Effect of Carboplatin Combined with Whole Body Hyperthermia on Normal Tissue and Tumor in Rats

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ABSTRACT

The antitumor activity and normal tissue toxicity of cis-diammine-1,1-cyclobutanedicarboxylate platinum (II) (carboplatin) in combination with whole body hyperthermia (WBH) (41.5°C, 120 min.) were examined in an F344 rat model. Carboplatin data were compared with those of cis-diaminedichloroplatinum (II) (cisplatin). At 37°C, carboplatin showed minimal activity against a rat fibrosarcoma, but when combined with WBH, the antitumor effect of the drug was greatly enhanced. The major carboplatin-induced acute toxicity at both normothermic and hyperthermic temperatures was marked hypocalcullerity of the bone marrow. A significant decrease in peripheral blood platelet counts was caused by the maximum tolerated doses (MTD) of carboplatin alone and with WBH. While the lethal dose of carboplatin alone caused only minimal renal damage, mild acute tubular necrosis was observed at the MTD of carboplatin with WBH, although no significant increase in blood urea nitrogen occurred. Therapeutic ratios of the combined chemotherapy and WBH modalities were calculated by comparing tumor growth response at the MTD of drug alone and drug combined with WBH. The combination of the nephrotoxic cisplatin with WBH resulted in a therapeutic ratio of only 0.8, whereas when carboplatin was combined with WBH, a value of 3.0 was obtained, representing a 3- to 4-fold increase over cisplatin in the therapeutic ratio. These data indicate that the less nephrotoxic carboplatin in combination with WBH improves therapeutic gain and may provide a more promising clinical combination for cancer treatment than cisplatin combined with WBH.

INTRODUCTION

Cisplatin is a widely used anticancer drug against a broad range of malignancies (1, 2). Hyperthermia enhances the cytotoxicity of cisplatin in vitro (3, 4) and in vivo (5, 6), and increasing attention has been given to this combination as a powerful anticancer therapy. Unfortunately, simultaneous application of cisplatin and WBH (3) produces unacceptable renal toxicity in humans (7, 8), as well as in experimental animals (9, 10). Our previous study showed that administration of cisplatin combined with WBH caused a 3-fold increase in renal injury, as measured by renal function and histopathological examination (11). It was concluded that thermal enhancement of cisplatin-mediated renal dysfunction would limit the clinical utility of the combined modality.

Carboplatin is a less nephrotoxic analogue of cisplatin that retains significant clinical antitumor effect against a variety of cancers (12, 13). In vitro, the antitumor activity of carboplatin is significantly enhanced when combined with hyperthermia (14, 15). Although the dose-limiting toxicity of this analogue is bone marrow suppression (12, 13), there are no in vivo reports about carboplatin-induced antitumor activity or normal tissue toxicity under hyperthermic conditions.

The purpose of this study is to examine the normal tissue toxicities and antitumor effect induced by carboplatin when combined with WBH. In addition, the therapeutic gain of this combination was compared with that of cisplatin combined with WBH.

MATERIALS AND METHODS

Animals. Experiments were performed on female Fischer 344 rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), 11–13 weeks old, with a body weight ranging from 140 to 170 g, which were cared for in accordance with NIH standards. The rats were fed a diet of standard laboratory chow, allowed free access to water, and housed five/cage in a controlled environment with a 12-h light/dark cycle. All animals were allowed a 1-week environmental adaptation period before their experimental use.

Drugs. Carboplatin was synthesized in-house by the published procedure as described by Khokhar et al. (16) and Baer et al. (17). Carboplatin was dissolved in 5% dextrose in water (Injection USP; Travenol Laboratories Inc., IL), and cisplatin (Platinol; Bristol Myers, Syracuse, NY) was reconstituted in sterile water (USP) according to manufacturer's recommendation, immediately prior to use. The final concentration was 10 mg/ml for carboplatin and 1 mg/ml for cisplatin. Carboplatin and cisplatin were injected by i.v. bolus through the lateral tail vein of halothane-anesthetized rats. In rats undergoing WBH, drugs were given simultaneously with WBH when the rectal temperature first reached 41.5°C. Animals not given carboplatin or cisplatin received the drug-reconstituting vehicle.

WBH. Whole body hyperthermia was induced by immersing halothane-anesthetized rats into a thermostatically controlled circulating water bath maintained at 41.5°C by a Haake model E 12 circulator/heater as described previously (9). An average of 30 min was required for the rectal temperature to reach 41.5°C, after which time the rats were maintained for 2 h at a temperature of 41.5 ± 0.1°C. Animals not receiving WBH were given normothermic (37°C) treatment by placement on a circulating warm water blanket (Blanketrol, Cincinnati Sub-Zero Products, Inc., Cincinnati, OH) where they were maintained at a core temperature of 37°C, which is within the reported range of normal rectal temperature for rats (18, 19). General anesthesia of 1% halothane in pure oxygen as described previously (20) was used for all treatments.

Tumor Studies. The antitumor effect of carboplatin and cisplatin with or without WBH was investigated using a transplantable fibrosarcoma (9). Viable tumor cells (10⁶) were injected s.c. into the left flank of rats, which produced tumors in 100% of the animals. Treatment was administered when tumors reached a volume of 300–500 mm³ (9–14 days after implantation). Tumor size was measured every 2 days by using a vernier caliper to determine three perpendicular diameters (d_i), and the tumor volume was calculated by using the formula, \( V = \frac{4}{3} \pi d_1 \times d_2 \times d_3 \). The response of the tumors to the treatments was determined by using the parameter of TGD, which was calculated as the tumor growth time necessary to reach 10 × treatment volume (9).
Normal Tissue Toxicity Evaluated during Dose-Response Studies. Lethality, during the first 14 days posttreatment, was used as a measure of general acute toxicity. The LD₅₀ was calculated using the method of Litchfield and Wilcoxon (21) and the highest dose, at which no deaths were observed within 2 weeks after treatment, was determined as the MTD. Body weight was measured every 2 days after treatment, and evidence of gastrointestinal toxicity, as determined by the presence of diarrhea or blood in the stool, was recorded at that time. Presence of fecal occult blood was confirmed by the guaiac test (Hemoccult slide; SmithKline Diagnostics, Inc., Sunnyvale, CA). Histopathological analysis was performed on rats that died during the dose-response study. Major organs, including lung, heart, spleen, liver, intestine, pancreas, sciatic nerve, femur, brain, and kidney, were fixed with 10% buffered formalin. From each paraffin-embedded sample, 4-μm thick sections were prepared and stained with hematoxylin and eosin for light microscopic evaluation. All histopathological examinations were performed by one of the authors (L. C. S).

Specific Normal Tissue Toxicity Studies. Histopathological examination was performed on the femoral bone marrow, the spleen, the gastrointestinal tract, and the kidneys in non-tumor-bearing rats that received the MTD of carboplatin with and without WBH (70 mg/kg for normothermic rats and 30 mg/kg for WBH-treated rats, see Fig. 2B), iso-dose of carboplatin alone (30 mg/kg), and carboplatin vehicle (37°C and WBH control). These rats were sacrificed on days 3, 5, or 7 after treatment, and the organs were fixed in 10% buffered formalin and processed for microscopic examination as already described in the previous section. Each group consisted of 9 rats (3 rats at each time point) in these histopathological studies.

Hematological toxicity mediated by treatment was evaluated on day 5 posttreatment functionally by measurement of peripheral blood counts in the same rats used for the day 5 histopathological examination. For blood count determinations, rats were lightly anesthetized with ether and 0.1 ml of blood was obtained from the ventral tail artery. RBCs, WBCs, and platelets were quantitated electronically on a Coulter Counter (model ZM; Coulter Electronics, Inc., Hialeah, FL), using Isoton II as the diluent for blood and Zapoglobin II as the RBC-lysing agent. The final blood dilutions for WBC, RBC, and platelet counts were 1:1,000, 1:200,000, and 1:20,000, respectively.

To examine the effects of treatment on renal function, blood was collected from the tail artery of non-tumor-bearing rats on day 5 posttreatment and serum BUN and creatinine were measured using a Beckman BUN analyzer II and Sigma creatinine kit 555, respectively.

Statistics. The data from each treatment group were compared using a two-sided Student’s t test to determine statistical variation.

Calculation of Therapeutic Ratio. The therapeutic ratio was calculated as follows.

\[
\text{Therapeutic ratio} = \frac{TGD_{\text{MTD of drug at } 41.5^\circ C}}{TGD_{\text{MTD of drug at } 37^\circ C}}
\]

Therapeutic gain is concluded when the therapeutic ratio > 1.0. Conversely, therapeutic loss occurs at a ratio < 1.0.

RESULTS

Carboplatin and WBH

Tumor Studies

The fibrosarcoma used in this study is relatively resistant to carboplatin. Fig. 1 shows the tumor growth curves after treatment with a fixed carboplatin dose (40 mg/kg). Neither carboplatin alone or WBH alone had a significant effect on tumor growth. When carboplatin was given during WBH, the tumor growth delay was significantly enhanced \((P < 0.01)\). Fig. 2A shows the TGD as a function of carboplatin dose. There was no statistical difference in tumor growth time to reach 10× initial tumor volume between control and carboplatin alone, up to 60 mg/kg. Administration of 70 mg/kg carboplatin alone, the MTD, caused a modest antitumor response with a TGD of 1.7 ± 0.5 days. In contrast, carboplatin in combination with WBH produced a 3-fold increase (TGD of 5.1 ± 1.4 days) in antitumor response at the MTD of 30 mg/kg.

Normal Tissue Toxicity Observed in Dose-Response Studies

Lethality. The LD₅₀ of carboplatin combined with WBH was 43.5 mg/kg (95% confidence limits, 37.6–50.3 mg/kg), as compared to 84.9 mg/kg (79.7–90.5 mg/kg) for carboplatin alone at 37°C (Fig. 2B).

Body Weight Loss and Diarrhea. Table 1 shows body weight loss and diarrhea following carboplatin administration with or
without WBH. Minimal weight loss occurred in the control and WBH alone groups. Administration of carboplatin alone led to significant loss of body weight at doses exceeding 40 mg/kg. Enhancement of body weight loss resulted when carboplatin was administered during WBH: a significant body weight loss occurred at a dose of only 20 mg/kg, and a greater than additive decrease in body weight was apparent when comparing the iso-dose at 40 or 50 mg/kg. At a dose of up to 80 mg/kg carboplatin alone and 30 mg/kg carboplatin combined with WBH, minimal or no diarrhea was observed. Acute diarrhea during the first 10 days posttreatment occurred in >50% of the animals when carboplatin was administered at a dose of 90 mg/kg alone or 40 mg/kg combined with WBH. Diarrhea, at lethal doses of either carboplatin alone or carboplatin combined with WBH, had a dark pasty appearance, due to gastrointestinal tract bleeding, as indicated by a positive guaiac test.

Histopathology. Rats receiving carboplatin at doses exceeding the MTD, either alone (80, 90, and 100 mg/kg) or combined with WBH (40 and 50 mg/kg), died between 4 and 8 days after treatment. Histopathologically, severe lesions were seen in the bone marrow and gastrointestinal tract of rats given either carboplatin alone or carboplatin combined with WBH at lethal doses. Marked hypocellularity due to erythroid, myeloid, and megakaryocyte atrophy was observed. Additionally, mild decreased extramedullary hematopoiesis in the spleen was observed in these rats. No histopathological changes were observed in any organs obtained from rats receiving 30 mg/kg carboplatin alone or WBH alone, as compared with the normal control group.

Table 2 shows the peripheral blood cell counts on day 5 posttreatment determined in the rats used for histopathological analysis of bone marrow. Carboplatin, 70 mg/kg, alone caused a significant decrease in platelet counts to 3.9 \times 10^{10}/ml, compared to 6.8 \pm 0.07 \times 10^{10}/ml in control animals (P < 0.05), while no reduction in blood cell counts was observed in rats given 30 mg/kg carboplatin alone. WBH alone caused an increase in platelets to 10.0 \times 10^{10}/ml, whereas when 30 mg/kg carboplatin was combined with WBH, a significant decrease in WBC and platelet counts occurred, as compared to the control group.

Gastrointestinal Toxicity. Histopathological examination of the GI tract on day 3 posttreatment revealed that mild to moderate lesions in the ileum, characterized by atrophy of villi and necrosis of crypts, were observed in rats receiving 70 mg/kg carboplatin alone or 30 mg/kg carboplatin combined with WBH. Analysis of sections of the GI tract on days 5 or 7 posttreatment showed mild erosion and ulceration of the gastric mucosa in rats receiving 70 mg/kg carboplatin alone or 30 mg/kg carboplatin in combination with WBH, but no lesions were observed in the small intestine at these times. No significant alterations were observed in the GI tract of rats given 30 mg/kg carboplatin alone and WBH alone, as compared to the control group.

Renal Toxicity. Microscopic examination of the kidney showed that the MTD of carboplatin combined with WBH caused mild renal injury, while the MTD of carboplatin alone caused no renal damage. Renal lesions consisting of mild acute necrosis of groups of tubules were seen in rats receiving 30 mg/kg carboplatin combined with WBH and sacrificed on day 5 posttreatment, while no alterations were observed in kidneys of rats given 70 mg/kg carboplatin alone. A separate experiment was conducted using non-tumor-bearing rats to examine the effect of carboplatin alone or in combination with WBH on renal function (Table 3). Administration of carboplatin alone did not increase serum BUN or creatinine on day 5 posttreatment. When carboplatin was administered during WBH, renal function was not significantly reduced because only one of 6 rats at the greater than MTD (40 mg/kg) showed a mild increase in BUN up to 30 mg/dl and creatinine to 0.68 mg/dl. Additional analysis of blood collected on days 10 and 15 posttreatment did not reveal any significant changes.

### Table 1: Toxicities associated with administration of carboplatin alone or combined with WBH in tumor-bearing rats

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Carboplatin dose (mg/kg)</th>
<th>Weight change* (% of pretreatment body weight)</th>
<th>Diarrhea*</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0</td>
<td>-1.9 ± 0.4</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>-3.7 ± 0.8</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-4.1 ± 0.5*</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-5.6 ± 0.4*</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>-6.7 ± 0.8*</td>
<td>±, ±, ±, ±</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>-10.0 ± 0.8*</td>
<td>±, ±, ±, ±</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>-13.0 ± 0.6*</td>
<td>+, ±, +, +</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-15.8 ± 1.1*</td>
<td>+, +, +, +</td>
</tr>
<tr>
<td>41.5</td>
<td>0</td>
<td>-2.7 ± 0.2</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>-4.5 ± 0.9</td>
<td>0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>-6.2 ± 0.6*</td>
<td>±, ±, ±, ±</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>-8.4 ± 1.0*</td>
<td>±, ±, ±, ±</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>-11.4 ± 1.2*</td>
<td>+, +, +, +</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>-17.0 ± 1.4*</td>
<td>+, +, +, +</td>
</tr>
</tbody>
</table>

* Mean ± SE at 4 days after treatment.

** Occurrence of diarrhea in each rat is indicated as follows: 0, no diarrhea; ±, questionable or minimal diarrhea; +, significant diarrhea covering more than 1 cm of ventral surface around rectal orifice.

\* P < 0.01 as compared with the control group at 37°C.

\* P < 0.05 as compared with the control group at 37°C.

\* P < 0.01 as compared with the iso-dose group at 37°C.

### Specific Normal Tissue Toxicity Studies

Hematological Toxicity. Histopathological examination of non-tumor-bearing rats that were sacrificed after receiving the MTD of 70 mg/kg carboplatin alone or 30 mg/kg combined with WBH showed that the most prominent lesions occurred in the bone marrow. The most severe carboplatin-mediated damage was observed in the femoral bone marrow examined 5 days after treatment by either carboplatin alone or carboplatin combined with WBH. When the rats received 70 mg/kg carboplatin alone or 30 mg/kg combined with WBH, moderate general atrophy of the bone marrow was observed in the femur. This was characterized by loss of erythropoiesis, elimination of developing myeloid cells leaving a predominance of mature granulocytes, and decreased megakaryocytes (Fig. 3). On day 7 posttreatment, recovery of erythropoiesis, myelopoiesis, and megakaryocyte atrophy was observed. Additionally, mild decreased extramedullary hematopoiesis in the spleen was observed in these rats. No histopathological changes were observed in any organs obtained from rats receiving 30 mg/kg carboplatin alone or WBH alone, as compared with the normal control group.
Fig. 3. Photomicrographs of hematoxylin and eosin-stained femoral bone marrow of rats (x 400). In A, rat sacrificed 5 days after receiving 5% dextrose vehicle at 37°C as the control shows no significant difference in bone marrow cellularity from those given 30 mg/kg carboplatin alone or WBH alone. In B, rat sacrificed 5 days after receiving 70 mg/kg carboplatin alone shows moderate bone marrow hypocellularity. In C, rat that died 6 days after receiving 100 mg/kg carboplatin alone shows severe bone marrow hypocellularity. In D, rat sacrificed 5 days after receiving 30 mg/kg carboplatin combined with WBH shows moderate bone marrow hypocellularity similar to B. In E, rat that died 4 days after receiving 50 mg/kg carboplatin combined with WBH shows severe bone marrow hypocellularity similar to C.

Table 2 Blood cell counts of rats on day 5 posttreatment

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Dose (mg/kg)</th>
<th>No.</th>
<th>WBC (×10⁹/ml)</th>
<th>RBC (×10⁹/ml)</th>
<th>Platelet (×10⁹/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0</td>
<td>3</td>
<td>5.9 ± 0.03*</td>
<td>6.5 ± 0.1</td>
<td>6.8 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>5.1 ± 0.5</td>
<td>6.0 ± 0.3</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>3</td>
<td>5.4 ± 0.3</td>
<td>6.6 ± 0.1</td>
<td>3.9 ± 0.02*</td>
</tr>
<tr>
<td>41.5</td>
<td>0</td>
<td>3</td>
<td>4.9 ± 0.5</td>
<td>5.9 ± 0.03</td>
<td>10.0 ± 0.3*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3</td>
<td>3.7 ± 0.4*</td>
<td>6.1 ± 0.2</td>
<td>4.8 ± 0.3*</td>
</tr>
</tbody>
</table>

* Mean ± SE.
* P < 0.01 as compared with the control group at 37°C.
* P < 0.01 as compared with the WBH control group at 41.5°C.
* P < 0.05 as compared with the iso-dose group at 37°C.

not show any significant change in serum BUN or creatinine from normative values.

Cisplatin and WBH

Tumor Studies. The fibrosarcoma used in this study was more responsive to cisplatin than carboplatin and a TGD of 4.9 ± 0.6 days occurred at the MTD of 7 mg/kg cisplatin at 37°C (Fig. 4A). When cisplatin was combined with 41.5°C WBH, a TGD of 4.0 ± 0.7 days was observed at the MTD of 2 mg/kg.
Table 3 Renal function after administration of carboplatin alone or in combination with WBH

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Carboplatin dose (mg/kg)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>0</td>
<td>40</td>
<td>0.3 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>16.0 ± 1.2</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>16.0 ± 1.2</td>
<td>3 ± 0.02</td>
</tr>
<tr>
<td>41.5</td>
<td>0</td>
<td>18.3 ± 2.3</td>
<td>2 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.7 ± 0.9</td>
<td>3 ± 0.02</td>
</tr>
</tbody>
</table>

* BUN and creatinine were determined 5 days after the treatment and are means ± SE. Normal serum BUN and creatinine of our rats are 10–20 and 0.3–0.6 mg/dl, respectively, which falls within the range of normative BUN and creatinine values reported for rats (39).

One of seven animals given 40 mg/kg carboplatin during WBH died 4 days after treatment.

**DISCUSSION**

The present study demonstrates that whole body hyperthermia enhances both the carboplatin-mediated antitumor effect and normal tissue toxicities. Relatively, the increase in normal tissue toxicity was moderate, whereas the increase in antitumor effect of carboplatin was larger, resulting in a therapeutic gain.

Our *in vivo* tumor data are in agreement with the *in vitro* observation of Cohen and Robins (14) that hyperthermia enhanced the cytotoxicity of carboplatin. In their study, the thermal enhancement ratios at 40.5°C and 41.8°C for a 60-min exposure were 1.89 and 3.32, respectively, and cell killing increased exponentially with increasing duration of combined treatment. Therefore, like cisplatin, the cytotoxicity of carboplatin may be increased by moderately elevated temperatures attainable with WBH. With the combination of cisplatin and hyperthermia, the enhancement of cytotoxicity may be due partly to increased drug uptake in the cells (22, 23), increased DNA cross-link formation (24), alteration in drug metabolism (25), and inhibition of DNA repair by heat (26). Mechanisms for the WBH-induced enhancement of carboplatin cytotoxicity may be similar. Additionally, our study shows that, when carboplatin is combined with WBH, tumor resistance to carboplatin is overcome, as has been demonstrated for other drugs in combination with hyperthermia (27, 28).

Administration of carboplatin during WBH led to an increase in normal tissue toxicity. The most prominent histopathological alterations posttreatment were observed in the bone marrow for both carboplatin alone and carboplatin combined with WBH and were characterized by decreased erythrocytes, granulocytes, and megakaryocytes. Myelosuppression, primarily as thrombocytopenia, has been reported as a dose-limiting toxicity of carboplatin both in human (12, 13, 29) and animal studies (30, 31). In our study, the MTD of carboplatin with and without WBH caused a significant decrease in platelet counts, while WBH alone resulted in increased platelet counts, likely due to acute platelet depression soon after WBH followed by a rebound a few days after treatment as previously observed by Nakayama and Nakamura (32) and Wondergem et al. (33).

With regard to gastrointestinal toxicity, carboplatin produces moderate nausea and vomiting in patients (13, 33). Kravovanszyk et al. (34) investigated the intestinal toxicity caused by cisplatin and carboplatin by measuring enzyme activities which are characteristic for cell proliferation and function. In that study, carboplatin induced less but significant damage in the intestine, compared to cisplatin. In our study, the MTD of carboplatin alone or in combination with WBH caused mild to moderate transient lesions in the ileum. However, at the lethal dose of carboplatin with or without WBH, increased severity of diarrhea and marked atrophy of villi and necrosis of crypt epithelium in the small intestine were observed.

An important advantage of using carboplatin is that it causes less nephrotoxicity than cisplatin. There have been few cases in which nephrotoxicity became a serious problem in the patients treated with carboplatin (12, 13, 33). Nonclercq et al. (35) investigated the extent of nephrotoxic injury and the tissue therapeutic ratio for the drug in combination with WBH was calculated as 3.0 ± 1.2 for carboplatin and only 0.8 ± 0.2 for cisplatin.

**Table 4 Therapeutic ratio of carboplatin or cisplatin in combination with WBH**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Temperature (°C)</th>
<th>MTD (mg/kg)</th>
<th>TGD (days)</th>
<th>Therapeutic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>37</td>
<td>7</td>
<td>4.9 ± 0.6</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>41.5</td>
<td>2</td>
<td>4.0 ± 0.7</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>37</td>
<td>70</td>
<td>1.7 ± 0.5</td>
<td>3.0 ± 1.2*</td>
</tr>
<tr>
<td></td>
<td>41.5</td>
<td>30</td>
<td>5.1 ± 1.4</td>
<td>3.0 ± 1.2*</td>
</tr>
</tbody>
</table>

* Therapeutic gain was calculated as the ratio of TGDs at MTD of drug alone or drug combined with WBH. The SE were estimated according to the method of Begg and Terry (40).

**Lethality.** The LD50 for cisplatin combined with WBH was 3.0 mg/kg (95% confidence interval, 2.5–3.7 mg/kg), compared to 7.5 mg/kg (7.1–8.3 mg/kg) for cisplatin alone (Fig. 4B).

**Therapeutic Ratio**

The MTD and TGD for drugs alone or combined with WBH are summarized in Table 4. From this information, the therapeutic ratio for the drug in combination with WBH was calculated as 3.0 ± 1.2 for carboplatin and only 0.8 ± 0.2 for cisplatin.
repair reaction occurring in the kidney of rats given 40 mg/kg carboplatin. Histopathological findings were characterized by focal acute tubular necrosis in proximal tubules, followed by a mild proliferative response. Siddik et al. (36) reported that a high dose (greater than MTD) of carboplatin (160 mg/kg) was necessary to cause an increase in BUN as a result of impairment in renal function in rats. In our study combining carboplatin with WBH, histopathological examination revealed that only the LD_{100} dose of carboplatin alone (100 mg/kg) in normothermic rats caused mild focal acute tubular necrosis, whereas, when 40 mg/kg carboplatin (LD_{20} dose) was combined with WBH, a more disseminated moderate acute tubular necrosis was observed. Although WBH enhanced the carboplatin-induced renal injury to some extent, the lesions were not severe enough to significantly reduce renal function as measured by serum BUN and creatinine. In contrast, the most severe toxicity associated with the simultaneous combination of cisplatin and WBH has been an unacceptable increase in renal toxicity. A 3-fold enhancement of cisplatin-mediated renal injury has been reported at the MTD of cisplatin combined with WBH (9, 11).

The pharmacological basis for thermal enhancement of carboplatin-mediated cytotoxicity is not clear but may partly relate to the following: the kidney is the major route of platinum excretion in patients receiving carboplatin, and carboplatin plasma clearance is linearly related to glomerular filtration rate (31). It has been reported that changes in renal function can alter the severity of carboplatin-induced myelosuppression (12). Since hyperthermia induces a reduction in renal blood flow and glomerular filtration rate (22, 37), a decreased plasma clearance of carboplatin during WBH (38) may contribute to an increase in the toxicity and antitumor effect of this platinum complex.

When we compared the therapeutic ratios of carboplatin and cisplatin in combination with WBH in this rat tumor model, the combination of carboplatin and WBH proved to be superior. Assessment of antitumor response at the MTD of carboplatin with and without WBH revealed that WBH enhanced cisplatin-mediated normal tissue toxicity to a greater extent than antitumor effect, resulting in a therapeutic ratio of only 0.8. This index of <1.0 is suggestive of a therapeutic loss for the combination of cisplatin and WBH, probably due to the large thermal enhancement of cisplatin-mediated renal toxicity (9, 11). In contrast, evaluation of tumor response at the MTD of carboplatin with and without WBH showed that WBH enhanced carboplatin-mediated normal tissue toxicity to a lesser degree than antitumor effect, resulting in an increase in the therapeutic ratio to 3.0. This improvement of the therapeutic ratio for combined carboplatin and WBH was due in large part to decreased normal tissue toxicity, as measured by lethality, which is likely the result of a decrease in renal toxicity associated with the carboplatin combined with WBH as compared to the severe renal toxicity reported for cisplatin combined with WBH.

In summary, this study demonstrates a potentially useful strategy of using a less nephrotoxic analogue of cisplatin such as carboplatin in combination with WBH. The simultaneous combination of carboplatin with WBH resulted in a 3- to 4-fold increase in therapeutic gain over that of combined cisplatin with WBH. We conclude that therapy with combined carboplatin and WBH may be a promising anticancer treatment in the clinical setting.

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CARBOPLATIN IN COMBINATION WITH WHOLE BODY HYPERTHERMIA


Effect of Carboplatin Combined with Whole Body Hyperthermia on Normal Tissue and Tumor in Rats

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