An Update on the Anticancer Effects of a Combination of Chemotherapy and Hyperthermia

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Abstract

Hyperthermia combined with chemotherapy is a complicated and poorly understood area, but one that portends great biological and clinical interest. The interrelationship of heat to vascular supply of tumor and normal organs that metabolize chemotherapy agents is complicated. The evocation of thermotolerance by chemical agents is a largely unexplored area. A class of chemotherapy agents that increase in cytotoxicity linearly with increasing temperature are the alkylating agents cisplatin, mitomycin C, and mitoxantrone. A class of chemotherapy agents that increase in cytotoxicity only after a threshold temperature above 42°C are Adriamycin, bleomycin, and actinomycin D. Time sequencing of heat and Adriamycin affects cytotoxicity, implying a membrane effect of the heat and drug interaction. A new class of drugs thought not to be chemotherapy drugs because they are not cytotoxic at 37°C but are cytotoxic at >41.5°C provides a new area of research. Chemotherapy agents that do not change in cytotoxicity with heat include the Vinca alkaloids, amscarcine, and 5-fluorouracil. In order to examine the complex interaction of heat and chemotherapy agents, whole animal models of tumor and normal tissue effects are extremely important.

Introduction

Chemotherapy combined with systemic hyperthermia is a logical and potentially powerful treatment for metastatic disease. The combination of chemotherapy with local and regional hyperthermia is a promising potent therapeutic possibility. To use these modalities successfully, however, requires a clearer understanding of their biological interaction.

The biological interaction of hyperthermia and radiation in tumor and normal tissue can be synergistic, but the therapeutic interaction is complex. The combination of heat and chemotherapy can also be synergistic (3, 5, 13), but the interactions with different agents may be more complicated, and there is less known about the biology.

All chemical agents potentially interact with heat in diametrically opposite ways. Either the compound can evoke a cellular protective mechanism that results in the production of heat shock proteins that correlate with cellular thermal tolerance (28), or the compound acts as a cellular toxin when combined with heat (24). Heat-drug time sequence may partially dictate how the chemical compound will affect the cell.

Heat-Drug Time Sequence

Heat-drug time sequencing affects the cytotoxicity of the combination because of the evocation of thermal tolerance and because of changes in cellular metabolism (12), cell membrane permeability, or membrane transport (13). These factors potentially allow for a differential effect on tumor and normal tissue. In addition, drug pharmacokinetics may change, with heat affecting drug metabolism and excretion.

Cellular susceptibility to heat damage is affected by nutrient state (25) and by the pH (9), and these conditions rely, in part, on vascular perfusion, which is itself affected by heat. Heat can cause capillary stasis but before stasis occurs the first physiological response to heat opens the capillary cascades to the skin, and at the same time decreases visceral blood flow. A change in liver or kidney perfusion changes drug metabolism and excretion. The changes in capillary blood flow also change distribution of the drug to tissue. While the studies documenting changes in visceral perfusion are of whole-body hyperthermia at 42°C (23), it is apparent that regional hyperthermia can also change perfusion patterns by the body's homeostatic attempt to dissipate heat.

There are very little data available on heat-drug sequencing and little detailed information on the effect of differing temperatures between 41.5 to 45°C on heat-drug interaction (2). There is sparse information about the effect of differing temperatures on the physiology and metabolism of the known chemotherapy agents.

A most fascinating new area of investigation is opened by the emergence of a whole new group of chemical agents that are cytotoxic with heat. These agents were not thought of as chemotherapy agents previously because they are not cytotoxic at normal temperatures (8, 21, 22, 24).

Heat-drug sequencing is important in affecting the biological interaction of heat and drug through several interrelated mechanisms. Heat changes the vascular perfusion of tumors as well as the perfusion of normal organs (23, 36). Heat changes capillary blood flow to normal organs because of physiological homeostatic mechanisms of the organism to dissipate heat. This occurs in response to regional heat applications and certainly to systemic heat. A decrease or cessation in tumor perfusion decreases or ends drug delivery to tumor if the chemotherapeutic agent is given after vascular collapse occurs in response to overwhelming heat. Decreased drug uptake by tumor can increase the quantity of the agent delivered to normal tissue, potentially increasing normal tissue toxicity. Changes in perfusion to liver and kidney will change the metabolism and excretion of the drug. This can change activation of the parent compound to an active drug moiety but can add to toxicity by causing a persistence of active drug in the circulation by slowing metabolic degradation and excretion. To study the bottom line effect of heat and drug on tumor and normal tissue requires whole animal models with pharmacokinetic, toxicity, and tumor studies.

Pharmacokinetics of Heat-Drug Interaction

A second interaction with heat and drug is a change in metabolism of the drug by hepatic and tumor microsomal processes
by temperature that can affect the transformation of the parent compound to an active metabolite or the inactivation and excretion of the active moiety by the liver or kidney. This potential pharmacokinetic alteration has been examined only in several instances. Cyclophosphamide is a monofunctional alkylating agent that requires hepatic microsomal metabolism to an active alkylating moiety. This cytotoxic metabolite, once produced, is also inactivated in the liver and then excreted by the kidney. Ostrow et al. (33) compared the pharmacokinetics of cyclophosphamide and systemic hyperthermia to 41.8° with cyclophosphamide at normal body 37° temperature and showed that hyperthermia increased the urinary excretion of unmetabolized cyclophosphamide and decreased the urinary excretion of alkylating activity. The comparative clinical results of these balanced pharmacokinetic changes were not described. The effect of heat on microsomal and hepatic slice metabolism of parent compound to active compound showed a cessation of microsomal metabolism of cyclophosphamide with heat but a continuation of liver slice metabolism of the drug (7). The importance of this information would be clarified with an animal tumor model (18).

Mimnaugh et al. (32) looked at the effect of whole-body hyperthermia on Adriamycin disposition and metabolism in the rabbit. Adriamycin was given by i.v. bolus at the start of a 1-hr exposure to 42.3° or to control animals at 37°. They showed minimal effects on the tissue distribution, metabolism, and excretion of Adriamycin, even though the distribution of Adriamycin to peripheral tissues occurred more rapidly. They did demonstrate increased tissue concentrations of Adriamycin and its metabolites in skeletal muscle and duodenum but surprisingly not in cardiac tissue, although cardiac toxicity was increased.

**Classification of Heat-reactive Agents**

While our information about the interaction of heat and chemotherapy remains severely limited, there are more data to consider than existed several years ago. While much information is rudimentary observation, there also exists some elegant elucidation of effect.

Hahn has pointed out the pitfalls of working with drugs in *vitro* that have certain implications in vivo. Some drugs interact with glass or plastic, changing their effect. Other drugs exist in excess intracellularly while the affected cell dies and, when the dead cells lyse, more drug is released to surrounding cells to increase overall cell kill (11).

The solvents of certain drugs, such as ethanol, may also be cytotoxic with heat (27). Also, these solvents can elicit thermal tolerance in either the tumor or the normal tissue by targeting the heat shock proteins, as described by Li et al. (28), and may induce thermal tolerance (24). Finally, drugs may be unstable and decay rapidly with increased temperature (2).

Some of the most sophisticated work on the interaction of heat and chemotherapy are the *in vitro* experiments by Hahn and others. Hahn describes 4 classifications of chemical agents by their interaction with heat.

The first of Hahn's chemical categories are the chemotherapy agents that undergo a linear increase in cytotoxicity with increasing temperature. Agents of this category are the alkylating agents. A linear increase in biological effect with heat is characteristic of the simple alkylators such as thiotepa (19), as well as the bifunctional alkylators such as the nitrosoureas (20). Other agents that also show a linear increase in cytotoxicity with increased heat are mitoantrone mitomycin C and cisplatinum (3, 15, 23, 31). Agrawal and Srivasan (1) have shown that heat increases in linear fashion the formation by hydrolysis of an intermediary moiety of the nitrosoureas, which more rapidly alkylates DNA with a temperature of >41°. The *in vitro* synergism of heat and thiopeta was demonstrated in vivo by Marmor using local microwave-induced hyperthermia to 43° for 30 min simultaneously with thiopeta in an *in vivo* model. She also demonstrated the synergetic effect of local hyperthermia with the nitrosoureas (29).

A second classification of heat-interactive chemotherapy agents is drugs that do not exhibit a linear increase in cytotoxicity with heat but show a threshold temperature effect. These agents in *vitro* exhibit little change in cytotoxicity above 37° until a threshold of 42 to 43°, when a synergistic interaction occurs. The drugs of this category include Adriamycin, bleomycin, and actinomycin D (4, 11, 12).

In the example of hyperthermia with Adriamycin, there is a time dependency requiring simultaneous heat-Adriamycin exposure for synergism (14). Different cells exhibit a different response to heat and Adriamycin. EMT6 cells show a temperature threshold effect (10) but little time dependency, whereas, if Chinese hamster ovary cells are exposed to heat prior to Adriamycin, the cytotoxic effect of Adriamycin is reduced (14). This is information that may predict a therapeutic gain factor. It has been demonstrated that intracellular Adriamycin concentration increases with simultaneous heat exposure of 43°. This increased concentration correlates with cytotoxicity and may be an increased inward flux of Adriamycin or may represent a block of Adriamycin efflux from the cell. On the other hand, heating the Chinese hamster ovary cells prior to Adriamycin exposure decreases Adriamycin concentration intracellularly. A hypothesis about this time sequence effect describes membrane permeability or transport as critical to the Adriamycin-heat toxicity. The mechanism of Adriamycin cell kill is thought to be DNA intercalation; however, a DNA effect of Adriamycin may not be critical, because Tritton and Yee (37) have shown that Adriamycin is cytotoxic, even when the drug is kept extracellularly and does not penetrate the cell membrane. Therefore, by implication, Adriamycin can kill cells by an effect on the cellular membrane. It is likely that prior heat exposure changes the ability of the membrane to mediate cytotoxicity by Adriamycin by interfering with this direct membrane-Adriamycin interaction. Similar examination of the time sequencing effect of heat and bleomycin or heat and actinomycin D have not been described.

**In vivo,** Overgaard (34) has demonstrated that simultaneous heat plus Adriamycin increases Adriamycin antitumor effect at 41.5° in a rat model. An explanation of this effect compared to the *in vitro* temperature dependency described by Hahn is not clear. Marmor et al. (30) showed no therapeutic advantage and, from the work of Mimnaugh et al. (32) and clinical observations (6), Adriamycin with heat appears to increase cardiac toxicity at 42°.

The third group of heat-interactive drugs is perhaps the most fascinating. These are drugs that have no cytotoxicity at 37° but that become cytotoxic at higher temperatures. Cysteamine, amphotericin B, and lidocaine are examples (11, 21, 35). The existence of this group of agents is newly recognized and represents a great unexplored resource.
It would be useful to understand the mechanistic interaction to predict a classification of agents that interact with heat to become cytotoxic. Clearly, some agents are directly cytotoxic with heat (26), and others act to potentiate cytotoxicity of known chemotherapy agents within the milieu of heat. Glucose, presumably by increasing tumor lactic acid production leading to decreased tumor pH, increases the therapeutic gain of cyclophosphamide and regional heat to 41.5°C in a murine fibrosarcoma (38). An example of another potentiator is superoxide dismutase, which increases tumor regression in an in vivo rat model when combined with 42°C heat plus cisplatinum.2 Similarly, paparavine increases the therapeutic gain of chemotherapy in a murine neuroblastoma model (39). Quercetin causes hyperthermia cytotoxicity presumably by decreasing tumor cell pH by inhibiting lactate transport (22).

There is a fourth group of chemotherapy agents that have no change in cytotoxic effect between 37 to 45°C. These agents include the antimetabolites, methotetate, 5-fluorouracil, amasa-crine, and the Vinca alkaloids vincristine and vinblastine (11, 15, 16).

The implications of the cytotoxic heat interaction are potentially of great importance. While several agents have been documented, there are obviously a host of agents that have not been examined at physiologically elevated temperatures. While the cytotoxic effect must be examined in normal tissue versus tumor tissue, still, this array of potential antitumor agents with heat is an extremely important potential tool.

In discussing the interaction of heat and chemotherapy, the concept of a therapeutic index is of basic importance. As in radiation therapy or in the clinical application of combination chemotherapy, a cancer is successfully treated by using an agent or a combination of agents that cause greater tumor injury than injury to normal tissue. This concept results primarily to the exploration of the interaction of heat with radiation or chemotherapy. A therapeutic index is referred to in radiation and hyperthermia biology as the therapeutic gain factor. Therefore, while the major data and often sophisticated work has been done combining heat and chemotherapy in vitro, the information must be examined further in an in vivo animal model that allows an evaluation of the interaction of heat and chemotherapy index on tumor and normal tissue (17, 22, 38, 39).2 Such fascinating phenomena as the evocation of heat shock proteins and their association with thermotolerance must be appreciated and possibly used to widen the toxicity gap between tumor and normal tissue. This concept has not been fully examined in relation to normal versus tumor tissue in the investigation of heat-chemotherapy effect. The field of hyperthermia and chemotherapy is extensive and is only beginning to be explored. A wealth of unrealized biological information and clinical therapy awaits exploration.

References

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Cancer Res 1984;44:4853s-4856s.

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