Recommendations for Future Research in Hyperthermia at the Molecular, Cellular, and Animal Levels

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Abstract

Recommendations are made for hyperthermia research to be carried out at the molecular, cellular, and animal levels. Effects of heat alone should be distinguished from effects of heat combined with radiation or chemotherapy. Factors to be considered include tumor cell environment, treatment sequencing, thermal tolerance, cell kinetics, any possible nonthermal effects, and therapeutic gain.

I have been asked by Dr. Kaplan to review briefly the hyperthermic research which I believe should be carried out at the molecular, cellular, and animal levels. These comments are based on discussions with several of my colleagues and, I hope, represent fairly accurately their opinions as well.

First, I wish to discuss molecular and cellular effects, not because they are the most important, but because they provide a basis for understanding effects observed in animals. In these studies, a clear distinction should be made between hyperthermic effects associated with cell killing and hyperthermic effects associated with radiosensitization. Also, several different normal and malignant cell lines from different species should be investigated to establish fundamental principles and to determine how much variability may be expected between different cell types. Furthermore, experiments should be carried out with both asynchronous cells, which mimic in vivo situations, and synchronous cells in order to understand the mechanisms associated with heat killing and heat radiosensitization. The use of synchronous cells is particularly important because heat kills S-phase cells selectively and will lead to a redistribution of viable cells in the cycle. This redistribution in itself will lead to an alteration in radiation response.

An investigation of the interplay between environmental factors is particularly important since these same factors are probably quite important in tumors in vivo. For example, many tumor cells probably exist in a radioresistant hypoxic compartment, which is also characterized by low pH and nutritional deprivation. As Dr. Suit pointed out previously, the effects of low pH are particularly relevant. For example, several studies indicate that lowering the pH from 7.4 to 6.7 has a dramatic effect on heat killing. An important question to address is whether this lowering of pH will have much effect on the radiation response when survival is normalized for heat killing itself. Specifically, will the survival curve for cells heated and irradiated at a low pH have a reduced extrapolation number and/or increased slope relative to the curve for cells heated and irradiated at the normal pH of 7.4? These same questions should be addressed when the cells are heated and irradiated under nutritionally deprived conditions or under low-oxygen tension. In other words, as data in the literature suggest, will heat have an effect on the oxygen enhancement ratio if cells irradiated under hypoxic conditions are also maintained at normal pH and under adequate nutrition? The interplay of these factors should be carefully investigated for different temperatures and durations of heating and for different sequences between the heat treatments and X-ray exposure.

The question of sequencing needs to be investigated when several different heat treatments consisting of different temperatures and durations of heating are used. Studies in the literature indicate that in general the greatest degree of killing is obtained when heat and irradiation are delivered simultaneously. However, the relationship between changes in lethality and changes in sequencing probably depends on the amount of heat damage as well as on the conditions of pH, oxygen tension, and nutritional status during the time of treatment. These sequencing studies are particularly important in understanding the results obtained in vivo. For example, the repair of radiation or heat damage from a second treatment may be different at low pH than at normal pH and may explain some of the observations in vivo.

A good example of the importance of sequencing is illustrated by the data of Henle and Leeper. These investigators showed that, when cells were exposed to hyperthermic treatment and then incubated at 40°C prior to a subsequent hyperthermic treatment or radiodose, survival decreased dramatically compared to the survival with either no incubation or incubation at 37°C between the 2 treatments. Also, if the order was reversed, with radiation given before heat, incubation at 40°C did not potentiate the damage.

The phenomenon of thermal tolerance may have important implications in vivo and should be carefully investigated for different temperatures and durations of heating. For example, when cells are heated continuously at a relatively low temperature of 42–42.5°C they become resistant to heat after about 3 hr of heating. This resistance is manifested by a flattening in the survival response plotted as a function of duration of heating. Furthermore, tolerance has been demonstrated when a second heat treatment is delivered after incubating the cells for a few hr at 37°C following a first heat treatment. This tolerance, which is transient and is observed with fractionated heat doses, may be particularly important in the clinic, and an important question to address is whether tolerance, in terms of radiosensitization, will also be observed with either continual heat treatment or fractionated doses of heat combined with radiation.

All of the factors mentioned above should be considered in relation to heat combined with chemotherapeutic agents, electron-affinic compounds, and agents which affect the membrane. As Dr. Yatvin will discuss, agents such as anesthetics, which affect the membrane, have a dramatic effect in sensitizing cells to heat. Reports in the literature also indicate that...
electron-affinic compounds cause dramatic heat sensitization of hypoxic cells.

As cell kinetics is thought to be important in radiation therapy and combined radiation and chemotherapy, cell kinetics also is probably important for hyperthermic treatments alone or when combined with X-irradiation. For example, delays in the cell cycle are very long following heat treatment and can have definite effects on the redistribution of viable cells in the cycle. Thus, in fractionated therapy involving heat alone or heat combined with radiation or chemotherapy, the perturbations in cell kinetics may be important and should be investigated in in vitro systems.

To understand the mechanism for hyperthermic effects observed at the cellular level, molecular studies should be carried out. These studies should compare thermal effects from water-bath heating with those observed from ultrasound, radio-frequency currents, or microwave heating. Carefully designed experiments should determine if, indeed, there are nonthermal effects associated with ultrasound, radio frequency, or microwaves. Membrane changes should be investigated and correlated with survival, and a distinction must be made between reversible and irreversible membrane changes leading to cell lethality and other secondary effects. For example, there may be both secondary and primary effects at the level of chromatin involving DNA and the chromosomal proteins. Effects on replication, breaks in the DNA, base damage, and denaturation of repair enzymes and structural proteins should be investigated in an attempt to identify molecular lesions leading to chromosomal aberrations, mutations, and sister chromatid exchanges. In these studies, an attempt should be made to relate molecular lesions with the mode of cell death and the phase of the cell cycle during which death occurs.

In my opinion, the most important studies to be carried out are those involving hyperthermic effects in animals, specifically effects on tumors and normal tissues. These studies should be carried out with both regional and whole-body heating of small animals, preferably those carrying nonimmunogenic tumors, and of large animals carrying spontaneous tumors. For these studies, good heating equipment and good thermometry will be needed in order to produce adequate uniformity in heating and to determine temperature distributions in both normal tissues and tumors. For example, a recent study indicated that water-bath heating was more effective than ultrasonic heating, but the difference was due apparently to the nonuniform temperature distribution in the tumor following ultrasonic heating. In these studies, as for the in vitro studies, investigations should involve heat alone; heat combined with X-irradiation; and heat combined with chemotherapy, electron-affinic compounds, and agents known to affect membranes. Also, different sequences between heating and the other agents should be investigated.

In these animal studies, the important parameter to investigate is therapeutic gain, in which the effect on the tumor is compared to the effect on normal tissues. As Dr. Gillette presented previously, the effects can be plotted in terms of percentage of effect on the ordinate versus dose of X-irradiation on the abscissa. The important parameter to investigate is whether hyperthermia will shift the curve for tumor cures more than it shifts the curve for effects on normal tissues. Ideally, the values plotted on the ordinate should be the percentage of tumors cured and the percentage of animals manifesting deleterious late effects in normal tissues. The effects of fractionated doses involving hyperthermia combined with radiation should be emphasized.

The therapeutic gain factor is defined as the thermal enhancement ratio for tumor divided by the thermal enhancement ratio for normal tissue, when determined at the same temperature. The thermal enhancement ratio is defined as the dose of X-irradiation alone divided by the dose of X-irradiation combined with hyperthermia necessary to produce a given biologic effect. Since the dose-response curve for radiation alone may have a different shape than that for heat plus radiation, the therapeutic gain factor should be determined over the same dose range for tumor and normal tissue. Also, the therapeutic gain factor should be determined for a number of tumors and normal tissues treated with fractionated regimens of heat and X-rays delivered with different sequences. In some practical situations, currently in use, the observable normal tissue (for example, skin) is deliberately cooled so that it is at a lower temperature than the tumor. In this case, the concept of therapeutic gain factor is not applicable. In its place, the term "therapeutic efficacy" may be used, which refers to the response of the tumor relative to the response of normal tissue under conditions where a temperature differential may exist between the two. Thus, an important question to answer is whether the therapeutic gain factor will be greater than or equal to 1 when the tumor and normal tissues are at the same temperature. If these studies in animals indicate that the therapeutic gain factor will not be greater than 1, then we will know that heating techniques must be developed to raise the tumor to a higher temperature than that of the limiting normal tissue. For such differentials in temperature distributions, the physiology involving metabolic rates and blood flow in normal tissues and tumors may be particularly relevant and should be carefully investigated.

In summary, hyperthermia combined with irradiation or chemotherapy will be of value only if the hyperthermic treatment results in an increase in therapeutic gain or therapeutic efficacy. Ultimately, results in vitro and in small and large animals should lead to the design of rational clinical trials to determine if hyperthermic treatments will provide an improvement in the therapeutic index, defined as the percentage of a group of treated cancer patients who remain both free of recurrence and free of severe complications for a given period.
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