Potential for Therapy of Drugs and Hyperthermia[1]

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Abstract

The interaction of hyperthermia (41–45°C) and chemotherapeutic agents frequently results in increased cytotoxicity over that predicted for an additive effect, although to date only a very limited number of drugs have been examined for such a possible interaction. At 42°C, the upper limit of temperature useful for whole-body hyperthermia, the most promising agents of those examined to date appear to be the nitrosoureas and cis-platinum. Insufficient data exist for cyclophosphamide, whose long plasma half-life makes it an attractive candidate. Localized heating seems optimum at higher temperatures (43–45°C). At these temperatures, not only those drugs effective at 42°C but particularly bleomycin and possibly amphotericin B become candidates. No data exist in the literature on possible "thermic sensitizers," i.e., drugs which are noncytotoxic at 37°C but which become effective at elevated temperatures. Two special cases are Adriamycin and actinomycin D. These drugs may be contraindicated for clinical use, since not only synergism but also protection by hyperthermia have been demonstrated, depending upon the time-sequence relationships of the heat and drug treatments.

Introduction

Other papers at this meeting deal with the biochemical and biophysical mechanisms of cell inactivation by heat or by the combination of heat and X-irradiation. Although considerable effort has gone into elucidating these, it is obvious that we are far from having a firm understanding of exactly why or how cells die after exposure to temperatures in the range of 41–45°C or why cells so treated become more sensitive to sparsely ionizing radiations. Interactions between heat and drugs are even less well understood, particularly because each drug, or at least each class of drugs, may have its own mode of interaction. Furthermore, surprisingly little effort has been expended in trying to establish these. Probably as a result, reported clinical uses of thermochemotherapy are few indeed (7). In this review, I am going to concern myself primarily with available in vitro data and their relevance to the potential clinical application of the combination of heat and drug to cancer chemotherapy. I will only touch upon mechanisms whenever these give clues to the clinical use or to point out needs for specific additional information required preparatory to such use. Obviously, only those agents can be discussed for which some data have been reported in the literature, or which have been examined in the laboratory but for which the data have not as yet been published.

The organization of the paper is designed to fit the stated purpose. First, I discuss those drugs which may be useful for whole-body hyperthermia; here the need is for agents which show increased activity at or below 42°C; particularly advantageous would be those which have relatively long plasma half-lives. Then I look at drugs which appear useful for localized hyperthermia; here treatments are characterized by higher temperatures (43–45°C) and by shorter exposure times. Finally, I discuss some currently used cytotoxic drugs which are useful at normal body temperatures but whose use in combination with heat is questionable or possibly even contraindicated.

Whole-Body Thermochemotherapy

The current practice of whole-body hyperthermia is based on the concept that malignant cells are more sensitive to mild hyperthermia (42°C) than are normal cells. However, such differences in sensitivities, whenever they have been reported (2, 3, 9), are small and hence the duration of patient exposure must be many hr before an appreciable differential cell inactivation results. The maximum safe temperature of whole-body hyperthermia is determined by the most sensitive normal tissue of the body, which is perhaps the liver. In practice, it has been shown that the safe upper limit is 42°C or somewhat less (10, 13).

Several agents show increased activity at such temperatures. Thiotepa, an alkylating agent, was shown by Johnson and Pavelec (8) to interact with hyperthermia in a manner consistent with that predicted by reaction kinetics of alkylation, i.e., the rate of cell inactivation was approximately linear with increasing temperature. Furthermore, the activation energy inferred from thermodynamic analysis was one appropriate for such a reaction. Very probably thiotepa can be regarded as a prototype for most bifunctional alkylating agents. Of more clinical interest, perhaps, are the nitrosoureas, which also show increased reaction rates with increased temperatures. Charts 1 to 3 show the cytotoxicity of 3 of these compounds against both proliferating and nonproliferating cells exposed at 37°C and at elevated temperatures. In these graphs, the abscissa is presented in units of "relative dose," a term coined to account for changes in free drug concentration during exposure (4). Chart 4 shows the temperature dependence of the cytotoxicity of cis-diamminedichloroplatinum, another agent whose inactivation rate is proportional to increased temperature. (Very similar data have also been obtained by Dr. Peter Corry, Department of Physics, University of Texas, M. D. Anderson Tumor Institute, Houston, Texas.) As a curious aside, at 37°C both the nitrosoureas and cis-diamminedichloroplatinum are somewhat more effective against nonproliferating cells (plateau) than against actively proliferating ones, a property they share with very few other drugs (14).

Of interest, perhaps, is a quantitative comparison of the changes in cytotoxicity for the various agents as the temperature is increased from 37 to 42°C. A convenient quantity for
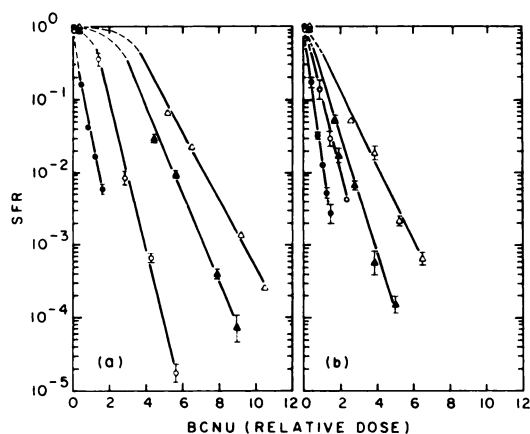
Chart 1. *In vitro* thermochemotherapy with BCNU of HA1 Chinese hamster cells. Cells were grown as monolayers on plastic Petri dishes. Prior to drug exposure, the growth medium was replaced with serum-free medium. Graded doses of drug were added to individual dishes. Exposure time was 1 hr; temperature control was to within ±0.1°. Following this treatment, cells were rinsed twice, then trypsinized and plated in appropriate dilutions for colony formation. Relative dose is defined as the average amount of active drug present during the 1-hr treatment interval. *a*, exponentially growing cells; *b*, plateau-phase cells; △, 37°C; ▲, 39°C; ○, 41°C; ●, 43°C.
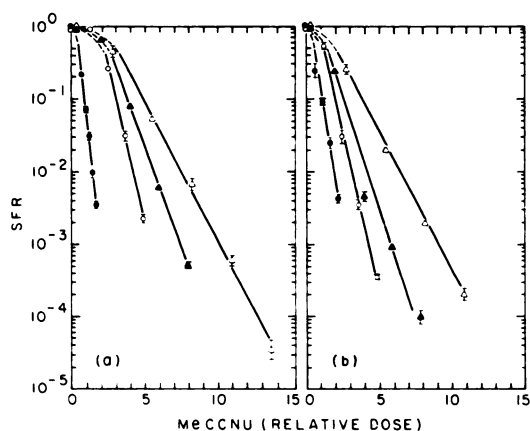

Chart 2. *In vitro* thermochemotherapy with 1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea of HA1 Chinese hamster cells. Experimental procedure as in Chart 1. *a*, exponentially growing cells; *b*, plateau-phase cells. Symbols as in Chart 1.

such a comparison is the TDMF[2], at temperature T. This factor is defined as the ratio of dose (or relative dose) required to achieve a certain end point (*e.g.*, 50 or 10% survival) at 37°C to the dose required to achieve the same end point at the temperature of interest. The TDMF at temperature T is usually independent of survival level only if the dose-response curves are exponential both at 37°C and at temperature T. This situation is the case, for example, for the killing of plateau-phase cells by the nitrosoureas. A table of TDMF at 42°C is shown for 3 survival values (50, 10, and 1%) for BCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, and 1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Table 1).

A drug which perhaps needs special discussion is cyclophosphamide. This agent, as is well known, needs activation by the liver before its cytotoxicity manifests itself. Its plasma half-life is relatively long, presumably because of its inertness prior to activation. This long persistence in the blood plus its presumed mode of action as an alkylating agent make it an

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[2] The abbreviations used are: TDMF, thermal dose-modifying factor; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

attractive candidate for whole-body hyperthermia. However, there are no data in the literature indicating whether or not, at the higher body temperature, the liver is able to activate cyclophosphamide or perhaps show an increased rate of activation nor on the thermal stability of the active compound. In the absence of such data, the usefulness of the drug as a thermochemotherapeutic agent cannot be judged.

Thermochemotherapy for Localized Lesions

In contrast to total-body hyperthermia, localized hyperthermia is not limited to temperatures below 42°C; in fact, current data indicate that for 30- to 60-min exposures, temperatures of about 44–45°C may be most useful (12). At these temperatures, the nitrosoureas and *cis*-platinum are also highly effective, as is obvious from Charts 1 to 4. In addition to these agents, there are drugs whose interactions with elevated temperatures do not follow simple Arrhenius kinetics. Between 37 and 41°C, these agents show little or no dependence on temperature in their abilities to kill cells. However, at about 43°C, there is a threshold change in their cytotoxicity, and they become highly efficient as cytotoxic agents. A good example of these is bleomycin. Dose response of cells exposed to this drug either at 37 or 41°C do not differ appreciably (Chart 5*a*). However, at 43°C, the survival curve shows quite a dramatic change, with TDMF's at 43°C becoming very large, particularly at low survival values (Table 2). Clearly, if hyperthermia is locally induced, the differential cytotoxicity and hence antitumor activity between heated and unheated volumes should be quite substantial.

Another agent of interest is the polyene antibiotic amphotericin B, not generally considered to be an effective agent against neoplastic diseases. Indeed, as shown in Chart 5*b* at 37° (or for that matter, at 41°C), it is not cytotoxic even at doses where its nephrotoxicity would make it impossible to use clinically. However, at 43°C it becomes a potent killer of cells at doses which might be useful clinically (5).

Amphotericin B illustrates one fact about thermochemotherapy: therapy need not necessarily be restricted to agents which are useful at 37°C. For instance, Chart 6 shows survival curves of cells exposed to S-(2-aminoethyl)isothiouronium bromide hydrobromide, a drug developed as a radiation-protection com-
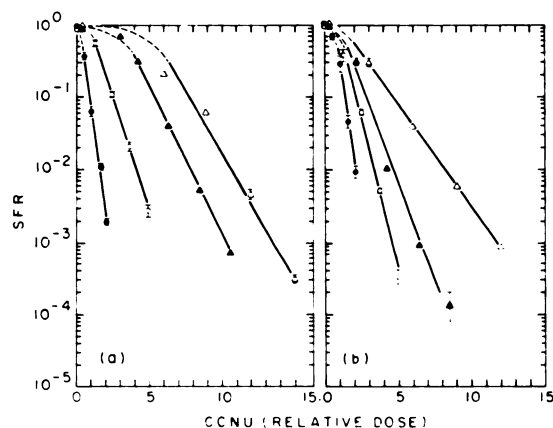

Chart 3. *In vitro* thermochemotherapy with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea of HA1 Chinese hamster cells. Experimental procedure as in Chart 1. *a*, exponentially growing cells; *b*, plateau-phase cells. Symbols are as in Chart 1.

Chart 4. *In vitro* thermochemotherapy with *cis*-diamminedichloroplatinum of HA1 Chinese hamster cells. Experimental procedure as in Chart 1. *a*, exponentially growing cells; *b*, plateau-phase cells.
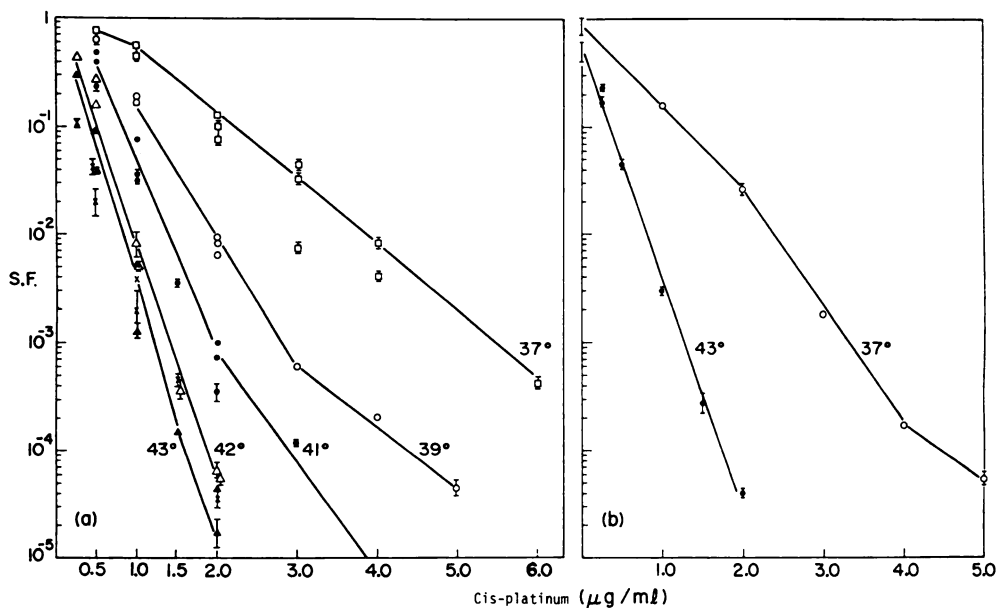
Table 1
TDMF at 42 °C

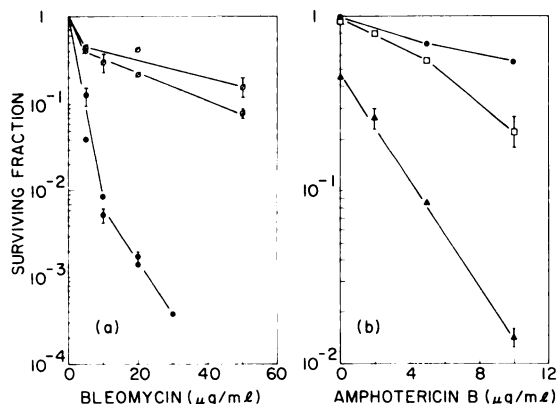| Survival values (%)	BCNU		1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea		1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea	
	Exponential	Plateau	Exponential	Plateau	Exponential	Plateau
50	5.1	3.0	6.9	2.5	2.3	2.5
10	3.9	2.9	4.5	2.6	2.7	2.5
1	3.3	2.6	3.9	2.9	2.8	2.6

Chart 5. Thermochemotherapy of Chinese hamster cells with drugs showing threshold behavior. Experimental procedure as in Chart 1. *a*, O, 37°C; Ø, 39°C; ●, 43°C. Exponentially growing cells exposed to bleomycin. *b*, exponentially growing cells exposed to amphotericin B. There is no statistically significant difference between 37 or 41°C results (*a* and *b*).

pound. At 37°, and over the dose-response range tested, it also shows little if any cytotoxicity, much like amphotericin B; it too becomes quite efficient at higher temperatures.[3] I am not suggesting that *S*-(2-aminoethyl)isothiouronium bromide hydrobromide is necessarily a good candidate for thermochemotherapy; in fact, preliminary studies have shown that it is not very effective against the KHT sarcoma growing in C3H mice.[4] Obviously, no systematic search has ever been initiated for

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[3] D. Kapp and G. M. Hahn, unpublished observations.
[4] J. B. Marmor and G. M. Hahn, unpublished observations.

agents which at physiological doses are cytotoxic only at temperatures well above 37°C. Finding such a "hyperthermic sensitizer" could be very rewarding, since it could make hyperthermia potentially much more useful in treating localized disease, much like effective radiosensitizers of resistant cells might make radiation therapy much more effective. Such putative heat sensitizers would be particularly useful for patients suffering from recurrence of disease in previously heavily irradiated fields. If such patients are still without evidence of metastases, localized hyperthermia might still be used with curative intent. Chances of success would surely be greatly enhanced if a sensitizer could be used.

Heat-induced Drug Tolerance

A biologically very interesting phenomenon and one of potential clinical importance is that of thermally induced thermal tolerance: cells sublethally heated become resistant to subsequent heat exposure. Not only do they become resistant to heat, but they also become quite resistant to at least 2 chemotherapeutic agents, Adriamycin (6) and actinomycin D (1). To my knowledge, whether or not the mechanism of induced thermal tolerance is the same as that of heat-induced drug tolerance is not known at the present time. The literature contains no data on the possibility that hyperthermia may protect against other drugs. However, for the nitrosoureas at least, a lack of protection is demonstrated by the results shown in Chart 7. In that experiment, heating cells for up to 4 hr prior to drug exposure did not make these cells more resistant to BCNU than were unheated controls.

The interaction of hyperthermia and Adriamycin is particularly complex. During short exposure times, cells heated in the presence of Adriamycin show marked synergism. However, as can be seen from Chart 8*a*, as the duration of exposure is increased, the heat plus drug curve becomes parallel to the heat-only control. This implies that, after an initial sensitive state, cells become highly resistant to Adriamycin. In another experiment, cells were heated for various lengths of time at 43°C and then exposed to the drug at 37°C. Cells became

Table 2
TDMF at 39–43°C

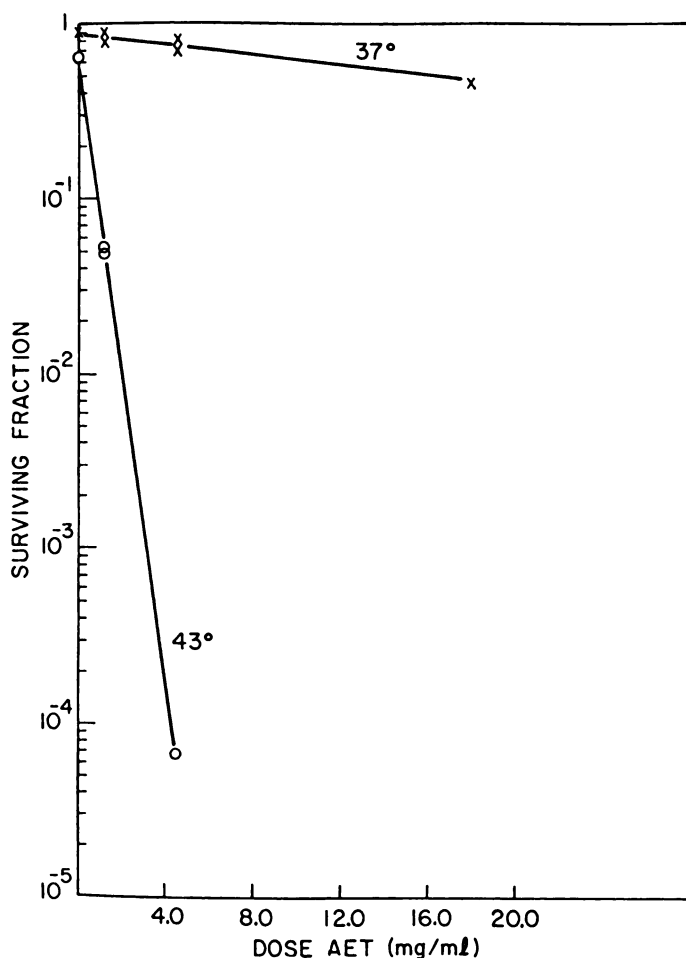|	TDMF at 39°C			TDMF at 41°C			TDMF at 43°C		
	50%	10%	1%	50%	10%	1%	50%	10%	1%
BCNU									
Exponential	1.4	1.3	1.3	2.8	2.5	2.4	36.0	8.3	5.1
Plateau	1.8	1.6	1.6	3.0	2.1	2.1	9.0	3.8	3.8
1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea									
Exponential	1.6	1.4	1.4	3.9	3.1	2.6	13.8	8.7	6.4
Plateau	1.5	1.7	1.7	1.7	2.1	2.5	2.8	3.4	3.9
1-*trans*-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea									
Exponential	1.2	1.3	1.4	1.4	1.7	1.8	2.3	6.0	5.8
Plateau	1.4	1.5	1.5	1.7	1.9	2.0	4.0	3.5	3.4
cis-Platinum									
Exponential	1.9	2.0	1.9	2.7	2.8	2.8	8.2	5.5	4.8
Plateau							1.9	3.2	3.0
Bleomycin									
Unfed									
Plateau				1	2	2	6	14	18

Chart 6. *In vitro* thermochemotherapy with *S*-(2-aminoethyl)isothiouronium bromide hydrochloride of HA1 cells. Experimental procedure as in Chart 1.

Chart 7. Kinetics of interaction between BCNU and hyperthermia. Cells were exposed to BCNU (μg/ml; 1 hr). At various times before or after BCNU exposure, cells were heated at 43°C for 1 hr. The 0-hr point is combined heat and BCNU; negative time values refer to heat before drug; positive drug time values, drug before heat.

progressively more resistant with increasing length of prior heating (Chart 8b).

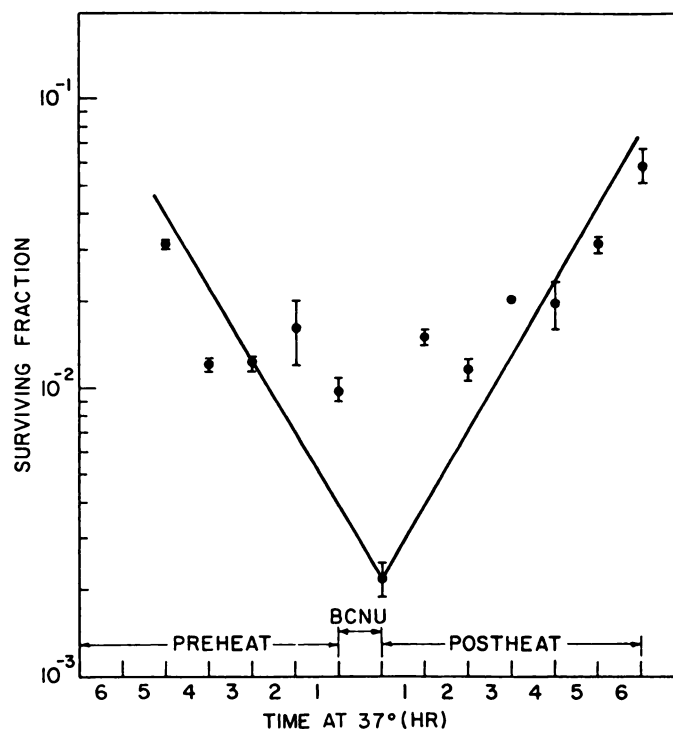The combined effect of heating and exposure to actinomycin D is somewhat similar to that found with Adriamycin; however, the initial period during which the cells are sensitized is shorter, and the amount of sensitization is not as pronounced (1, 6).

Thus, neither Adriamycin nor actinomycin D appears to be a good candidate for thermochemotherapy. This seems particularly true for whole-body heating where long-term exposures are mandatory. A saving feature may be that the lower temperatures involved delayed onset of drug resistance. For localized heating, actinomycin D appears to be useless, and Adriamycin should only be used for short-term exposures.

Chart 8. Thermochemotherapy with Adriamycin of HA1 cells. *a*, experimental procedure as in Chart 1. *b*, survival results of cells which were heated at 43°C for the indicated period and then exposed to Adriamycin (1.0 μg/ml; 1 hr).
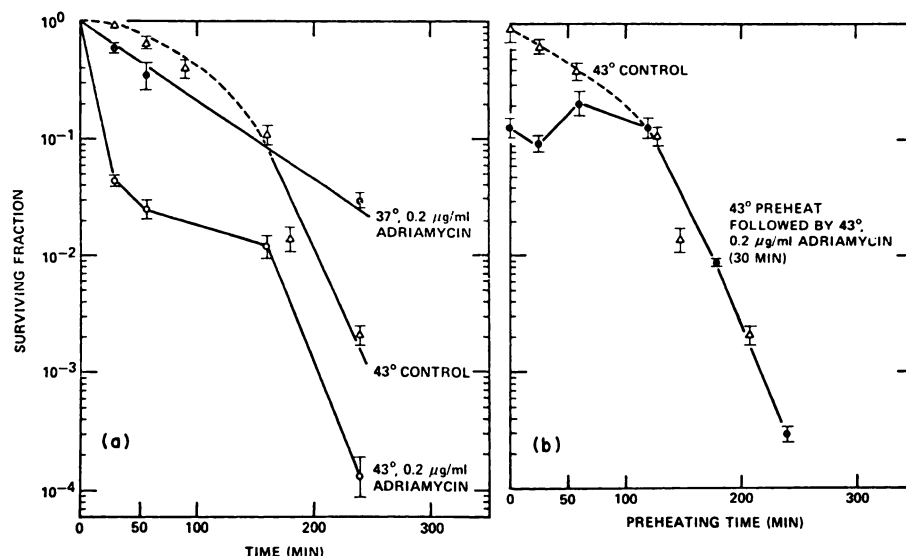
Conclusions

There is no question that the cytotoxic efficiency of several drugs is greatly increased at elevated temperatures. Furthermore, as shown in the companion paper by Marmor (11), the *in vitro* results are reflected *in vivo* in the KHT mouse-tumor system. There the simultaneous treatments by heating and by some drugs (BCNU and bleomycin) were found to be much more effective in inducing tumor growth delay than were those in which hyperthermia and chemotherapy were separated by 24 hr. Do the *in vitro* and the mouse results have any clinical relevance? Obviously, in the absence of actual clinical experience, rash predictions should be avoided. However, the *in vitro* and the *in vivo* animal data are consistent and strongly encouraging so that it appears that some predictions can be made with a reasonable degree of confidence.

It will almost surely be found that at elevated temperatures the cytotoxicity of many drugs (particularly that of the alkylating agents, of *cis*-platinum and, at or above 43°C, bleomycin) is equally as enhanced in humans as in model systems. Whether or not such increased cytotoxicity can be translated into an increased therapeutic ratio depends on a variety of factors. These are related both to the types of treatment carried out and to tumor biology. For whole-body thermochemotherapy, the question of normal tissue *versus* tumor toxicity is dominant; in the absence of extensive normal tissue data, particularly on bone marrow and on gastrointestinal stem cells, it is impossible to make meaningful predictions. However, increases in blood flow during hyperthermia may improve drug distributions in poorly vascularized tumors.

Chemotherapy in conjunction with localized hyperthermia seems to offer the best prospects, provided equipment becomes available for safely and predictably heating arbitrary tumor volumes. Once such equipment has been developed, the increased tumor temperature over that of normal tissue will almost surely yield improvements in therapeutic ratios. Two obvious strategies exist: lower drug concentrations could be utilized, thereby reducing normal tissue toxicity outside the treatment volume. Alternately, cell killing within the treatment volume could be vastly enhanced at currently used drug dosages without causing a concomitant increase in side effects outside the heated volume. Thus, the use of chemotherapy with curative intent could and should become a more realistic goal for a wider spectrum of tumors than is currently the case.

Acknowledgments

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