Enhancement of Bleomycin Activity against Lewis Lung Tumors in Mice by Local Hyperthermia

Richard L. Magin, Branimir I. Sikic, and Richard L. Cysyk

ABSTRACT

The cytotoxicity of the drug bleomycin in vitro has previously been shown to be enhanced by hyperthermia. This report demonstrates in vivo a more than additive interaction between local tumor hyperthermia (43°C, 60 min) and bleomycin (15 mg/kg s.c.) against s.c.-implanted Lewis lung carcinomas in mice. Local hyperthermia was produced by the application of 2450-MHz microwaves to the region of the tumor without induction of significant whole-body hyperthermia. The combined drug and heat treatments were applied to tumors on Days 4, 7, and 10 following implantation. The response of the tumors to simultaneous treatment was a 17-day growth delay compared with controls, whereas the local hyperthermia and bleomycin individually resulted in only 3- and 4-day growth delays, respectively. If the two treatments were given either 4 or 24 hr apart only an additive effect on growth delay was observed.

INTRODUCTION

The effectiveness of certain chemotherapeutic agents can be improved by combination with LTH. Recent reviews summarize the current understanding of the interaction of these 2 cancer treatment modalities (3, 6). Two drugs which display increased activity against normal and neoplastic tissues when combined with LTH are the antibiotics BLEO and Adriamycin (2, 4). Synergism between these drugs and LTH occurs only when temperatures are applied above a threshold of 42-43°C (3). The combination of BLEO and LTH has been carefully studied in vitro with respect to dependence upon the treatment conditions, dose, and schedule (1, 5, 9). The purpose of the present study was to pursue the reported in vitro synergism between LTH and BLEO in a solid tumor in vivo and to investigate the influence of scheduling on antitumor activity.

MATERIALS AND METHODS

Animals and Tumors. Male C56BL/6 x DBA/2 F1 (hereafter called B6D2F1) mice and Lewis lung carcinoma were obtained from the Mammalian Genetics and Animal Production Section, National Cancer Institute. Groups of 8 mice were housed in plastic cages and given pelleted food and water ad libitum. Lewis lung carcinoma was maintained in the C57BL/6 strain and transplanted into the B6D2F1 strain for experimentation. The tumors were implanted s.c. on the left flank anterior to the hind leg with a 0.2-ml inoculum of 10⁶ viable cells, as determined by trypan blue dye exclusion.

Microwave Hyperthermia Apparatus. A microwave system provided controlled and localized hyperthermia in the treated tumors. A complete description of the design and operation of the microwave heating apparatus has been presented previously (7); therefore, only a brief description will be given here. The microwave source (MW-150; ELMED, Inc., Addison, Ill.) generates 50 watts of microwave power at 2450 MHz. The power output of the generator is divided equally into 4 channels which provides the energy for heating 4 individual tumors simultaneously. Each channel utilizes a power regulator (Model A; ELTEK Corp., Gaithersburg, Md.) to control the power delivered to each tumor so that a constant temperature is maintained. The tumor temperature is measured by a 0.13-mm-diameter thermocouple wire imbedded at the center of the tumor. A digital thermometer (BAT6C; Bailey Instrument, Saddie Brook, N. J.) displays the temperature of the tumor and provides a reference signal for the power regulator. The microwaves are localized to the volume of the tumor by a 3.2-cm diameter microwave applicator (AT-502/7z; ELMED Inc.) which is directly coupled to the tumor by a bolus of muscle-equivalent dielectric material. The tumor encapsulation by the bolus reduces the intratumor temperature gradients to ±0.5°C in tumors less than 8 mm in diameter and increases the efficiency of the applicator.

Drug Dosage and Heat Treatment. BLEO was obtained from Nippon Kayaku, Inc., Lot UIIAS, through the courtesy of Dr. John Douros of the Natural Products Section, Developmental Therapeutics Program, National Cancer Institute. This material is known to be a mixture of 13 related glycopeptides elaborated by the actinomycete Streptomyces verticillus. BLEO was administered s.c. at a dose of 15 mg/kg immediately prior to tumor heating on Days 4, 7, and 10 following tumor implantation. This dose and schedule of BLEO was previously shown to exert antitumor effects versus Lewis lung carcinoma (10) and to be nonlethal in B6D2F1 mice (11). The tumors were heated to 43°C and maintained at that temperature for 60 min for each heat treatment. All mice were anesthetized with chloral hydrate (525 mg/kg i.p.) prior to heat, drug, or sham treatments. The rectal temperatures of the mice were measured before and after treatment to check for whole-body hyperthermia. The control and BLEO groups were kept under a heat lamp following anesthesia and drug administration to maintain constant rectal temperatures in the mice. Experimental groups of 8 mice were weighed, and their tumors were measured twice each...
Mice with Lewis lung carcinoma s.c. were treated on Days 4, 7, and following implantation. The conditions of local tumor heat treatment were 43° for 60 min. The drug BLEO was administered s.c. at a dose of 15 mg/kg. Tumor growth delay was determined from the tumor growth curves of the control and treatment groups on the day when the average size of the control tumors first exceeded 1 cu cm.

RESULTS

All mice survived the LTH and BLEO treatments. No burns or hair loss occurred at the sites of heating. The effects of the treatments on body weights were minimal. The results of rectal temperature measurements during these experiments showed an average change of less than ±1.0° for each experimental group.

The results of 3 separate experiments are summarized in Table 1. In each experiment, the LTH and BLEO treatments alone resulted in slightly retarded tumor growth and an increase in the median day of death. The tumor growth delays for the LTH- and BLEO-treated groups were 3 and 4 days, respectively, on the day when the control tumors reached 1 cu cm. The simultaneous application of the LTH and BLEO treatments resulted in a tumor growth delay of 17 days, much greater than could be accounted for by the addition of the individual treatment effects. The effect of these treatments on the tumor growth curves is illustrated by Chart 1. The median day of death in the groups given individual LTH or BLEO treatments was delayed 2 and 9 days, respectively, compared with controls, whereas the combination LTH + BLEO treatment resulted in a 12-day increase in life span compared with controls. The inclusion of a 4- or 24-hr gap between the LTH and BLEO treatments abolished the extra growth delay that was observed when the treatments were applied simultaneously. These results are clearly demonstrated by the tumor growth curves shown in Chart 2. The sequence of treatments did not appear

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor growth delay* (days)</th>
<th>Median day of death (day)</th>
<th>Survival (cures/treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27 ± 1b</td>
<td>0/24</td>
<td></td>
</tr>
<tr>
<td>LTH</td>
<td>3</td>
<td>29 ± 1</td>
<td>0/16</td>
</tr>
<tr>
<td>BLEO</td>
<td>4</td>
<td>36 ± 2</td>
<td>0/24</td>
</tr>
<tr>
<td>LTH + BLEO</td>
<td>17</td>
<td>39 ± 1</td>
<td>3/24</td>
</tr>
<tr>
<td>LTH/4 hr/BLEO</td>
<td>6</td>
<td>34</td>
<td>1/8</td>
</tr>
<tr>
<td>LTH/24 hr/BLEO</td>
<td>8</td>
<td>31</td>
<td>0/8</td>
</tr>
<tr>
<td>BLEO/4 hr/LTH</td>
<td>11</td>
<td>36</td>
<td>1/8</td>
</tr>
<tr>
<td>BLEO/24 hr/LTH</td>
<td>7</td>
<td>31</td>
<td>0/8</td>
</tr>
</tbody>
</table>

* Tumor growth delay was determined on the day when the average size of the control tumors first exceeded 1 cu cm.

b Average ± S.D., of 3 experiments.

Enhancement of BLEO by LTH

Table 1

Tumor growth delay and mouse survival

Figure 1. Tumor growth curves for experimental treatment groups: control, LTH, BLEO, and the combination of BLEO with LTH. Each point is the average for 24 tumors except for the LTH group which contained only 16 tumors. Bars, S.E. The treatments were administered on Days 4, 7, and 10 as indicated by arrows.
to significantly alter the results. Cures were defined as animals alive with no apparent sign of tumor on Day 75 following tumor implantation. Approximately 13% of the mice were cured when the LTH and BLEO treatments were administered within 4 hr of each other. No cures were observed in any of the other treatment groups.

**DISCUSSION**

Previous *in vitro* studies established a synergistic cytotoxicity threshold of 42–43°C for the combination of heat with BLEO (1, 4, 5, 9, 12). Braun and Hahn (1), Rabbani *et al.* (9), and Wust *et al.* (12) observed no synergism of BLEO activity with heat at 40–41°C. Preheating immediately prior to BLEO for 1 hr at 43°C but not at 41°C also produced a marked sensitization of BLEO cytotoxicity (1) suggesting that a cellular injury was occurring and not just an increased intracellular accumulation of drug. This result was confirmed by [14C]BLEO uptake experiments (1) which indicated that accumulation of BLEO into Chinese hamster cells was actually less at 43°C than it was at 37°C or 41°C. Further studies (1) of the repair of BLEO-induced damage concluded that most cells exposed to BLEO received potentially lethal damage which could be repaired unless the cells were heated to 43°C. The results shown in Table 1 demonstrate that the synergism reported previously *in vitro* also occurs *in vivo* in the Lewis lung carcinoma. These data indicate that a significant response against a highly resistant and well-established solid tumor can be obtained by combining 2 agents which were relatively ineffective when used alone. No effect greater than an additive one occurred when the LTH and BLEO treatments were separated by 24 or 4 hr. Rabbani *et al.* (9) also reported a loss of synergism between BLEO and heat *in vitro* when the 2 treatments were separated by 3 hr. The differences in tumor growth observed in our sequence experiments could be due to the time course of the repair of BLEO damage (5), the duration of repair inhibition by LTH (1), or the delayed absorption of the s.c. administered drug.

The relatively few cures obtained and the result that the median day of death was additive and not synergistic with LTH and BLEO treatments are due in part to the aggressive metastatic properties of the Lewis lung carcinoma and to the fact that the heat treatments were localized to only the primary tumor. Previous results using the Lewis lung carcinoma (8) have demonstrated that, by Day 4 following tumor implantation, metastasis to the lung had occurred in 60% of the animals studied. In spite of this limitation, which applies to all local cancer treatment methods, these encouraging results demonstrate that LTH can play a significant role in improving the effectiveness of systemically administered anticancer drugs.

**REFERENCES**


2. Hahn, G. M. Metabolic aspects of the role of hyperthermia in mammalian cell inactivation and their possible relevance to cancer treatment. Cancer
Enhancement of BLEO by LTH


Enhancement of Bleomycin Activity against Lewis Lung Tumors in Mice by Local Hyperthermia

Richard L. Magin, Branimir I. Sikic and Richard L. Cysyk