD of the various tumor models used for the calculation of net tumor cell kill were: i.p. and i.v. P388, 0.57 day; i.p. L1210, 0.45 day; i.p. LL, 1.0 day; i.p. B16, 1.4 days and s.c. B16, 1.3 days; i.p. M109, 1.2 days. Values for s.c. M109 and i.p. C26 were not determined, since no suggestion of drug activity was apparent. The TD for P388 and L1210 leukemias were estimated from a best-fit straight line from a log-linear plot of the MST (of dying mice only) versus log 10 of the leukemia cells in untreated mice. Similar values for M109 (8) and B16 (3) were obtained from the literature or, for LL, estimated from unpublished data.

Drug-treated mice dying prior to the first death among parallel, untreated, tumor control mice were presumed to have died from drug toxicity and were excluded from calculations of MST. No result of therapy is reported in which deaths attributable to drug toxicity exceeded 17% in the treated group (i.e., allowing for one early death among a group of 6 mice).

Toxicity Testing. The evaluation of the toxicities of BBM 928A was performed on the water-soluble formulation only. An acute LD$_{50}$ was determined following single i.p. injections into mice (groups of 10 mice/dose). The mice were observed for 30 days, and the LD$_{50}$ was calculated according to the method of Weil (11).

Hematology and serum chemistry studies were performed using methods which have been described previously (1). Briefly, 3 groups of mice, 50/group, were bled from the retroorbital plexus 3 days prior to dosing to determine their individual pretreatment total WBC and differential leukocyte counts (Group 1), BUN values (Group 2), and SGPT values (Group 3). The total WBC counts were made using a Model S Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), and the differential leukocyte counts were done manually. The BUN and SGPT values were determined using a Centrifichem System 400 (Union Carbide Co., Rye, N. Y.). BBM 928A was administered as a single i.p. injection at several dose levels of the leukemias used and were compared to the untreated control mice.

Tumor wt (mg) = $a \times b^2/2$

where $a$ and $b$ are the tumor length and width (mm), respectively (2, 8). A drug was considered active if it produced T/C of $\geq 125\%$ ($\geq 135\%$ versus s.c. B16) or T-C of $\geq 4$ days.

Additionally, an attempt was made to quantitate the degree of tumor cell kill achieved with each drug regimen using methods described earlier (9) and more recently (2). Briefly, the log$_{10}$ net cell kill achieved was calculated as

$$\log_{10} \text{net cell kill} = \frac{\text{T-C value (days)} - \text{[duration of treatment (days)]}}{(3.32) \text{(TD)}}$$

where T-C value was the MST for drug-treated dying mice only, minus the MST for control mice (the T-C value could also reflect tumor growth delay, as described above for s.c. M109; but since BBM 928A was inactive in the s.c. M109 model, this particular parameter was never used). Such calculations were performed only with data from experiments where drug activity (as defined above) was observed. If the log$_{10}$ net cell kill was positive, there were fewer cells present at the end of therapy than at the start. The TD of the various tumor models used for the calculation of net tumor cell kill were: i.p. and i.v. P388, 0.57 day; i.p. L1210, 0.45 day; i.p. LL, 1.0 day; i.p. B16, 1.4 days and s.c. B16, 1.3 days; i.p. M109, 1.2 days. Values for s.c. M109 and i.p. C26 were not determined, since no suggestion of drug activity was apparent. The TD for P388 and L1210 leukemias were estimated from a best-fit straight line from a log-linear plot of the MST (of dying mice only) versus log$_{10}$ dilutions of the leukemia cells in untreated mice. Similar values for M109 (8) and B16 (3) were obtained from the literature or, for LL, estimated from unpublished data.

Activity and Toxicity of BBM 928A

A. BBM-928 A

B. ECHINOMYCIN (QUINOMYCIN A)

Chart 1. Structures of BBM 928 A (A) and echinomycin (B).

$^4$ W. C. Rose, unpublished data.
RESULTS

Antitumor Testing. BBM 928A was evaluated against 6 murine tumors (9 tumor models). The optimal doses and maximum therapeutic effects achieved in each of these models (using the drug as a suspension) are summarized in Table 1.

Against L1210 leukemia, BBM 928A was only weakly active, regardless of the dosing regimen used. In terms of tumor cell kill, only single-drug injections on Day 1, which yielded maximum T/C values of 136 to 138%, produced a net reduction (of 1 log_{10}) in the leukemia body burden. In one of the L1210 experiments, a comparison was made between BBM 928A in 0.9% NaCl solution only, versus the drug in DMSO plus 0.9% NaCl solution. BBM 928A in 0.9% NaCl solution only caused a maximum T/C of 129%, whereas BBM 928A in DMSO plus 0.9% NaCl solution caused a T/C of only 114% (data for BBM 928A in other than 0.9% NaCl solution are not included in Table 1), indicating no advantage to using DMSO to initially dissolve the drug.

BBM 928A was evaluated in 21 experiments involving P388 leukemia implanted i.p. Single i.g. injections of BBM 928A (on Day 1) yielded maximum T/C values of between 128 and 206%, which were consistent with net decreases in leukemia body burden of between 0.8 and 4.5 log_{10} (mean of 2.4 log_{10}). Daily dosing for 5 consecutive days resulted in a mean maximum T/C of 167%, which reflected a minimal net decrease in P388 leukemia by the end of treatment. Nine daily injections of BBM 928A resulted in a mean maximum T/C of 163%, but such treatment failed to achieve a net reduction in leukemia body burden following the last dose.

As part of the P388 testing, we sought to determine which of 2 vehicles, 0.9% NaCl solution or DMSO plus 0.9% NaCl solution, served as a better vehicle (data not shown). No essential differences were noted in the maximum effects obtained with BBM 928A in these 2 vehicles; but in 0.9% NaCl solution only, the optimal doses were 32 to 128 µg/kg/injection, whereas in DMSO plus 0.9% NaCl solution, they were 8 to 32 µg/kg/injection (both treated each day, Days 1 to 9).

We also evaluated the use of fresh daily preparations of BBM

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### Table 1
Summary of experimental antitumor activity of BBM 928A

<table>
<thead>
<tr>
<th>Tumor inoculum and route</th>
<th>No. of experiments/schedule</th>
<th>Range of optimal i.p. doses (µg/kg/injection)</th>
<th>Treatment schedule</th>
<th>Range of maximum % T/C</th>
<th>Net log_{10} cell kill</th>
<th>Mean % T/C</th>
<th>Mean log_{10} cell kill</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210, 10^6, i.p.</td>
<td>2</td>
<td>192–256</td>
<td>Day 1</td>
<td>136–138</td>
<td>1.0</td>
<td>163</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>96–192</td>
<td>Days 1, 5, and 9</td>
<td>129–138</td>
<td>-4.4 to -4.7</td>
<td>163</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>64–128</td>
<td>qd 1–9^2</td>
<td>129–146</td>
<td>-4.0 to -4.7</td>
<td>131</td>
<td>-4.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>48</td>
<td>2/qd 1–9</td>
<td>131</td>
<td>-4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P388, 10^6, i.p.</td>
<td>15</td>
<td>96–512</td>
<td>Day 1</td>
<td>128–206</td>
<td>0.8 to 4.5</td>
<td>163</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>128–256</td>
<td>Days 1 and 5</td>
<td>144–156</td>
<td>-0.3 to 0</td>
<td>163</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>64–192</td>
<td>Days 1, 5, and 9</td>
<td>159–194</td>
<td>-2.1 to -0.3</td>
<td>163</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>192</td>
<td>Days 1, 3, 5, 7, 9</td>
<td>172</td>
<td>-1.3</td>
<td>163</td>
<td>-1.8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>64–128</td>
<td>qd 1–9</td>
<td>150–189</td>
<td>-0.3 to 1.6</td>
<td>167</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>32–192</td>
<td>qd 1–9</td>
<td>144–183</td>
<td>-2.8 to -0.6</td>
<td>163</td>
<td>-1.8</td>
</tr>
<tr>
<td>P388, 10^6, i.v.</td>
<td>1</td>
<td>256, i.v.</td>
<td>Days 1, 5, and 9</td>
<td>128</td>
<td>-3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>All, i.p.</td>
<td>Days 1, 5, and 9</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>256, i.v.</td>
<td>Days 1, 3, and 5</td>
<td>125–141</td>
<td>-1.3 to -0.8</td>
<td>163</td>
<td>-1.8</td>
</tr>
<tr>
<td>Lewis lung, 10^6, i.p.</td>
<td>1</td>
<td>40</td>
<td>qd 1–9</td>
<td>137 (2/10)^a</td>
<td>-1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>160</td>
<td>Days 1, 5, and 9</td>
<td>127 (1/10)</td>
<td>-1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B16, 0.5 ml of a 10% brei, i.p.</td>
<td>3</td>
<td>192–256</td>
<td>Days 1, 5, and 9</td>
<td>179–251 (0–5/10)</td>
<td>1.3 to 4.7</td>
<td>207</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>128–192</td>
<td>qd 1–9</td>
<td>208–290 (0–5/10)</td>
<td>2.5 to 6.7</td>
<td>250</td>
<td>4.6</td>
</tr>
<tr>
<td>B16, 25-mg fragments, s.c.</td>
<td>1</td>
<td>All, i.v. and i.p.</td>
<td>qd 1–9</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>All</td>
<td>qd 1–9</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109, 0.5 ml of a 2% brei, i.p.</td>
<td>3</td>
<td>96–192</td>
<td>Days 1 and 4</td>
<td>116–159</td>
<td>-1.6 to 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>32–64</td>
<td>qd 1–4</td>
<td>116–156</td>
<td>-1.8 to 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M109, 0.1 ml of a 2% brei, s.c.</td>
<td>1</td>
<td>All</td>
<td>qd 1–4</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>All</td>
<td>qd 1–4</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon 26, 0.5 ml of a 1% brei, i.p.</td>
<td>1</td>
<td>All</td>
<td>Days 1 and 4</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>All</td>
<td>qd 1–4</td>
<td>Inactive</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a MST for control mice receiving the various tumor inocula were: L1210 i.p., 6.5 to 7.0 days (2 experiments); P388 i.p., 9.0 days (19 experiments), 8.5 days (one experiment), and 11.0 days (one experiment involving a National Cancer Institute line of P388); P388 i.v., 8.5 to 10.0 days (4 experiments); Lewis lung i.p. 15.0 days (one experiment); B16 i.p., 19.0 to 21.0 days (5 experiments); B16 s.c., 29.5 days (one experiment); M109 i.p., 16.0 to 17.5 days (3 experiments); M109 s.c., median time to reach a 1-g tumor was 18.0 days, and MST was 37.5 days (one experiment); Colon 26 i.p., 29.0 days (one experiment). There were no survivors among any control mice.

^b Percent T/C values reflect MST of dying mice only; cures shown separately. Group sizes for both drug-treated and control mice are described in "Materials and Methods."

^c Calculated as described in "Materials and Methods" and in Refs. 2 and 8. TDs for the various tumor models used are also provided in "Materials and Methods."

^d qd 1–X, each day, Days 1 to X.

^e Numbers in parentheses, number of cures per total.
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928A suspensions (in 0.9% NaCl solution only) versus the (typical) use of one preparation for as many as 9 days (with refrigeration during non-use). In a single experiment (data not shown), use of the standing preparation yielded a maximum T/C of 183% (64 μg/kg/injection) compared to 150% (achieved with both 32 and 64 μg/kg/injection) with drug suspensions prepared fresh daily (both treated each day, Days 1 to 9).

Four P388 experiments conducted involved i.v.-implanted leukemic cells. Although inactive by the i.p. route, BBM 928A achieved maximum T/C of 125 to 141% when given i.v. on intermittent or consecutive daily dosing schedules. In terms of cell kill, however, no net reduction of leukemia was accomplished.

Against LL, the drug displayed modest activity (T/C of 137% and 2 of 10 mice cured), but there was actually an increase in the calculated tumor body burden by the end of therapy.

Five experiments were performed with B16 implanted i.p. Substantial increases in life span with occasional cures occurred following either intermittent or consecutive daily dosing of BBM 928A. For example, in one study, BBM 928A given either on Days 1, 5, and 9 or each day, Days 1 to 9, caused 50% cure rates with T/C values, for dying mice only, of 251 and 241%, respectively. Included in this same experiment were groups of mice receiving BBM 928A in DMSO plus 0.9% NaCl solution, given each day, Days 1 to 9, and the maximum effect achieved was 3 of 10 mice cured and a T/C of 234% (data not shown). In terms of tumor cell kill, a net reduction in tumor body burden was achieved in each experiment (e.g., a mean cell kill of 4.6 log₁₀ following drug dosing daily, Days 1 to 9).

One experiment involving s.c.-implanted B16 was performed, but BBM 928A, as a suspension in 0.9% NaCl solution, was inactive either i.p. or i.v.

BBM 928A was found to be active against i.p. M109 in 2 experiments (maximum T/C of 137 and 159%), but in a third study no activity was observed. Even with the most successful of these treatments, the surviving tumor burden at the end of therapy was essentially the same as had been present initially.

As part of one of the aforementioned M109 experiments, separate groups of mice received s.c. implants of tumor and were used to evaluate BBM 928A. Using the same treatment regimens that had been proved mildly effective (maximum T/C of 156 to 159%) against i.p. M109, BBM 928A failed to either increase the MST or delay s.c. tumor growth compared to that in tumor control mice.

One experiment involving i.p. C26 was performed, and BBM 928A was inactive using both treatment schedules evaluated.

Comparison of BBM 928A and Echinomycin. Except for the s.c. M109 and B16 models, BBM 928A and echinomycin were evaluated concomitantly at least once against each of the other tumor models. A summary of the maximum therapeutic effects achieved by both drugs (as suspensions in 0.9% NaCl solution), using treatment schedules of equivalent overall duration, is shown in Table 2.

Against L1210 leukemia, both BBM 928A and echinomycin caused similar maximum T/C values (129 to 136%) in the single experiment performed.

In only 1 of 8 studies involving i.p. P388 leukemia did the

### Table 2

Comparison of the antitumor activity of BBM 928A and echinomycin based upon concomitant testing

<table>
<thead>
<tr>
<th>Tumor inoculum and route</th>
<th>BBM 928A</th>
<th>Echinomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Optimal dose, i.p. (μg/kg/injection)</td>
<td>Treatment schedule</td>
</tr>
<tr>
<td>L1210, 10⁶, i.p.</td>
<td>128</td>
<td>qd 1-9</td>
</tr>
<tr>
<td>P388, 10⁶, i.p.</td>
<td>512</td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td>128 and 256</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>qd 1-9</td>
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<tr>
<td></td>
<td>128</td>
<td>qd 1-9</td>
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<td></td>
<td>128</td>
<td>qd 1-9</td>
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<td></td>
<td>128</td>
<td>qd 1-9</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>qd 1-9</td>
</tr>
<tr>
<td>P388, 10⁶, i.v.</td>
<td>256, i.v.</td>
<td>Days 1, 5, and 9</td>
</tr>
<tr>
<td>Lewis lung, 10⁵, i.p.</td>
<td>40</td>
<td>qd 1-9</td>
</tr>
<tr>
<td>B16, 0.5 ml of a 10% brei, i.p.</td>
<td>192</td>
<td>Days 1, 5, and 9</td>
</tr>
<tr>
<td></td>
<td>256</td>
<td>qd 1-9</td>
</tr>
<tr>
<td></td>
<td>192</td>
<td>qd 1-9</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>qd 1-9</td>
</tr>
<tr>
<td>M109, 0.5 ml of a 2% brei, i.p.</td>
<td>150</td>
<td>Days 1 and 4</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>qd 1-4</td>
</tr>
<tr>
<td>C26, 0.5 ml of a 1% brei, i.p.</td>
<td>150</td>
<td>Days 1 and 4</td>
</tr>
</tbody>
</table>

* MST for control mice receiving the various tumor inocula were: L1210 i.p., 7.0 days; P388 i.p., 8.5 to 11.0 days; P388 i.v., 9.0 days; Lewis lung i.p., 15.0 days; B16 i.p., 20.0 to 21.0 days; M109 i.p., 16.0 to 17.5 days; Colon 26 i.p., 28.0 days.

* Treatment schedule (and dose) yielding the maximum net tumor cell kill for echinomycin in each experiment was selected for presentation. Maximum therapeutic effect of BBM 928A (in each experiment), obtained using a treatment schedule of duration equal to that found optimal with echinomycin, was selected for comparison. Each line of data represents a result from a different experiment.

* T/C percentage values reflect relative MST values of dying mice only with cures shown separately, in both drug-treated and control groups of mice.

* Calculated as described in "Materials and Methods" and in Refs. 2 and 8. T/Ds for the various tumor models used are also provided in "Materials and Methods."

* qd 1-9, each day. Days 1 to 9;

* Highest dose tested with no obvious indication (i.e., early deaths) of drug-associated lethality.

* Numbers in parentheses, number of cures per total.
maximum therapeutic effects caused by each drug differ appreciably. In general, the dose of echinomycin required to achieve the maximum effect in these experiments was 2- to 4-fold less than that of BBM 928A. Two of the P388 experiments were performed concomitantly in order to evaluate the relative sensitivity of our P388 line with one obtained from the National Cancer Institute tumor bank (Table 2, last 2 lines of data involving i.p. P388). Both leukemia lines displayed similar sensitivities toward BBM 928A and echinomycin, and echinomycin caused slightly greater effects than did BBM 928A against both P388 lines. Both drugs were also evaluated in these studies using DMSO and 0.9% NaCl solution as the vehicle (data not shown), and similar maximum effects were obtained with each of them.

Against i.v.-implanted P388 leukemia, neither drug was active by the i.p. route, but BBM 928A achieved a borderline positive effect (T/C of 128%) when given i.v. Echinomycin was inactive by the i.v. route, but an obvious lethal dose was not apparent; therefore, no advantage for BBM 928A in this model can be assumed.

In the only LL experiment performed, echinomycin and BBM 928A achieved similar maximum T/C values (133 and 137%, respectively), but 2 of 10 mice treated with BBM 928A were cured of their tumors.

The maximum therapeutic effects achieved by BBM 928A in 4 experiments involving B16 were greater than those caused by echinomycin. In the first study, not only did BBM 928A cure 50% of the treated mice compared to a 20% cure rate for echinomycin, but the life span of the dying mice was also increased appreciably more by BBM 928A (T/C of 251%) than by echinomycin (T/C of 180%). Although no mice were cured in the second experiment, BBM 928A again achieved a noticeably greater T/C (220%) than did echinomycin (T/C 185%), a difference, in terms of tumor cell kill, of 1.5 log10.

The final 2 B16 experiments were performed concomitantly in order to evaluate the relative sensitivity of our own B16 line with one obtained from the National Cancer Institute tumor bank (Table 2, last 2 lines of data involving i.p. B16). Both tumor lines displayed a marked sensitivity toward BBM 928A, and BBM 928A displayed greater therapeutic activity than did echinomycin (>4 log10 net tumor cell kill advantage) against both B16 lines. This advantage of BBM 928A over echinomycin was also observed when DMSO plus 0.9% NaCl solution was used as the vehicle (data not shown).

The advantage for BBM 928A over echinomycin seen in the B16 model was reversed in the i.p. M109 model. Whereas BBM 928A was inactive on one occasion and caused a maximum T/C of 137% (0.6 log10 net tumor cell kill), on another occasion, echinomycin yielded consistent maximum T/C values of 144 to 151% (0.8 to 1.3 log10 net tumor cell kill) in the same 2 experiments.

Finally, both drugs were evaluated in the single C26 experiment performed, and both were found to be inactive.

**Comparison of BBM 928A as a Suspension and Soluble Formulation.** The final group of experiments to be described included a comparison of BBM 928A as a suspension and as a soluble formulation. The final 2 B16 experiments were performed concomitantly in order to evaluate the relative sensitivity of our own B16 line with one obtained from the National Cancer Institute tumor bank (Table 2, last 2 lines of data involving i.p. B16). Both tumor lines displayed a marked sensitivity toward BBM 928A, and BBM 928A displayed greater therapeutic activity than did echinomycin (>4 log10 net tumor cell kill advantage) against both B16 lines. This advantage of BBM 928A over echinomycin was also observed when DMSO plus 0.9% NaCl solution was used as the vehicle (data not shown).

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involves the evaluation of a BBM 928A soluble formulation. A summary of the antitumor testing performed using the formulation, as well as the concomitant results obtained using BBM 928A in suspension, are presented in Table 3.

Three different lots of the soluble formulation were evaluated in a total of 5 i.p. P388 experiments. Each lot was found to be active, and each produced fairly comparable degrees of leukemia cell kill. The formulation was also judged to be comparable in activity to the drug administered as a suspension, although the formulation was noted to have a superior potency (active at lower doses).

One experiment (Experiment 6745) addressed itself to the issue of drug stability. Groups of mice were treated with formulated BBM 928A that was prepared at various times in relation to its use. Although no significant differences in maximum therapeutic effects were observed, the doses of BBM 928A required to achieve these effects were 2- to 4-fold lower with the freshly prepared solutions. In addition (data not shown), a frankly lethal dose of freshly prepared soluble BBM 928A was well tolerated when the drug was dissolved 1 week prior to use. An evaluation of the soluble BBM 928A using thin-layer chromatography indicated a decomposition of approximately 10% after a solution was held at room temperature for 4 hr.

One experiment involving s.c.- and i.p.-implanted B16 was conducted to evaluate the antitumor activity of the soluble BBM 928A and to compare it to the drug as a suspension. Against i.p. B16, similar maximum therapeutic effects were obtained with each preparation when they were administered on a daily, Days 9-12 schedule but, using intermittent dosing schedules, the suspended drug preparation was more effective than was the soluble preparation. Against s.c. B16, however, the suspended drug was inactive, whereas the soluble preparation achieved a maximum T/C of 151% (1.4 log10 net cell kill).

**Toxicity Testing (of the Formulation).** The single-dose i.p. LD50 for the soluble formulation of BBM 928A was 0.109 mg/kg with 95% confidence limits of 0.090 to 0.133 mg/kg. The results of the hematometry and serum chemistry studies are summarized in Tables 4 and 5. BBM 928A did not cause a reduction in the total WBC of ≥35% at any time point, not even at lethal doses (Table 4). However, with regard to the differential leukocyte counts (Table 4), BBM 928A caused an increase in the absolute neutrophil counts and a decrease in the absolute lymphocyte counts. These changes were generally dose related up through the LD50 level and, with the exception of the lowest dose, were still evident on Day 7. BBM 928A caused one incident of elevated BUN at 0.145 and 0.109 mg/kg on Day 4, but it had no effect on SGPT at any dose level (Table 5).

**DISCUSSION**

BBM 928A, a new antibiotic derived from fermentation of A. luzonensis (7, 10), was found to possess antitumor activity in several murine tumor models. In qualitative terms, it demonstrated good to excellent activity (T/C values > 200% with occasional cures) versus i.p. B16 melanoma, moderately good activity (T/C values > 150%) versus i.p. P388, and fair activity (T/C of 125 to 150%) versus i.v. P388 and i.p. L1210, LL, and M109; BBM 928A was inactive against only i.p. C26 and s.c. M109. In terms of tumor cell kill, consistent reductions in net tumor body burden were achieved against only i.p. B16 (1.3 to 6.7 log10), i.p. P388 following single-drug injections (0.8 to 4.5 log10), and i.p. L1210, also following single-drug injections (1.0 log10).

In comparison to echinomycin, which it resembles in structure, BBM 928A behaved very similarly against the various murine tumors. Against i.p. B16, BBM 928A displayed superiority (mean 3.7 log10, net tumor cell kill advantage), but it was comparable against i.p. L1210, i.p. P388, i.p. LL, and i.p. C26, and only slightly inferior (=1.0 log10, net tumor cell kill disadvantage) in the i.p. M109 model. No conclusion could be reached regarding their relative activities against i.v. P388. A soluble formulation of BBM 928A was also evaluated. It was found to be equivalent in activity to the suspension in tests performed against i.p. P388 and i.p. B16 and superior in activity against s.c. B16.

Regarding BBM 928A suspensions, no therapeutic advantage was discerned when either fresh daily preparations were used (as opposed to one suspension used for 9 days) or when the drug was administered in DMSO plus 0.9% NaCl solution, as opposed to 0.9% NaCl solution only, against L1210, P388, or B16.

With respect to the soluble BBM 928A formulation, there was
a definite indication from a P388 experiment that maximum effects were obtained at lower dose levels with freshly prepared solutions, thus suggesting some degradation of the drug despite refrigeration of the solutions. Also, in the i.p. B16 experiment performed with the formulation, a substantial therapeutic advantage was obtained when consecutive daily dosing was used as opposed to intermittent dosing. This may be due to a shorter biological half-life of the soluble drug, necessitating more frequent administrations.

BBM 928A has recently been reported by Ohkuma et al. (7) to have good antitumor activity in several murine i.p. tumor models, including P388, L1210, Sarcoma 180, LL, and B16. Their therapeutic results were consistent with our own data; for example, against P388, a single dose of BBM 928A yielded a maximum T/C of 163%, and dosing daily, Days 1 to 9, yielded a T/C of 200%; against B16, daily, Days 1 to 9, dosing caused a maximum T/C of 214%. In general, the optimum doses for BBM 928A in Ohkuma’s studies were lower than those reported in the present series of experiments, but that may be due to their use of DMSO plus 0.9% NaCl solution as a vehicle.

The results of toxicity testing of the soluble formulation of BBM 928A are quite interesting. BBM 928A has a single-dose i.p. LD₅₀ of about 0.1 mg/kg, indicating that it is very potent in terms of lethality. However, BBM 928A did not produce leukopenia, nephrotoxicity, or hepatotoxicity based on its lack of effect upon WBC, BUN levels, or SGPT levels, respectively. Therefore, the cause of death does not appear to be related to effects on these organs and remains to be determined.

Although BBM 928A had no apparent effect on the total WBC, it did have an effect on the differential leukocyte counts in that it produced a dose-related neutrophilia along with a dose-related lymphopenia. We have not yet determined whether the increase in neutrophil counts reflects demargination, release from reserve pools, or an increase in neutrophil production, nor do we know if the neutrophils have normal function. Likewise, we do not yet know the reason for the lymphocyte reduction. Studies to determine these factors are currently in progress.

In conclusion, BBM 928A was most active against B16 and P388 murine tumor models (of those tested). The fact that this antibiotic has a novel structure, strong binding to DNA (4), and an apparent lack of myelotoxicity, nephrotoxicity, or hepatotoxicity suggests that further development of this compound or its analogs is warranted.

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Experimental Antitumor Activity and Toxicity of a New Chemotherapeutic Agent, BBM 928A


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