Effect of Adriamycin Combined with Whole Body Hyperthermia on Tumor and Normal Tissues

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ABSTRACT

Thermal enhancement of Adriamycin-mediated antitumor activity and normal tissue toxicities by whole body hyperthermia were compared using a F344 rat model. Antitumor activity was studied using a tumor growth delay assay. Acute normal tissue toxicities (i.e., leukopenia and thrombocytopenia) and late normal tissue toxicities (i.e., myocardial and kidney injury) were evaluated by functional/physiological assays and by morphological techniques. Whole body hyperthermia (120 min at 41.5°C) enhanced both Adriamycin-mediated antitumor activity and toxic side effects. The thermal enhancement ratio calculated for antitumor activity was 1.6. Thermal enhancement ratios estimated for "acute" hematological changes were 1.3, whereas those estimated for "late" damage (based on morphological cardiac and renal lesions) varied between 2.4 and 4.3. Thus, while whole body hyperthermia enhances Adriamycin-mediated antitumor effect, normal tissue toxicity is also increased, and the potential therapeutic gain of the combined modality treatment is eroded.

INTRODUCTION

ADR is a commonly used antineoplastic agent with proven efficacy in acute leukemias, lymphomas, and a number of solid tumors (1). However, except for some reversible acute toxic effects (i.e., bone marrow suppression and gastrointestinal toxicity) (1), cardiotoxicity is the major dose-limiting toxicity in the clinic (1). ADR also induces severe nephrotoxicity in experimental animals, such as the rat (2-4) and rabbit (5, 6). There are few reports of this problem in humans (7, 8).

Hyperthermia has been shown to potentiate the actions of some anticancer drugs (9). Enhancement of the cytotoxicity of ADR in vitro was demonstrated first by Hahn et al. (10, 11) at temperatures between 41 and 43°C. Several preclinical papers have been published indicating thermal enhancement of ADR-mediated antitumor effects in vivo using local hyperthermia (12-16) or WBH (16, 17). There is scant information about the effects of combined ADR and heat on normal tissues. Hinkelbein et al. (18), studying the influence of WBH on the myelotoxicity of ADR and irradiation in rats, reported an increase of bone marrow toxicity as a result of WBH (10 or 20 min at 41.5°C). Birmelin and colleagues (19) reported thermal enhancement of ADR-mediated cardiotoxicity by WBH. Kim et al. (20) observed a potentiation of ADR-related cardiotoxic acute effects during a combined ADR-WBH session in a patient treated for metastatic squamous cell carcinoma. Gerard et al. (21), treating patients with WBH combined with ADR and CF, observed unusual and enhanced drug-mediated toxicities during and after hyperthermia, including reversible neuropathy, cardiac arrhythmias, and diarrhea.

With increasing clinical interest in WBH combined with chemotherapeutic agents such as ADR, dose-limiting toxicities may be expected. The aim of the present study was therefore to determine the effect of combined ADR and WBH on several normal tissues in the rat, including heart, kidneys, peripheral blood, gastrointestinal tract, and the neurological system, as well as on an experimental tumor model in the same rat.

MATERIALS AND METHODS

Animals

Female Fischer 344 rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing 140 to 170 g were given standard laboratory chow and water ad libitum. The animals were housed 5 to a cage and were kept under conventional laboratory conditions (22). All treatments were performed with rats under general anesthesia using halothane (1%) inhalation anesthetic in pure oxygen as previously described (23).

Adriamycin

Adriamycin (doxorubicin hydrochloride; Farmitalia, Milan, Italy) was obtained from Adria Laboratories (Columbus, OH) and dissolved in 0.9% NaCl resulting in a final concentration of 2 mg/ml and administered i.v. as a single bolus injection through the lateral tail vein. Because of a possible circadian effect on ADR-mediated toxicities, ADR injections were always administered between 1400 and 1600 h. In rats undergoing WBH, ADR was given simultaneously with heat when the rectal temperature first reached 41.5°C. Rats not treated with ADR received the same volume of saline vehicle as used for ADR inoculation.

Whole Body Hyperthermia

Systemic hyperthermia of 41.5 ± 0.1°C was induced by immersing the rats in a thermostatically controlled water bath maintained at a temperature of 41.5°C as previously described (22). The core body temperature of each rat was monitored continuously by a thermistor probe (YSI model 402; Yellow Springs Instrument Co., Yellow Springs, OH) inserted into the rectum to a depth of 6 cm from the anal sphincter (24, 25) and secured with tape to the tail. The water bath and rat core body temperature were displayed on a YSI model 49TA digital thermometer, with a resolution of 0.01°C, connected to a YSI model 4002 12-channel switchbox. The body temperature was recorded every 5 min. All probes were calibrated against a standard thermometer certified by the National Bureau of Standards. A 20- to 30-min heat-up time was required to reach a rectal temperature of 41.5°C, and the rats were maintained at that temperature for 2 h.

Tumors

Tumor studies were performed using a transplantable rat fibrosarcoma. The tumor, originally induced by methylcholanthrene, is moderately well-differentiated slightly antigenic fibrosarcoma. Viable tumor cells (10⁶ in 100 μl) were inoculated s.c. into the left flank of each rat as described previously (22). When tumors reached the desired volume...
were sacrificed 60 days after treatment, and chronic morphological sclerosis and vacuolization of glomeruli. The severity of microscopic extent of deposition of proteinaceous material within the tubular lam

the quantitative method of Herman et al. (26) and was based on the affect many or most areas). 4+, severe (marked morphological changes affecting many or most areas).

0, no lesions; 1+, modest (very slight, occasional, or rare alterations); 2+, mild (slight, few, or scattered lesions); 3+, moderate (medium degree or more pronounced type of damage with a less focal, more disseminated appearance); 4+, severe (marked morphological changes affecting many or most areas).

In particular, the scoring of nephropathy was adapted largely from the quantitative method of Herman et al. (26) and was based on the extent of deposition of proteinaceous material within the tubular lumen, degeneration and loss of tubules, interstitial inflammation, and sclerosis and vacuolization of glomeruli. The severity of microscopic myocardial damage was graded according to the quantitative scoring method proposed by Perkins et al. (27): 1+, modest (scattered single myocardial fibers with vacuolation or degenerative changes); 2+, mild (scattered small groups of altered myocardial fibers throughout the atrial and ventricular myocardium); 3+, moderate (disseminated myocardial fiber vacuolation or degeneration with only occasional focal

unaffected areas); 4+, severe (confluent groups of affected myocardial fibers; most myocardial fibers affected).

Statistical Procedures. The LD50 and ED50 and the corresponding 95% confidence intervals for these values were calculated as described by Finney (28).

From the dose-response curves of ADR with and without WBH, TERs for antitumor and normal tissue effects were estimated by calculating the ratio of the ADR dose without WBH to the ADR dose with WBH, causing the same effect (isoeffect ratio):

\begin{equation}
\text{TER}_{\text{isoeffect}} = \frac{\text{ADR} \text{ dose without WBH}}{\text{ADR} \text{ dose with WBH}}
\end{equation}

The estimate of TER was expressed as the ratio of slopes (29) of the dose-response curves when the curves could be fit to a linear regression (Graphpad Software; ITI Press, Philadelphia, PA). Only curves with a correlation coefficient $r > 0.95$ were judged to be a linear fit. For other cases with nonlinear dose-effect curves, the method of using an arbitrary level of isoeffect (22, 30) was chosen for the estimate of TER. SEM of the TERs were calculated as

\begin{equation}
f^2 = f_1^2 + f_2^2
\end{equation}

where $f_1$ and $f_2$ are fractional errors of the TER, the dose with WBH, and the dose without WBH, respectively (31). Differences in effect caused by combined ADR and WBH compared to ADR alone were evaluated by $\chi^2$ and Student’s $t$ test.

RESULTS

Tumor Studies. The fibrosarcoma tumor used in this study was moderately sensitive to ADR, whereas WBH alone (120 min at 41.5°C) caused only a minor tumor response (TGD of 1.2 days). Fig. 1 shows the effect of WBH on the dose-response curve for ADR. At doses greater than 2.5 mg/kg, WBH clearly enhanced the ADR-mediated antitumor effect. Five mg/kg ADR in combination with WBH caused a TGD of 18 days compared to a 10-day TGD in response to ADR alone ($P < 0.05$).

Acute Toxicities: Lethality, Body Weight Change, and Diarrhea. Administration of ADR during WBH led to an increase of acute lethality (<14 days posttreatment). The LD50 calculated for combined ADR plus WBH decreased from 9.4 (8.4–10.3) mg/kg to 5.8 (4.9–6.7) mg/kg (numbers in parentheses indicate 95% confidence intervals) when compared to ADR administration.
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### Table 1 Acute toxicity as a result of ADR treatment with or without WBH

<table>
<thead>
<tr>
<th>Temperature</th>
<th>ADR dose (mg/kg)</th>
<th>N</th>
<th>Mortality* (%)</th>
<th>Weight change† (% of pretreatment body wt)</th>
<th>Bloody diarrhea* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>Saline</td>
<td>9</td>
<td>0</td>
<td>-1.3 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>6</td>
<td>0</td>
<td>-3.8 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>6</td>
<td>0</td>
<td>-5.0 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>6</td>
<td>0</td>
<td>-5.8 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>6</td>
<td>0</td>
<td>-8.2 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8.75</td>
<td>6</td>
<td>0</td>
<td>-11.0 ± 0.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>6</td>
<td>100</td>
<td>-15.4 ± 1.2</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>11.0</td>
<td>6</td>
<td>100</td>
<td>-17.2 ± 1.0</td>
<td>83</td>
</tr>
<tr>
<td>41.5°C</td>
<td>Saline</td>
<td>9</td>
<td>0</td>
<td>-3.2 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>6</td>
<td>0</td>
<td>-4.4 ± 0.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>6</td>
<td>0</td>
<td>-6.7 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>6</td>
<td>0</td>
<td>-7.5 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6.25</td>
<td>6</td>
<td>83</td>
<td>-15.1 ± 2.0†</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6</td>
<td>100</td>
<td>-16.4 ± 0.7†</td>
<td>100</td>
</tr>
</tbody>
</table>

* Mortality was evaluated by the survival within 14 days after treatment.
† Weight change (mean ± SEM) was determined at day 6 posttreatment.
†† Percentage of animals (incidence) with bloody diarrhea during the first 2 weeks. The most severe diarrhea usually occurred from day 6 to day 8.
* P < 0.05 when compared to 7.5 mg/kg at 37°C.
†† P < 0.05 when compared to 8.75 mg/kg at 37°C.

Table 1 shows the acute dose-dependent body weight loss and the incidence of bloody diarrhea after ADR administration with and without WBH. When ADR was combined with WBH, the ADR-induced decrease in body weight was more severe [the doses of ADR with and without WBH producing a >10% loss in body weight on day 6 in 50% of the rats were 5.5 (4.9–6.2) and 7.9 (7.0–8.9) mg/kg, respectively], and the incidence of bloody diarrhea was increased compared to ADR alone. With ADR alone, 50% of the animals showed bloody diarrhea at a dose of 10.1 (9.1–11.0) mg/kg, whereas the ED50 after combined ADR plus WBH decreased to 6.7 (5.7–7.7) mg/kg.

**Acute Hematological Toxicity.** Examples of the hematological changes after ADR, WBH, and combined ADR plus WBH administration are shown in Fig. 2. The acute hematological effects of combined ADR plus WBH are complex. WBH by itself caused a different time to nadir compared to the nadir following ADR alone. As shown in Fig. 2A, WBH alone caused a marked (50%) decrease in peripheral platelet count resulting in a nadir of platelet counts on day 2 posttreatment, followed by a strong rebound above initial levels (approximately 150%) by day 4–6. In contrast, ADR (5 mg/kg) alone resulted in a platelet count nadir on day 6, followed by a strong rebound beginning on day 8. When ADR was given in combination with WBH, a bimodal curve resulted, with an initial WBH-mediated platelet count depression on day 2, followed by a brief rebound on day 4, which was followed in turn by a second, ADR-induced platelet count depression on day 6. As seen with ADR alone, the decrease in platelet counts caused by combined ADR plus WBH resulted in a strong rebound increase in platelets above control values beginning on day 8 after treatment. The depth of the day 6 nadir platelet count after combined 5 mg/kg ADR plus WBH was almost identical to the day 6 nadir count after 5 mg/kg ADR alone.

The effect of therapy on the peripheral WBC count is somewhat different from its effect on peripheral platelet counts. As shown in Fig. 2B, WBH alone caused a relatively mild WBC depression resulting in a nadir of WBC counts on day 4, followed by a mild rebound, whereas ADR at a dose of 5 mg/
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Chronic Toxicities: Body Weight, Hematological Effects, and Renal Function. A notable general chronic toxicity resulting from ADR administration was a dose-dependent slowing of expected normal body weight gain at lower ADR doses. At higher ADR doses, actual body weight loss occurred. The weight loss was enhanced by the combined therapy of WBH plus ADR. As shown in Fig. 4A, a relative decrease in body weight occurred in rats given ADR at doses >5 mg/kg at 8 weeks posttreatment. When WBH was combined with ADR at doses >2.5 mg/kg, a similar weight loss was observed.

Neither leukopenia nor thrombocytopenia was observed as a late effect of ADR treatment with or without WBH; however, chronic thrombocytosis (platelet count >150% of control) and anemia (RBC count <80% of control) were seen from weeks 4 through 8 posttreatment as a result of both ADR alone and combined ADR plus WBH administration. These effects occurred at a lower dose of ADR in combination with WBH than with ADR alone. The ED50s for ADR with and without WBH were 2.2 (1.5–3.2) and 5.2 (4.1–6.4) mg/kg for anemia, and 2.2 (1.5–3.2) and 4.2 (3.6–5.0) mg/kg for thrombocytosis, respectively.

ADR-treated rats did not show a significant change in plasma BUN during the first 2 weeks after treatment. However, during weeks 3 through 8, a dose-dependent increase in BUN values was observed, and enhancement of this effect was seen when WBH was combined with ADR. Fig. 4B shows that by 8 weeks kg caused a more severe decrease in WBC with the nadir of counts occurring on day 6, followed by a marked rebound overshoot above control values within 48 h of the nadir. The combination therapy (5 mg/kg ADR plus WBH) caused a somewhat earlier decrease in WBC with the nadir of counts occurring on day 4, which did not differ significantly in severity from the WBC nadir associated with ADR alone. The WBC count depression caused by combined ADR plus WBH was followed by a WBC rebound above control values that was similar in timing and degree compared to that seen with ADR alone. Neither ADR alone nor combined ADR plus WBH caused a significant acute effect on RBC or hematocrit.

In order to demonstrate a dose-response relationship between the acute depression of the platelet and WBC counts and the ADR dose with and without WBH, the mean nadir values (day 6 for platelets; average of days 4 and 6 for WBC) were taken as a measure of acute toxicity. A slight leftward shift of the dose-response curves for platelet and WBC counts caused by combined WBH plus ADR, as shown in Fig. 3, suggests a relatively mild thermal enhancement of ADR-mediated acute myelosuppression.

Fig. 3. Acute depression of platelet counts (A) and WBC counts (B), expressed as a percentage of control, plotted as a function of the dose of ADR. Animals received ADR alone (C) or ADR combined with WBH (●). Points, mean of 5–6 rats and represent nadir values of platelet counts (day 6) and WBC counts (average of day 4 plus day 6), respectively; bars, SEM (shown where they exceed the diameter of the points). The percentage control values were calculated based on normothermic controls: platelets, 7.3 ± 0.2 x 10^8/ml; WBC, 6.0 ± 0.2 x 10^6/ml) for ADR alone, and WBH controls: platelets, 11.3 ± 0.3 x 10^8/ml; WBC, 4.6 ± 0.1 x 10^6/ml) for combined ADR plus WBH. P < 0.05 for ADR plus WBH compared to ADR alone at isodose 2.5, 3.75, and 5 mg/kg (A) and 2.5 mg/kg (B).

Fig. 4. Late effects of treatment on body weight (A) and BUN (B) plotted as a function of the ADR dose, at 8 weeks posttreatment. Animals received ADR alone (C) or ADR combined with WBH (●). Body weight change is a percentage of pretreatment body weight. Points, mean of 5–6 rats; bars, SEM (shown where they exceed the diameter of the points). P < 0.05 for ADR plus WBH compared to ADR alone at isodose 3.75 mg/kg.

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Fig. 5. Chronic morphological changes in the kidney. Light micrographs of the renal cortex from animals 60 days after (A) treatment with 3.75 mg/kg ADR alone showing minimal changes of the kidney such as hyaline casts in the tubules (arrow) and (B) treatment with 3.75 mg/kg ADR combined with WBH showing severe glomerulonephropathy with extensive alterations of the glomeruli, loss of tubules, and interstitial inflammation (arrows, two of the atrophic and sclerotic glomeruli). × 100.

Fig. 6. Quantitation of chronic nephropathy (score of renal lesions) plotted as a function of ADR dose, at 60 days posttreatment. Animals received ADR alone (○) or ADR combined with WBH (●). Points, mean of 5–6 rats; bars, SEM (shown when they exceed the diameter of the points). P < 0.05 for ADR plus WBH compared to ADR alone at isodose 2.5 and 3.75 mg/kg.

posttreatment, an increase in BUN to more than 50 mg/dl (325% of control) resulted from a dose of >2.5 mg/kg of ADR combined with WBH. Only at doses >7.5 mg/kg did ADR alone increase the BUN to this level. Plasma collected from ADR-treated rats during weeks 3 through 8 posttreatment exhibited a turbid, milky appearance indicative of the development of a hyperlipidemia that has been associated with nephrotic syndrome, which is a consequence of ADR-induced chronic renal toxicity in rats (2, 3, 32). A marked hypercholesterolemia (32) to >400 mg/dl (Cobas Auto-analyzer, procedure 455) was observed in randomly selected rats exhibiting milky plasma (compared to 75–90 mg/dl for normal controls). Consistent with nephrotic syndrome (33), abdominal ascites, as indicated by a grossly swollen belly, was also observed in some ADR-treated rats with and without WBH 4 to 5 weeks after treatment. Both the incidence and degree of ADR-mediated hyperlipidemia and ascites appeared to be dose dependent, and these changes were not qualitatively different in appearance between normothermic and WBH-treated rats; however, these symptoms were enhanced by the combination of WBH with ADR inasmuch as they occurred at a lower dose of ADR plus WBH than with ADR alone. The ED50 for ADR with and without WBH were 1.7 (1.2–2.4) and 5.2 (4.2–6.4) mg/kg for moderate to severe hyperlipidemia (by visual assessment of the degree of milkiness of plasma) and 3.6 (2.9–4.6) and 8.1 (7.0–9.4) mg/kg for ascites, respectively.

Chronic Renal Toxicity: Morphological Changes. Histopathological examination of kidneys 60 days after treatment showed dose-dependent ADR-mediated morphological changes. The renal lesions were diagnosed as progressive chronic glomerulonephropathy characterized by sclerosis and vacuolization of glomeruli, deposition of hyaline casts within tubular lamina, atrophy, dilatation, loss of tubules, and interstitial inflammation and fibrosis. Although there were no qualitative differences in morphological kidney damage between rats treated with combined ADR plus WBH and those receiving ADR alone, WBH enhanced the severity of the ADR-mediated lesions. Fig. 5A shows only modest renal changes caused by ADR alone (3.75 mg/kg) 60 days after treatment. In comparison, rats treated with isodose ADR (3.75 mg/kg) in combination with WBH developed severe glomerulonephropathy (Fig. 5B). A quantitative representation of renal nephropathy scores at day 60 (Fig. 6) as a function of ADR dose shows that moderate lesions occurred with ADR alone at doses of 5 to 7.5 mg/kg, compared to 2.5 mg/kg for combined ADR plus WBH, respectively.

Chronic Cardiac Toxicity: Morphological Changes. Histological examination of the heart also revealed significant ADR-induced morphological alterations that increased in terms of severity and frequency in a dose-dependent manner. The myocardial injury was diagnosed as multifocal cardiomyopathy, characterized by the presence of necrotic, degenerated, and
vacuolated myocytes; infiltration of lymphocytes and macrophages; and fibrosis. As with the kidney, morphological damage to the heart produced by ADR alone was not qualitatively different from that caused by ADR plus WBH; however, the severity of ADR-induced myocardial lesions was enhanced by WBH. Fig. 7A is a representative photomicrograph showing only modest cardiac changes 60 days after treatment with 3.75 mg/kg of ADR alone. In contrast, moderate to severe cardiomyopathy developed in some rats that received isodose ADR (3.75 mg/kg) in combination with WBH (Fig. 7B). As seen in Fig. 8, a quantitative representation of myocardial lesion score as a function of ADR dose shows that the maximally tolerated dose of ADR alone (8.75 mg/kg) led to the development of only modest to mild lesions by 60 days posttreatment, whereas combined ADR plus WBH caused mild to moderate myocardial lesions at a dose of ≤3.75 mg/kg.

**Chronic Neurological Toxicity.** Rats treated with the maximal tolerated dose of ADR alone (8.75 mg/kg) also developed neuropathies by 7 to 8 weeks posttreatment manifested by hindlimb ataxia (abnormal gait, flexation, or posture). Histopathological examination revealed lesions of the sciatic nerve (large numbers of mast cells) and of the distal part of the spinal cord (dilation of the myelin sheath of nerve fibers in the cauda equina). Interestingly, neuropathies were not observed in any rats treated with combined WBH plus ADR.

**DISCUSSION**

Our data demonstrated that WBH improved ADR-mediated antitumor activity; however, it also increased overall ADR-mediated toxicity.

**Antitumor Efficacy.** In our experimental tumor system, WBH in combination with ADR doses over 2.5 mg/kg significantly increased the TGD (Fig. 1). We believe the increased tumor response to be caused by thermal enhancement of ADR-mediated cytotoxicity, because WBH by itself led to a modest TGD of only 1.2 days.

Rotstein et al. (17), using the same rat strain (Fischer 344) and two different tumor models, a methylcholanthrene-induced sarcoma and a transitional cell carcinoma, reported no effect of single treatment on the response of their experimental tumors with WBH alone (30 min at 41.5°C), ADR alone (2 mg/kg), or WBH combined with ADR. However, when ADR combined with WBH was given 3 times at weekly intervals, a small reduction of the growth rate of the methylcholanthrene-induced sarcoma could be observed. The other tumor did not respond. It is possible that the duration of the heat treatment was too short and/or the ADR dosage was too small to adequately evaluate an interaction between WBH and ADR. On the other hand, Overgaard (16), using a murine mammary carcinoma, observed a large thermal enhancement of the antitumor effect of ADR (25 mg/kg) after WBH at 40.5°C for 120
min. In his model, WBH increased the TGD from 13 to 28.6 days. However, statistical evaluation of his results is important because 90% of the tumor-bearing animals died as a result of ADR alone. Rose et al. (34) failed to demonstrate an effect of WBH on ADR-mediated antitumor response using two different murine tumor models. However, the temperature and the duration used (41°C for 30 min) may have been inadequate to demonstrate thermal enhancement of ADR-mediated cytotoxicity.

Several investigators studied local hyperthermia combined with ADR on experimental tumor models in vivo. [See the review by Engelhart (37).] Hahn et al. (10) reported thermal enhancement of ADR-mediated cytotoxicity in vivo using a mouse mammary carcinoma (EMT6). This observation was confirmed by several other investigators (12-16). However, not all investigators observed an increased effect of heat on ADR-mediated tumor response (38, 39). In the latter study both an ADR-resistant and an ADR-sensitive subline of a mammary 16C mouse tumor were used. Because most investigators used a fixed ADR dose, no thermal enhancement ratios can be calculated. The estimated TER for antitumor effect in our study was 1.6 (Table 2).

Normal Tissue Effects. Our data show that WBH also enhanced ADR-mediated side effects. Administration of ADR during WBH led to a decrease of the "acute" LD50 from 9.4 to 5.8 mg/kg, when compared to administration of ADR at a body temperature of about 37°C. The estimated TER, using lethality as an end point, was 1.6. From data presented by Rose et al. (34) on the influence of WBH on ADR-mediated toxicity in mice, we calculated a TER of 1.1-1.2 using LD10 as an end point. It is likely that the low TER reflects the mild heat treatment used in their combined heat-chemotherapy studies. On the other hand, Overgaard (16) reported that WBH (120 min at 40.5°C) combined with ADR (25 mg/kg) decreased the number of toxic deaths from 90% (18 of 20) to 50% (7 of 14) compared to ADR alone (25 mg/kg). However, a protective effect of heat has not yet been confirmed by other investigators.

Our hematological studies show that WBH alone induced marked acute changes in peripheral platelet counts, whereas the changes in WBC were relatively mild. A heat treatment of 2 h at 41.5°C caused an approximate 50% depression in platelet count with a nadir occurring on day 2, followed by a strong rebound above control values, with maximum platelet numbers (approximately 150% of control) occurring on day 6. A similar drop and rebound of platelets after WBH alone was observed by Nakayama and Nakamura (40) using an experimental mouse model. In their study, the maximum decrease in platelets occurred 3.5 h posttreatment, and a maximum platelet overshoot (twice the value of controls) occurred on day 3. Their experiments also indicate that the acute platelet drop shortly after WBH results from platelet aggregation. Clinical studies also demonstrate an acute decrease in platelet count after WBH alone (41).

Our study also shows that WBH enhances the ADR-mediated acute depression of WBC and platelet counts. The actual pattern of ADR-mediated hematological toxicities observed in this study was very similar to that found in other studies reporting on ADR-mediated hematological effects observed at normal temperatures (35, 36). In order to estimate the magnitude of the WBH enhancement of ADR-mediated acute myelosuppression, we calculated TER values based on dose-response curves of the nadir of WBC and platelet counts shown in Fig. 3. At the arbitrary isoeffect level of a 50% reduction of the blood cell count, a relatively low TER of 1.3 was estimated for the decrease in both WBC and platelet counts (Table 2). Bone marrow toxicity of ADR (6 and 8 mg/kg) and WBH (41°C) were studied in rats by Hinkelbein et al. (18). Histological examination of bone marrow of the femur 21 days after combined treatment demonstrated thermal enhancement of ADR-mediated bone marrow toxicity. Moreover, potentiation of ADR-mediated myelotoxicity increased when the duration of the heat treatment was increased from 10 to 20 min. Gerard et al. (21), treating patients with WBH (120 min at 41.8-43°C) in combination with ADR and CF, observed thermal enhancement of ADR-mediated hematological toxicity 24 h post-WBH. The mean platelet count nadir dropped from 242,000/μl for euthermic treatment to 177,900/μl when ADR and CF were combined with WBH. They also observed a fall in hemoglobin of 1 to 2 g/dl in all patients over the first 48 h post-WBH. In contrast to our study, however, they did not observe a thermal enhancement of ADR/CF-mediated decrease in leukocyte counts.

Thermal enhancement of ADR-mediated chronic late toxicities 60 days posttreatment was estimated using the dose-response curves presented in Fig. 4. The estimated TER values were 2.4 and 3.0 for body weight loss and increase in BUN, respectively.

Late histopathological changes in the kidney, after ADR administration, alone and combined with WBH, were found primarily in the cortex and diagnosed as chronic glomerulonephropathy. There was no qualitative difference in glomerular damage between rats treated with ADR alone or ADR combined with WBH. The manifestation of renal damage was reflected by the development of symptoms of nephrotic syndrome (ascites and hyperlipidemia; Refs. 2, 3, 32, and 33) and an increase in BUN (Fig. 4B). From the quantitative data on morphological renal damage as a result of ADR alone or combined with WBH (Fig. 6), a TER of 2.4 can be estimated for chronic nephropathy. It is known that ADR administration causes chronic glomerulonephropathy in at least several species such as the rat and rabbit (2-6, 33). Since the rat kidney is a target organ for ADR-mediated toxicity under normothermic conditions, it is not surprising that the combination of ADR with WBH would enhance the renal injury in a rat model. ADR-mediated renal

| Table 2 Summary of TERs estimated for ADR-mediated antitumor effect and normal tissue toxicities |
|---------------------------------------------|---------------------------------------------|
| Toxicity index | TER ± 0.3* | Isoeffect for TER calculation |
| Acute toxicities (0–14 days)     |                |                        |
| Lethality                     | 1.6 ± 0.2    | LD10                   |
| Body weight                   | 1.5 ± 0.1    | 10% body weight loss   |
| Diarrhea                      | 1.5 ± 0.1    | ED10                   |
| WBC count                     | 1.3 ± 0.1    | 50% decrease           |
| Platelet count                | 1.3 ± 0.1    | 50% decrease           |
| Chronic toxicities (60 days)   |                |                        |
| Lethality                     | 2.3 ± 0.2    | LD10                   |
| Body weight                   | 2.2 ± 0.2    | 10% body weight loss   |
| BUN                           | 3.0 ± 0.2    | 325% increase to 50 mg/dl |
| Nephropathy                   | 2.4 ± 0.5    | Slope of lesion score curves* |
| Cardiopathy                   | 4.3 ± 0.9    | Slope of lesion score curves* |

* TER = ADR dose without WBH that caused a specified effect
* Errors are ± SEM (see statistics in "Materials and Methods").
* Slopes were fit to a linear regression, and the ratio of the slopes was used for TER estimation (also see "Materials and Methods").

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toxicity, however, has rarely been reported in humans, and thus the clinical use of combined ADR and WBH is not likely to be nephrotoxic.

Under normothermic conditions, chronic cardiac toxicity (congestive heart failure) is the major dose-limiting complication as a result of ADR administration in the clinical treatment of cancer patients. In this preclinical study combining ADR with WBH, ADR-mediated cardiac damage was diagnosed as chronic multifocal cardiomyopathy. No qualitative difference in the cardiac lesions was observed between the animals treated with ADR alone and those treated with ADR plus WBH. From the dose-response curves presented in Fig. 8, a relatively large enhancement of ADR-mediated late cardiac injury by WBH is shown, and a TER of 4.3 can be estimated. Birmelin et al. (19) have also reported thermal potentiation of ADR-mediated morphological heart damage. In their study, they observed a large increase in morphological damage after a mild WBH treatment of 10 to 20 min at 41°C combined with ADR. However, in their study microwave-induced heat alone also induced lesions which were morphologically similar to those found after ADR administration. In contrast to the observations by Birmelin et al. (19), we did not detect any cardiac damage after WBH alone (120 min at 41.5°C) (Fig. 8). Kim et al. (20) and Gerard et al. (21) reported thermal enhancement of ADR-mediated acute cardiotoxic effects in clinical hyperthermia. Kim et al. observed electrocardiographic disturbances, ventricular irritability, and cardiac dysfunction during the combined treatment at ADR dosages (10 and 20 mg/m²) that without heat did not cause acute cardiac effects. Gerard et al. also encountered acute cardiac problems during combined WBH plus ADR treatments. Cardiac problems occurred during 20% of the WBH plus ADR treatments. All cardiac symptoms were transient functional abnormalities that disappeared after medication or cooling. Although it is arbitrary to estimate TERs for normal tissue damage at a fixed time point posttreatment, when morphological change might be still progressive, the results obtained 60 days posttreatment give an indication of thermal enhancement of ADR-mediated “late” side effects. It is interesting that calculated TERs are larger for chronic toxicities in the rat than for acute toxicities (2.4–4.3 versus 1.3–1.6). The early lethality (<14 days posttreatment) caused by ADR administration in combination with WBH is probably the result of hematological toxicity and injury to the gastrointestinal tract. These acute toxicities appeared to be transient and reversible in nature, and as determined by weekly blood counts and histological examination after 60 days, did not appear to contribute to “late” mortality caused by combined ADR plus WBH. In contrast, the late lethality (>14 days posttreatment) of combined ADR plus WBH was most likely due to a combination of several different toxicities, i.e., injury to heart and kidney. These late toxicities appeared to be progressive in nature, and there was a greater thermal enhancement of chronic toxicity than of acute toxicity. Therefore, the TER calculated for late lethality increased to 2.3 as compared to 1.6 for early mortality (Table 2).

The thermal enhancement of ADR-induced acute toxicities by WBH was fairly mild in these preclinical studies, and such acute side effects may be manageable by appropriate strategies in the clinical situation. On the other hand, late normal tissue toxicities are more profound, would be more difficult to treat, and could have deleterious consequences.

Because calculated TERs are larger for ADR-mediated chronic toxicities than for antitumor effects (2.4–4.3 versus 1.6), caution is advised when applying ADR in combination with WBH (Table 2). The therapeutic index of combined cis-diaminedichloroplatinum (II) (cisplatin) plus WBH was improved in preclinical studies by employing the use of a renal protective agent such as o-(β-hydroxyethyl)-rutoside (venoruton) (42) or modification of the heat/drug schedule (43). In both studies, WBH enhancement of cisplatin-mediated normal tissue toxicity was selectively reduced while the supraadditive antitumor effect was retained. Further experiments which explore alterations in heat-drug sequence and the use of potential normal tissue “protective” agents such as ICRF-187, an agent that protects against ADR-mediated toxicities under normothermic conditions (26, 27), are necessary to attempt to reduce or prevent severe side effects without interfering with the enhanced antitumor effect of combined ADR plus WBH treatment.

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REFERENCES


ADRIAMYCIN/HYPERTHERMIA EFFECTS ON TUMOR AND NORMAL TISSUE


Effect of Adriamycin Combined with Whole Body Hyperthermia on Tumor and Normal Tissues

Jan Wondergem, L. Clifton Stephens, Frederick R. Strebel, et al.


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