Interactions of Hyperthermia and Chemotherapy in Animals

Jane B. Marmor

Department of Radiology and Medicine (Oncology), Stanford University School of Medicine, Stanford, California 94305

Abstract

In tissue culture, the cytotoxicity of a number of commonly used chemotherapeutic drugs is greatly enhanced at elevated temperatures. However, pharmacokinetics, drug concentrations, oxygen tension, and pH in tumors in animals can all vary widely from those in cell cultures. In addition, tissue culture studies do not give vital information on the effect of combinations of drugs and hyperthermia on normal tissues or metastases. Available studies of drugs and hyperthermia in animals are reviewed, and they yield clinically useful information. One study indicated that the activity of methotrexate was not enhanced by hyperthermia in vivo. Results for alkylating agents were not conclusive. Antitumor effects of 1,3-bis(2-chloroethyl)-1-nitrosourea, bleomycin, and cis-diamminedichloroplatinum, however, were significantly potentiated by local hyperthermia. 1,3-bis(2-chloroethyl)-1-nitrosourea effects were enhanced between 41 and 42°C, and thus, it is potentially of use in the setting of systemic hyperthermia. Bleomycin, however, was enhanced significantly only near 43°C, suggesting that its clinical use is more appropriate with local hyperthermia. Adriamycin and S-(2-aminoethyl)isothiouronium dihydrobromide, although potentiated in vitro, were not potentiated in vivo in doses which were clinically tolerable. Discrepancies between the in vitro and in vivo findings are likely due, at least in part, to differences in drug concentration. The combination of local primary tumor hyperthermia and chemotherapy did not adversely affect the incidence or severity of spontaneous lung metastases in KHT tumor-bearing mice.

Very few studies of the pharmacology of drugs in vivo in the presence of hyperthermia have been reported. Uptake of chemotherapeutic drugs may be enhanced in hyperthermic tissue. Further in vivo studies both of effectiveness and drug pharmacology for combined hyperthermia and chemotherapy are advisable to define optimum time-dose schedules for clinical trials.

Introduction

Studies on a variety of cells in vitro have shown that the cytotoxic efficiency of a number of commonly used chemotherapeutic drugs is greatly enhanced at elevated temperatures (8, 13). These findings have raised the possibility that hyperthermia can be used clinically to increase the effectiveness of systemically administered chemotherapeutic agents. However, relatively few studies have attempted to see whether the interaction between chemotherapy and hyperthermia is useful in tumor-bearing animals treated systemically with drugs. Even fewer clinical studies have attempted to take advantage of the potential interaction (13).

There are several reasons why results from studies of combinations of hyperthermia and chemotherapy in vivo may differ from the in vitro results. The first of these is that drug doses to which cells in a tumor (in vivo) are exposed may differ from those to which cells in vitro are exposed. Drug levels in tissue cultures are likely to remain relatively stable during the experimental period (often only 1 hr), and drug doses are easier to quantitate, usually being the concentration times the time. Drug doses to which cells in tumors in vivo are exposed are much more complex and are a function of the distribution, metabolism, and excretion of the drugs by the animals being tested as well as the tumor blood supply. The situation in animals is made even more complex by the probability that hyperthermia itself affects the distribution, metabolism, and excretion of the drug and could markedly affect drug levels to which cells in the tumor are exposed (1, 17). Even local hyperthermia may affect blood flow (and thus the drug delivered to the tumor), local drug transport, and metabolism. Unfortunately, very few studies are available on the effect of hyperthermia on the pharmacology of antineoplastic drugs. Finally, the effects of heat on tissues or organs may differ in some respects from those on isolated cells in vitro. Although mechanisms of cell killing due to hyperthermia may be the same in vitro as in vivo, the situation in vivo is more complex because of the interaction of structural components of a given organ or tissue. For example, recent studies have shown that hyperthermia has a profound effect on the vasculature of an experimental tumor in vivo and that clinical effects observed in the tumor following hyperthermia are in part secondary to the effect on blood vessels and not direct effects of the heat on tumor cells (2).

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1 These studies were supported by Grant CA-19386, National Cancer Institute, Department of Health, Education, and Welfare. Presented at the Conference on Hyperthermia in Cancer Treatment, September 15 and 16, 1978, San Diego, Calif.

and drugs was in vivo are included in this review; studies where cells were exposed in vitro but assayed in vivo are omitted. In addition, only published work which included details of experimental protocol is included.

Antimetabolites

In 1973, Muckle and Dickson (18) reported on combined methotrexate and heating of VX-2 carcinomas in rabbits. Host animals were given 6 daily injections of methotrexate (0.4 mg/kg), and 4 days after starting the course, tumors were heated to 42°C for 1 hr by water bath. Response was analyzed by volume change and by rate of respiration and glycolysis of tumors. The effect of the combined therapy was not significantly different from that for methotrexate alone by either assay.

Alkylating Agents

Suzuki (24) studied the effect of the combination of Nitromin (mechlorethamine N-oxide) and hyperthermia on the Yoshida solid sarcoma in rats. The combination of 42°C local waterbath hyperthermia with Nitromin (5 mg/kg) caused local tumor regression, whereas either of these treatments alone only caused slowing of tumor growth. At higher doses of Nitromin (10 mg/kg), tumor regression occurred with or without heat, and the difference between them was not significant. Local toxicity in terms of swelling and loss of feet was worse in the group which received combined treatment. In a later study, Dickson and Suzanger (5) examined the effects of 42°C hyperthermia in conjunction with the alkylating agent MDMS3 on Yoshida sarcomas in rats. This study showed no improvement in clinical results from the combination of drug and heat. In fact, tumors treated with heat and MDMS regressed more slowly than did tumors treated with MDMS alone. When the authors assayed for inhibition of glycolysis, the 2 treatments were additive at 24 hr after treatment, but later the effect of heat and drug on tumor glycolysis was the same as that for drug alone. There were several flaws in the design of this experiment, however. In the first place, curative doses of MDMS alone were utilized. Secondly, drug and hyperthermia were not administered simultaneously; drug was followed 20 hr later by 42°C hyperthermia. Thus, a synergistic effect that required the 2 modalities to be given close together in time would have been missed.

Antitumor Antibiotics

Adriamycin. Hahn et al. (10) examined the combination of ADR and 43°C hyperthermia for 30 min in BALB/c mice bearing the EMT6 sarcoma. Analysis of the effects was by in vitro cloning of cells from tumors treated in situ. Effects on tumor growth or cure rates were not reported. In doses between 10 and 25 mg/kg, the combined cytotoxicity of ADR and 43°C hyperthermia was clearly superior to either modality alone.

Clinical observations of effects on tumor growth of the combination of ADR and heat in mammary carcinoma bearing C3H × DBA/2 F1 (hereafter called C3D2F1/BOM) mice were reported by Overgaard (19). Local hyperthermia was administered 5 min after drug at 40.5°C for 2 hr or 42.5°C for 1 hr; one group of animals underwent systemic hyperthermia at 40.5°C for 2 hr. Unfortunately, ADR was utilized in a dose that was not clinically tolerable (25 mg/kg). As a result, 18 of 20 control animals which received ADR alone died approximately 7 days after treatment. Both 40.5 and 42.5°C combined with ADR gave a higher proportion of cures than heat alone. However, since only 2 animals treated with ADR alone survived, it is not possible to tell whether this effect is greater than one would expect from ADR alone or from an additive effect of the 2 modalities. All animals treated with systemic hyperthermia and ADR died a toxic death. Surprisingly, the author noted a decreased number of toxic deaths in the animals given both local hyperthermia and ADR. This study might have been more definitive if it had been done at a practical clinical dose level of ADR.

Our laboratory has studied the combination of ADR and 43°C local hyperthermia in 2 experimental mouse tumor systems (16). The EMT6 tumor in BALB/c mice (22) utilized to study the effects of local tumor hyperthermia and i.v. administered ADR on tumor cell survival. The KHT mammary carcinoma in C3H mice (14) was utilized to study effects of single and combined therapies on growth delay, tumor cure, and incidence of lung metastases. Heating was by radio-frequency fields as described previously (15) and was for 30 min at 43°C begun immediately after injection of drug.

Results of cell survival studies on EMT6 tumors excised 2 hr after i.v. injection of graded doses of ADR with or without local heating of the tumor are shown in Chart 1. At the highest dose (10 mg/kg), there appeared to be significant enhancement of ADR cytotoxicity by 43°C. However, no significant potentiation was seen at doses of 5 mg or 2.5 mg/kg.

The results of experiments in which combinations of ADR and heat were administered to KHT tumor-bearing C3H mice are shown in Chart 2 and Table 1 (16). In these and subsequent experiments, effects on tumor growth were expressed as the median time (in days) which it took for tumors to double their mean tumor diameter (defined as the geometric mean of 3 orthogonal measurements). Doses of ADR (10 mg/kg) when combined with hyperthermia resulted in 14 of 25 deaths about 5 days after treatment. The same dose of drug alone caused 3 of 16 deaths. Thus, in contrast to result of Overgaard (19), combination treatment was more toxic than was either treatment alone. Clinically tolerable doses of ADR (2.5 and 5 mg/kg) were not significantly increased in effectiveness by simultaneous local hyperthermia (open circles) over that expected from simple additive effects of the 2 modalities (closed circles). In addition, tumor cures were not seen as a result of combined treatment (Table 1). Thus, despite the fact that the cytotoxic effects of ADR in tissue culture are markedly potentiated by local hyperthermia (10, 12), such potentiation was not demonstrated in vivo.

There are several possible explanations for the apparent discrepancy between the in vivo and in vitro results for ADR and for the apparent discrepancy between Overgaard's results and ours. The most probable is that suggested by Chart 1; drug doses may be critical, and sufficiently high concentrations...
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High toxicity of drug alone, he did believe some potentiation occurred at this high drug dose. In any case, if hyperthermic potentiation can only be demonstrated at doses which are toxic to the host it can be expected to be of little clinical use.

A second possible explanation for the discrepancy between in vivo and in vitro findings for ADA and heat is that timing of the drug and heat dose may not have been optimum in these studies. Studies on cells in vitro indicate that heating for too long a period or prior to drug can actually make cells resistant to ADR (12). It is possible, therefore, that a different heat fractionation might demonstrate sensitization.

BLEO. In vitro cell killing by BLEO is markedly potentiated by heat (3). Animal studies from our laboratory indicate that antitumor effects of BLEO are also potentiated by local heating (16). Chart 3 and Table 1 show the effect of combined BLEO and 43°C on KHT tumor-growth delay and cure rate. Cures were seen with combined BLEO and hyperthermia given together, although no cures were seen for the 2 modalities given 24 hr apart. BLEO and hyperthermia given together also caused a significantly greater growth delay than did either modality alone or than both heat and BLEO given 24 hr apart. The fact that the 2 modalities had to be given close together in time to achieve a synergistic effect suggests that there is a true "interaction." It also has implications for the clinical use of combinations of heat and BLEO. Further definition of the critical time interval between BLEO and heat administration in vivo is desirable.

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Table 1
Incidence of KHT flank tumor cure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 treatment</th>
<th>2 treatments</th>
<th>3 treatments</th>
<th>Total, 1–3 treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/15</td>
<td>0/12</td>
<td>0/12</td>
<td>0/22</td>
</tr>
<tr>
<td>43° for 30 min</td>
<td>0/12</td>
<td>0/6</td>
<td>0/6</td>
<td>0/24</td>
</tr>
<tr>
<td>ADR (2.5 mg/kg)</td>
<td>0/6</td>
<td>1/5</td>
<td>0/8</td>
<td>1/19</td>
</tr>
<tr>
<td>ADR (2.5 mg/kg) + 43°C (together)</td>
<td>0/6</td>
<td>0/5</td>
<td>0/6</td>
<td>0/17</td>
</tr>
<tr>
<td>ADR (5 mg/kg)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/2</td>
<td>0/14</td>
</tr>
<tr>
<td>ADR (5 mg/kg) + 43°C (together)</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/16</td>
</tr>
<tr>
<td>ADR (5 mg/kg) + 43°C (24 hr apart)</td>
<td>0/7</td>
<td>0/11</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>BLEO (7 mg/kg)</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/24</td>
</tr>
<tr>
<td>BLEO (7 mg/kg) + 43°C (24 hr apart)</td>
<td>0/7</td>
<td>0/8</td>
<td>0/7</td>
<td>0/22</td>
</tr>
<tr>
<td>BLEO (7 mg/kg) + 43°C (together)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/18</td>
</tr>
<tr>
<td>BLEO (15 mg/kg)</td>
<td>0/7</td>
<td>0/6</td>
<td>0/7</td>
<td>0/22</td>
</tr>
<tr>
<td>BLEO (15 mg/kg) + 43°C (24 hr apart)</td>
<td>1/12</td>
<td>3/14</td>
<td>4/26</td>
<td></td>
</tr>
<tr>
<td>BLEO (15 mg/kg) + 43°C (together)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/18</td>
</tr>
<tr>
<td>BCNU (10 mg/kg)</td>
<td>0/14</td>
<td>0/13</td>
<td>0/13</td>
<td>0/40</td>
</tr>
<tr>
<td>BCNU (10 mg/kg) + 43°C (24 hr apart)</td>
<td>0/15</td>
<td>3/15</td>
<td>2/17</td>
<td>5/47</td>
</tr>
<tr>
<td>BCNU (10 mg/kg) + 43°C (together)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/18</td>
</tr>
<tr>
<td>cis-DDP (2 mg/kg)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/18</td>
</tr>
<tr>
<td>cis-DDP (2 mg/kg) + 43°C (24 hr apart)</td>
<td>0/6</td>
<td>0/7</td>
<td>0/3</td>
<td>0/16</td>
</tr>
<tr>
<td>cis-DDP (2 mg/kg) + 43°C (together)</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/18</td>
</tr>
</tbody>
</table>

* BCNU given 24 hr before heat in 8 animals each group and 24 hr after heat in 8 animals each group.

Table 2
Ratio of expected/observed survival values for BLEO and BCNU with heat

<table>
<thead>
<tr>
<th>Drug</th>
<th>Temperature (°C)</th>
<th>Expected survival (clonogenic cells/tumor)</th>
<th>Observed survival (clonogenic cells/tumor)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLEO (15 mg/kg)</td>
<td>41</td>
<td>1700</td>
<td>500</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>1500</td>
<td>300</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>90</td>
<td>0.9</td>
<td>100.0</td>
</tr>
<tr>
<td>BCNU (5 mg/kg)</td>
<td>41</td>
<td>4500</td>
<td>1900</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>4000</td>
<td>100</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>400</td>
<td>10</td>
<td>40.0</td>
</tr>
</tbody>
</table>

The "expected" value is the product of cell survival for heat alone and drug alone and is the theoretic "additive" effect of the two modalities. Values are derived from Figs. 3 and 5.

dose of BLEO together with heat caused 5 of 6 deaths in treated animals. Autopsies on animals that died following heat and BLEO showed edema, necrosis, and perforation of bowel near the tumor (i.e., in an area that probably received some heating). The finding of increased normal tissue toxicity from the combination also has important clinical implications.

In cell survival studies with EMT6 tumors, the cytotoxicity of BLEO combined with 43°C hyperthermia was 10- to 100-fold greater than that expected from simple additive effects. Effects at lower temperatures, however, were less pronounced. At 41°C, cell survival after the combination treatment was within the expected additive range. At 42°C, survival was lower than expected from additive effects but much less dramatically so than at 43°C. Ratios of "observed" to "expected" survivals for the 3 temperatures are shown in Table 2. There is a temperature "threshold" for the combined effect of BLEO and hyperthermia in vivo between 42 and 43°C. This is consistent
with the finding of a similar temperature "threshold" in vitro (10). Since safe levels of systemic hyperthermia are limited to 41.8°C (20), this finding has important implications for clinical therapy. To obtain significant therapeutic benefit from the combination of heat and BLEO, it appears necessary to utilize temperatures above those that can be tolerated systemically. Thus, BLEO is not an appropriate drug to combine with systemic hyperthermia. A more appropriate use is combined with local hyperthermia where temperatures in the range of 43°C can safely be utilized.

Actinomycin D. Yerushalmi (26) studied the effect of actinomycin D and local hyperthermia on a solid SV10 fibrosarcoma in BALB/c × C57BL/6F, mice. A marked increase in median survival time was noted following combined treatment compared to actinomycin alone. Unfortunately, the study lacked information on the effect of heat alone, so one cannot tell if the effect was more than additive.

Nitrosoureas

BCNU. Twentyman et al. (25) studied BCNU and hyperthermia in the EMT6 mouse-tumor system. Animals bearing flank or leg tumors were treated with BCNU (20 mg/kg) immediately prior to hyperthermia by water bath at an average tumor temperature of 41.6°C for 1 hr. Both tumor regrowth and in vitro assay for survival of tumor cells were reported. In both assays, the effect of the combination treatment was clearly superior to that of either treatment alone. By the cell survival assay, the effect of the combination appeared to be greater than that expected from additive effects of the 2 modalities.

We have also studied the combined effects of BCNU and hyperthermia on both the EMT6 and KHT tumor systems and our results agree substantially with those of Twentyman.4 Considerable potentiation of the in vivo cytotoxic effects of BCNU was seen at both 42 and 43°C, although the effect was less significant at 41°C (Table 2). These results agree with the temperature response reported in vitro for BCNU (8). Since the potentiation threshold, if any, for BCNU is between 41 and 42°C, this drug is an appropriate one to consider for adjuvant use with systemic hyperthermia.

The effects of BCNU with or without heat on tumor growth delay and cure of the KHT carcinoma are shown in Chart 4 and Table 1. Considerable increase in the antitumor activity of BCNU was demonstrated both in growth delay and incidence of tumor "cure" when heat was used with the drug. Heat and BCNU had to be given close together in time (open circles) to observe this effect; when exposure to the 2 modalities was separated by 24 hr, the effects were much less (solid circles). For exposures separated by 24 hr, results were the same whether heat or drug was given first.

Other Agents Enhanced in Vitro

Cis-DDP. The enhancement of cytotoxicity in vitro of cis-DDP at elevated temperatures was described by Hahn (9). Studies in our laboratory have also shown that the antitumor effects of cis-DDP were also potentiated in vivo.4 The combined effect of hyperthermia and cis-DDP on KHT tumor growth delay is shown in Chart 5. For a single combined treatment, no increase in

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bined with heat in vitro. This compound was nontoxic to cells at 37°C but became markedly cytotoxic at 43°C. We have attempted to see whether this cytotoxicity at elevated temperatures was useful against animal tumors in vivo. Chart 6 shows the results of cell survival studies from tumors excised after treatment with AET in the maximum tolerated dose with or without 43°C hyperthermia. No cytotoxicity was demonstrated. If anything, a small amount of protection from the effects of heat occurred. Studies of the effects on tumor growth of the combination confirmed that there was no antitumor effect of AET at elevated temperatures (Chart 7). Thus, in the case of AET, the in vitro studies did not predict accurately the effect observed in animals, at least in these systems.

Effects of Local Hyperthermia With or Without Chemotherapy on Spontaneous KHT Lung Metastases

There has been considerable concern about the possibility that local hyperthermia to a primary tumor might increase the rate of metastasis. Suzuki (24), and Muckle and Dickson (18) noted that, with combinations of drug and local hyperthermia to a primary tumor, there was no survival advantage despite regression of the primary tumor because animals died at approximately the same rate as controls from lung metastases. Dickson and Ellis (4), in a later study, suggested that local hyperthermia at 42°C increased the rate of metastasis of the Yoshida tumor in rats. However, this study is not conclusive since the animals so treated had significant systemic hyperthermia and a high death rate from hyperthermia alone.

Yoshida tumor in rats. However, this study is not conclusive since the animals so treated had significant systemic hyperthermia and a high death rate from hyperthermia alone. Indeed, 151 of 167 animals died during treatment or within 24 hr and another 10 died 3 to 12 days later leaving only 6 survivors for pathological examination. Clearly the survivors of such a toxic regimen may have been systemically debilitated, which may have affected growth of metastases.

We have considered this problem in KHT tumor-bearing animals treated with local hyperthermia alone or with chemotherapy. This tumor metastasized spontaneously to the lungs in close to 100% of control mice. Metastasis first occurred between 10 and 13 days after implantation of intradermal flank tumors (16). Since the usual day of treatment for tumors was 11 days after implantation, it was possible to assess the effects of local hyperthermia on the incidence and severity of lung metastases. Lungs were examined for metastasis 23 to 25 days after treatment, and the severity of metastasis was graded from 0 (macroscopically free of metastases) to 4. The average grades for control and treated animals are shown in Table 3 (16)5-6. Heat alone did not appear to increase the average severity of metastases over control. For ADR and AET, treatments not potentiated by heat, the average grade for animals given heat with drug was similar to that for drug alone. Heat decreased the severity of lung metastasis when used with BLEO or BCNU. The most dramatic effect was seen with BCNU. Thirty-two of 47 lungs were negative for tumor following BCNU and heat given together compared to 2 of 18 negative with BCNU alone and 2 of 38 when BCNU and heat were given 24 hr apart (16). These data do not support the contention that local hyperthermia increases metastasis. In fact, it appears that those treatments which are most effective against the primary tumor decrease metastasis whether or not they include hyperthermia.

Studies of Pharmacokinetics of Drugs in the Presence of Hyperthermia

The plasma clearance, distribution, biotransformation, and excretion of drugs may be affected by hyperthermia either directly or as a result of changes in blood flow due to the hyperthermia (1, 17). However, few published studies address

the problem of alterations in drug pharmacokinetics in the presence of hyperthermia.

Rochlin et al. (21) reported on the rate of binding of 32p-labeled thiotepa in isolated perfused dog limbs held at different temperatures. Binding of drug was increased at increasing temperatures. Unfortunately, most of his observations were in the hypothermic temperature range; only 3 observations in the hyperthermic range were made, and no temperature greater than 38.9°C was studied.

Shingleton et al. (23) described uptake of 14C-labeled nitrogen mustard under conditions of regional normothermia, hyperthermia, and hyperthermia in normal dog legs and in VX-2 carcinomas in rabbits; 42°C hyperthermia was produced by either regional perfusion (abdomen) or by radio-frequency inductive heating (limbs). In both tumors and normal tissue, the uptake of drug was greatest under conditions of local hyperthermia. Local toxicity of the nitrogen mustard was also increased at the elevated temperature.

Finally, Mimnaugh et al. (17) studied the disposition and metabolism of ADR in normothermic and hyperthermic rabbits. In general, distribution of drug was the same to tissues at similar concentrations in both normothermic and hyperthermic animals. However, the authors did note increased concentrations of ADR in skeletal muscle and increased total ADR in the hearts of hyperthermic animals. There was no significant difference in urinary or biliary excretion of ADR or its metabolites. Plasma clearance was similar in both groups although the data suggested an expanded "peripheral" drug compartment in the hyperthermic animals. They stated that, theoretically at least, the problem of alterations in drug pharmacokinetics in the presence of hyperthermia.

Interaction of Hyperthermia and Chemotherapy in Animals

Conclusions

Tissue culture studies have raised the possibility that the effectiveness of a number of chemotherapeutic agents may be greatly enhanced by systemic or regional hyperthermia. The animal studies reviewed here suggest that some, but not all, drugs which are potentiated by heat in vitro also show clinically useful potentiation in vivo. Clinically useful data, which will be important in designing clinical trials of hyperthermia and chemotherapy, can be obtained from animal studies.

The results of the animal studies reviewed in this paper are shown in Table 4. Significant potentiation occurs with BCNU, BLEO, and cis-DDP. BCNU appears to be one of the most promising drugs for clinical trials. Its effect was potentiated significantly at doses well below the toxic range. Potentiation was near maximal between 41 and 42°C. Thus, BCNU is an appropriate drug to consider with systemic hyperthermia. BLEO, in contrast, was maximally potentiated only at temperatures between 42 and 43°C. Since systemic temperatures in this range cannot be safely achieved, BLEO is more likely to be useful as an adjunct to local hyperthermia.

Not all drugs that are potentiated in vitro show clinically useful potentiation in vivo. ADR failed to show significant potentiation by heat in clinically tolerable doses. Studies at high dose, however, did suggest potentiation. Thus, ADR does not appear to be a good candidate for hyperthermic potentiation of systemically administered drug. It might be appropriate, however, to utilize hyperthermia and ADR in regional perfusion where higher local concentration of drug could be achieved without systemic toxicity. AET is another example of a drug that is potentiated in vitro but did not show clinically useful potentiation in vivo.

In general, toxicity to normal tissues and death rates were increased in animals receiving combination therapy.

Local hyperthermia to primary tumors did not appear to adversely affect the incidence or extent of spontaneous lung metastases. In fact, combinations that were very effective against primary tumors, such as heat and BCNU, appeared to decrease the incidence of lung metastases.

Few studies of the effects of hyperthermia on pharmacological disposition of drug were available. Some of these indicated that tissue binding of alkylating agents is enhanced by local hyperthermia. More studies of the interaction of hyperthermia and drug metabolism are desirable, as such information will be extremely useful in designing appropriate clinical trials of hyperthermia and chemotherapy.
Table 4
Combined chemotherapy and hyperthermia in animal tumors

<table>
<thead>
<tr>
<th>Drug/class</th>
<th>Temperature (°C)</th>
<th>Assay</th>
<th>Effect</th>
<th>Investigators and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimetabolite</td>
<td>Methotrexate</td>
<td>42</td>
<td>Tumor growth</td>
<td>Not enhanced</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>Nitroin</td>
<td>42</td>
<td>Tumor size</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td>MDMS</td>
<td>42</td>
<td>Tumor size</td>
<td>Less than additive</td>
</tr>
<tr>
<td></td>
<td>Glycolysis</td>
<td>42</td>
<td></td>
<td>Additive</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Adriamycin</td>
<td>43</td>
<td>Tumor cell survival</td>
<td>Synergistic*</td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>40.5 and 42.5</td>
<td>Tumor cure and growth delay</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>43</td>
<td>Tumor cure and growth delay</td>
<td>Not enhanced</td>
</tr>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>42.3 and 43.6</td>
<td>Survival time</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td>BLEO</td>
<td>43</td>
<td>Tumor cure and growth delay</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Nitrosoureas</td>
<td>BCNU</td>
<td>41.6</td>
<td>Tumor regrowth</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td>BCNU</td>
<td>42 and 43</td>
<td>Tumor cure and regrowth</td>
<td>Synergistic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cell survival</td>
<td>Synergistic</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>cis-DDP</td>
<td>43</td>
<td>Tumor cure and regrowth</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cell survival</td>
<td>Additive or synergistic</td>
</tr>
<tr>
<td></td>
<td>AET</td>
<td>43</td>
<td>Tumor cure and regrowth</td>
<td>Not enhanced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cell survival</td>
<td>Not enhanced</td>
</tr>
</tbody>
</table>

* At doses greater than 10 mg/kg.
+ Unpublished observations.

References

Interactions of Hyperthermia and Chemotherapy in Animals

Jane B. Marmor


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