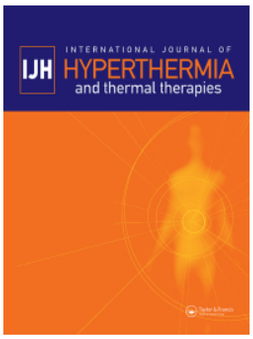

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Re-setting the biologic rationale for thermal therapy

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Abstract
This review takes a retrospective look at how hyperthermia biology, as defined from studies emerging from the late 1970s and into the 1980s, mis-directed the clinical field of hyperthermia, by placing too much emphasis on the necessity of killing cells with hyperthermia in order to define success. The requirement that cell killing be achieved led to sub-optimal hyperthermia fractionation goals for combinations with radiotherapy, inappropriate sequencing between radiation and hyperthermia and goals for hyperthermia equipment performance that were neither achievable nor necessary. The review then considers the importance of the biologic effects of hyperthermia that occur in the temperature range that lies between that necessary to kill substantial proportions of cells and normothermia (e.g. 39–42°C for 1 h). The effects that occur in this temperature range are compelling—including inhibition of radiation-induced damage repair, changes in perfusion, re-oxygenation, effects on macromolecular and nanoparticle delivery, induction of the heat shock response and immunological stimulation, all of which can be exploited to improve tumour response to radiation and chemotherapy. This new knowledge about the biology of hyperthermia compels one to continue to move the field forward, but with thermal goals that are eminently achievable and tolerable by patients. The fact that lower temperatures are incorporated into thermal goals does not lessen the need for non-invasive thermometry or more sophisticated hyperthermia delivery systems, however. If anything, it further compels one to move the field forward on an integrated biological, engineering and clinical level.

Keywords: *Thermal dose, clinical trials, hypoxia, hyperthermia biology*

Introduction

Although there have been references to the use of heat to treat human cancers, dating back to the writings of Hippocrates, the modern discipline of thermal therapy emerged from a number of radiation biology oriented laboratories in the mid-to-late 1970s.

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Leading scientists placed great emphasis on quantitative evaluation of the cytotoxic effects of elevated temperature [1, 2]. Striking consistencies were observed in the temperature dependence of cell killing rate between cell types as assessed *in vitro* or in similar analyses of tissue tolerances *in vivo* [3]. Two key papers, published in the mid-1980s, attracted attention to the opportunity to assess efficacy of cell killing with hyperthermia [4, 5]. These papers established the first concepts for thermal dosimetry and indicated that significant cell killing could occur if cells or tissues were heated to >42°C for 1 h or more. Consequently, clinical thermal goals and equipment performance design criteria [6] focused primarily on achieving cytotoxic temperatures.

Although the biology studies were successful in jump-starting the emerging field of thermal therapy, they had unintended consequences that stymied hyperthermia equipment design, led to un-optimized scheduling and sequencing of heat and radiation in thermo-radiotherapy trials. Overall, enthusiasm for thermal therapy waned significantly in the mid-to-late 1990s, partly as a result of the perceived difficulties in achieving adequate treatment as defined by the need to kill cells directly by heating [7]. The problem that was faced by the hyperthermia community at that juncture was multi-fold—starting with unrealistic thermal goals. Fueling the fires of frustration, however, were lack of what was perceived to be adequate equipment for delivering hyperthermia treatment and inability to measure the treatment delivered. The result of these problems led to inability to design clinical trials that could adequately test thermal dosimetry questions (Figure 1).

Importantly, powerful, yet subtle effects of mild temperature hyperthermia (MTH; herein defined as 39–42°C for 1–2 h) on tissues were largely ignored, until recently. It is now known that cytotoxic temperatures are only achieved in small sub-volumes of tumour during typical hyperthermia treatments with currently available heating technology (except with thermal ablation). However, the more subtle effects of MTH, including heat-mediated tumour reoxygenation [8–11] and inhibition of sub-lethal and potentially lethal damage repair [2, 12–15], provide very strong rationale for MTH when combined with radiotherapy. Additionally, physiological and cellular effects of MTH can improve nanotechnology-driven drug vehicle delivery [16, 17], activate promoters for heat-mediated

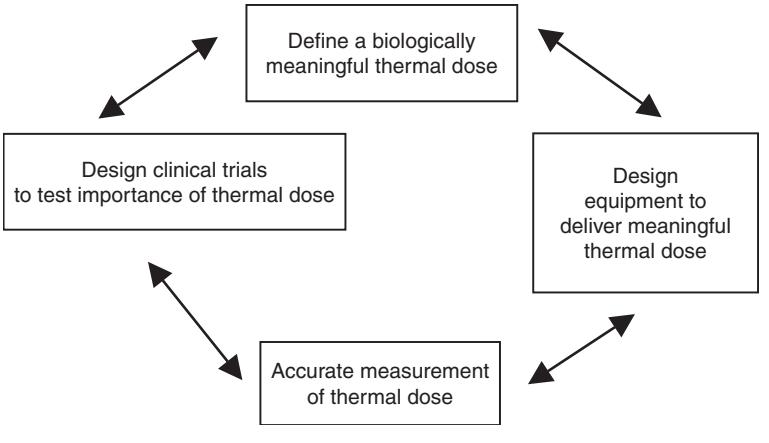

Figure 1. Challenges to development of hyperthermia. As described in the text, historical obsession with the perceived need to cause thermal cell killing led to unrealistic clinical goals that could not be met with available equipment. Additionally, constraints were placed on how to combine hyperthermia with radiotherapy that did not take full advantage of heat induced inhibition of DNA damage repair and reoxygenation.

gene therapy [18, 19] and augment immune responses to tumours via a variety of mechanisms that are discussed elsewhere in this Special Issue. Emphasis in the future should focus on these and as yet to be defined other effects of MTH. It is important to note that the biology of MTH is equally important to those involved in thermal ablation, as the thermal gradients at the margin of ablated tissue fall into the MTH range [20].

Two recently completed thermoradiotherapy trials point to the importance of MTH in controlling treatment outcome. In both trials, the 10th percentile of the temperature distribution (T_{90}) averaged between 39–40.5°C [21, 22]. Nevertheless, a 10× escalation of the cumulative thermal dose, as expressed in cumulative equivalent minutes at 43°C at the T_{90}, led to significant improvements in thermoradiotherapy outcome in both trials. The dosimetric term used in this context, CEM43°T_{90}, was originally conceived by Paul Stauffer at the University of California San Francisco [1].

The results of these two trials provide a powerful reminder of the relative importance of MTH in cancer therapy. Furthermore, they provide scaffolding for development of more sophisticated thermal dosimetry methods and they legitimize this form of therapy as being quantifiable. It is important to note that the thermal dosimetry for thermochemotherapy trials may be different, as different temperatures may be necessary to achieve the desired consequences. Additional trials are needed to further clarify these issues for both thermoradiotherapy and thermochemotherapy.

Unintended consequences of the directive to kill cells with heat

In 1984, Eric Hall made the following statement, which at the time was the mantra for thermotherapy ''Although biology is clearly on our side, the picture is considerably different for the physics of localized hyperthermia, the basic principles of which appear to be against us'' [23]. The original quote has often been shortened to 'The biology is with us and the physics is against us'. The comment came largely from four observations. First, it was possible to kill cells with heat, particularly when temperatures were in excess of 42°C for 1 h or more [1]. Secondly, heat killed cells that were preferentially resistant to radiation, such as cells in the S-phase of the cell cycle and hypoxic cells [2]. Thirdly, heat-induced significant thermoradiosensitization and chemosensitization [2], partly by inhibiting DNA damage repair and, fourthly, the vasculature of tumours appeared to be more thermally sensitive than normal vasculature, suggesting that tumour tissues would be more thermally sensitive than normal tissues [24]. While all of this seemed attractive, there were negative consequences of the focus on heat induced cytotoxicity.

Clinical consequences

*Hyperthermia prescriptions define therapy goals as reaching temperatures greater than 42*deg*C for periods up to 1 h per session.* A cursory examination of a 15 phase I/II clinical trial report conducted during the late 1970s and mid-1980s was done [25–39]. All of the trials except one had a thermal treatment goal of >42°C for 30–60 min. Half stipulated that $T > 43$°C. During this period of time the number of thermometry sensors was low (typically 1–4 points), so the true adequacy of heating was not accurate (with the exception of the report by Shimm et al. [39]). Often times, single probes were placed in the centre of or at the base of the tumour. The actual temperatures achieved were not reported in six papers. Of the remaining nine, three reported success in achieving the stated thermal goal. These reports, combined with two negative RTOG phase III trials in which adequate heating was rarely

if ever achieved [40, 41], seriously wounded the reputation of thermal therapy as being a quantifiable therapy that could be delivered precisely and accurately.

Heat treatment should be given only twice per week. The rationale for this recommendation came from fears of thermotolerance. When cells are heated to temperatures >43°C for periods of 30–60 min, many cells are killed, but the ones that survive acquire resistance to heat killing during the period after the heating is completed [42]. The higher the initial temperature, the greater the degree of thermotolerance is induced in surviving cells. Thermotolerance decays back to baseline thermosensitivity, but it requires a few days [43]. Thus, prescriptions for most clinical trials stipulated no more than two fractions of heat per week to avoid retreating when tissues were thermotolerant. Interestingly, Dewey [42] postulated in 1984 that thermotolerance would not be important when heat was combined with typical radiation fraction sizes of 2 Gy. He stipulated that caution should be used for larger doses per fraction. Nevertheless, the accepted convention was that heat should not be given more than twice per week, despite the strong biological rationale for taking advantage of thermoradiosensitization with every fraction of radiation. This tradition has been carried on in trials conducted at Duke University Medical Centre over the past 20 years [21, 44] and at nearly all other centres where hyperthermia is practiced. What is important in this context is that inhibition of DNA damage repair can occur at temperatures as low as 40°C, a temperature where thermotolerance is not going to be induced during or after heating [12]. This argues strongly for combining hyperthermia with more fractions of radiotherapy.

Vascular damage in tumours leads to transient hypoxia. Therefore, never give hyperthermia treatment before a radiotherapy fraction. It is now known that MTH actually causes tumour reoxygenation, whereas higher temperature heating leads to vascular damage and hypoxia (Figure 2). The reoxygenation that occurs during and after MTH lasts up to 24 h post-treatment and can, therefore, influence the efficacy of at least two radiation fractions—one given the day of hyperthermia treatment and one 24 h later [8–11]. The majority of the phase I/II trials referred to above administered hyperthermia after radiation and, therefore, did not take full advantage of reoxygenation. It is very clear from this discussion that it is better to give hyperthermia before radiation to take advantage of reoxygenation effects on the day of heating as well as the day after heating.

Effects on equipment design criteria

The goal of achieving $T > 43$°C also influenced design criteria for hyperthermia equipment [6]. In the early and mid-1980s, a dozen or more companies were established to manufacture hyperthermia equipment for clinical use. Some larger companies, such as Varian, were briefly invested in the idea as well [45, 46]. However, the perceived difficulties of achieving what was considered therapeutically effective temperatures, combined with technical difficulties of performing hyperthermia in general and lack of adequate reimbursement led to loss of commercial interest in the technology [7]. Today, only a handful of companies survive from that era.

Redefining thermal goals for hyperthermia treatment

Following the myriad of early clinical trials in which only a few temperature measurements were made, methods were developed for invasive thermometry that permitted multiple

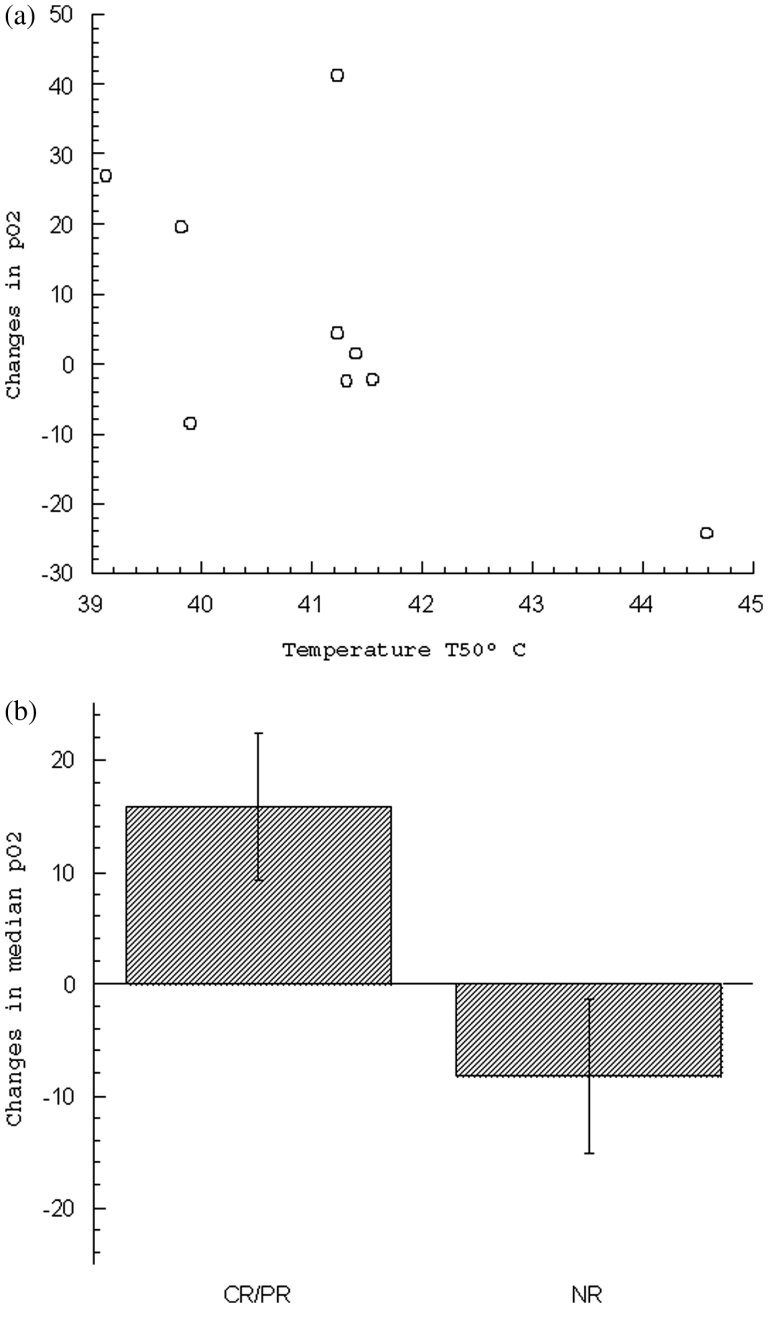

Figure 2. Effects of mild temperature hyperthermia on tumour oxygenation, 24 h post-treatment. These results are from a phase I/II trial that examined the feasibility of performing neoadjuvant thermochemoradiotherapy for locally advanced breast cancer. (a) Median temperatures required to cause reoxygenation at 24 h post-treatment were below 42°C. (b) Reoxygenation at 24 h after the first heat treatment was associated with greater likelihood for achieving a response to treatment. Data reproduced from Jones et al. [9] with permission of the author and publisher.

measurements during treatment. These were based largely on the idea of using multi-sensor probes or single sensor probes that can be moved inside a pre-placed catheter that is placed inside a tumour during treatment [47]. Guidelines for acquisition of multi-point thermometry data were published several years ago [48–53] and are in the process of being updated, under sponsorship of the thermal therapy societies in the US and Europe.

A cadre of clinical investigators has focused on the concept that quantification of thermal exposure is important for the validation of hyperthermia. The recognition that temperatures during heating are inevitably non-uniform led to the concept of using a descriptor of the temperature distribution, such as the median, minimum or a percentile of the distribution, such as the 10th percentile (often referred to as T_{90}). The biological consequence of the treatment, as described by cell killing, is a product of the temperature and time of exposure [3, 4]. Gerner [54] suggested that one consider using time above a threshold temperature as a descriptor. This unit has been evaluated with respect to the T_{90} (e.g. cumulative minutes that T_{90} exceeds a temperature of 40°C) and it is associated with treatment outcome in superficial tumours and soft tissue sarcomas, based on retrospective analysis of phase II clinical trial data [55, 56]. However, this is not a controllable parameter, since it is difficult to guarantee that a T_{90} can be maintained above an arbitrarily defined threshold. Under this system, a T_{90} of 39.9°C would be considered treatment failure, whereas a T_{90} of 40°C would be considered a success. This arbitrary distinction of failure vs. success does not make biologic sense.

The original formulation of Sapareto and Dewey [4] combined temperature and time together by converting all time-temperature histories into an equivalent number of minutes at 43°C (CEM43°C). The details of how this conversion was done are not discussed herein, but are examined in detail elsewhere [3]. Using this formulation, success or failure is not based solely on a temperature threshold. Several investigators used this formulation in retrospective analyses of phase II/III clinical trial data and showed that higher CEM43°C values are correlated with superior treatment outcome [44, 57–62]. Various descriptors of the temperature distribution were used in these analyses (i.e. CEM43°C$T_{\min}$, CEM43°CT_{90}, CEM43°CT_{50}, etc.; where $T_{\min}$ = minimum measured temperature and T_{50} = median measured temperature).

It is interesting to recognize that the first demonstration of the prognostic power of CEM43°C$T_{\min}$ was published by these authors over 20 years ago, following completion of a phase III trial in pet animals with spontaneous tumours. A hazard ratio of 2.5 was achieved when CEM43°C$T_{\min}$ exceeded 20 min, as compared with a radiation alone control group [57]. The value of the CEM43°C dose unit has been demonstrated in retrospective analyses of other human phase III clinical trials that compared thermoradiotherapy vs. radiotherapy [58, 59]. However, to truly test the value of CEM43°CT_{90} or $T_{\min}$ as a thermal dosimetric parameter, it is necessary to prospectively control the parameter and determine whether its escalation leads to improved tumour response. Oleson et al. [44] evaluated phase II human clinical trial data from Duke and predicted that a 10-fold difference in CEM43°CT_{90} would be needed to distinguish differences in treatment outcome between two thermal doses delivered in combination with radiotherapy.

This analysis led to the design and completion of two pivotally important clinical trials—one in superficial human tumours and the other, ironically, in canine soft tissue sarcomas. Multi-point invasive thermometry was used in both trials to assess T_{90}. The trial design was critically important for both, in that it involved a test heat treatment to determine whether a tumour was 'heatable' or not. If the tumour was deemed 'heatable', then the patient was randomized to a low thermal dose or a high thermal dose group. The definition of 'heatable' was based on whether the prescribed CEM43°CT_{90} could be

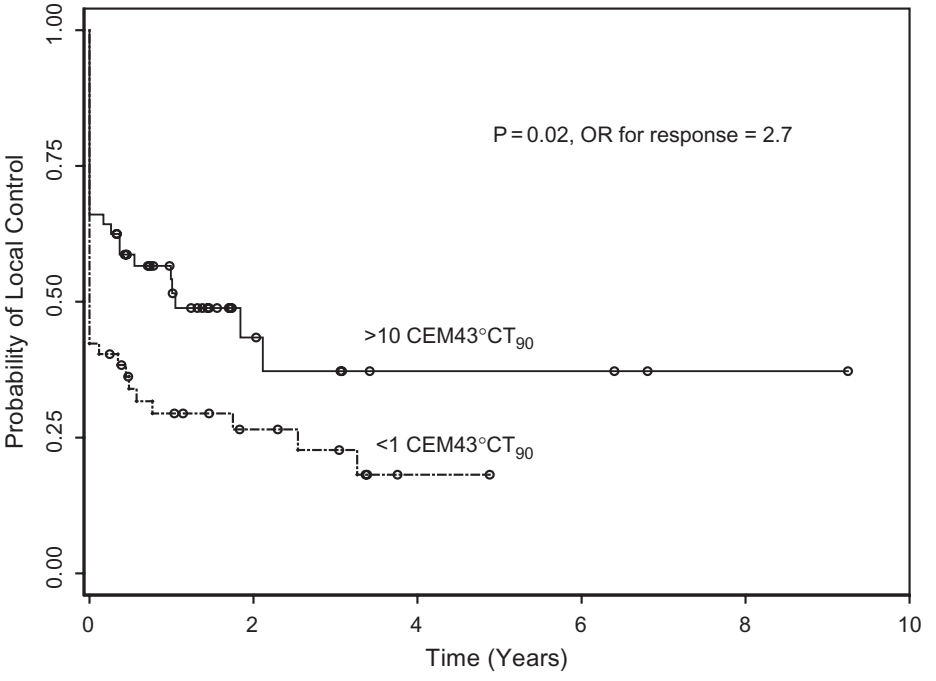

Figure 3. Summary of results of phase III trial of human superficial tumours treated with different thermal doses in combination with radiotherapy. Probability for local control as a function of time post-treatment shows that higher thermal dose, defined as Cumulative Equivalent Minutes at 43°C at the 10th percentile of the temperature distribution (CEM 43°C T_{90}), yields higher likelihood for achieving a complete response. Data modified from a paper by Jones et al. [21], with permission of the author and publisher.

achieved in five or 10 fractions of heat, delivered over no greater than 120 min per fraction for each treatment. If the tumour was deemed not 'heatable', then the patient went on to receive other treatments and was not enrolled onto the protocol. About 10% of patients on both trials were not randomized because their tumours were deemed unheatable.

The main difference between the two trials was the definition of low vs. high thermal dose. In the human trial, patients randomized to the low thermal dose group received only the test heat treatment with a target CEM43°CT_{90} between 0.5–1 min. The high dose group received a CEM43°CT_{90} between 10–100 min. To achieve the high dose, patients could receive up to two fractions of hyperthermia per week for up to 120 min each. In the canine trial, the number of hyperthermia fractions was five, scheduled once per week. The low dose group received treatment over a shorter period of time, per treatment, than the high dose group, where the target CEM43°CT_{90} was 2–5 and 20–50 min, respectively.

Key results of the human trial are shown in Figure 3. In both trials, the high thermal dose group achieved better outcome than the low dose group. The odds ratio for difference in response in the human trial was 2.7 ($p = 0.02$) and the hazard ratio for duration of local control in the canine trial was 2.8 ($p = 0.023$) [21, 22]. These two trials represent the first demonstration that prospective control of thermal dose affects outcome of thermoradiotherapy. Importantly, they provide a roadmap for eventual achievement of quantifiable thermal dosimetry on a more widespread basis.

Table I. Summary of key thermal parameters from two prospective randomized phase III clinical trials comparing the efficacy of low *vs.* high thermal doses when combined with radiotherapy. Data abstracted from Jones et al. [21] and Thrall et al. [22].

	Trial			
Temperature parameter (°C)	Canine		Human	
Dose arm	Low	High	Low	High
T_{50}	41.7*	43.2	40.7	41.1
T_{90}	39.6*	40.5	39.4	39.7

* Significantly different from high thermal dose arm.

Temperatures achieved in the phase III randomized trial comparing thermoradiotherapy to radiotherapy alone for locally advanced cervix cancer were in the same range as reported for both of these prospective trials. In a recent follow-up phase II trial of trimodality therapy for locally advanced cervix cancer, T_{50} values averaged $40 \pm 0.8^{\circ}$C [63] and maximum temperatures averaged $41.3 \pm 0.8^{\circ}$C (Van der Zee, personal communication).

Two prior randomized clinical trials were published previously where the intent was to compare the efficacy of differences in thermal dose when combined with radiotherapy. In both of these trials, the 'dose' was controlled by varying the number of hyperthermia treatments. Neither trial showed an improvement in response rates of superficial tumours treated with more fractions of hyperthermia (2 vs. 6 and 4 vs. 8 fractions were compared [64, 65]). Although the number of hyperthermia fractions was varied in these trials, there was no *a priori* attempt to control the temperature distributions, as was done in the two recently reported trials.

Tying clinical results back to underlying biology

At the outset of this review, it was stated that thermal goals set for thermal therapy in the early 1980s were too focused on thermal cytotoxicity and that this unrealistic expectation led to clinical goals that were not achievable and in many ways were counterproductive for optimizing thermoradiotherapy. So, it is perhaps confusing to now advocate thermal isoeffect dosimetry, which is derived from the kinetics of cell killing as a function of temperature.

What is important here is to go back to the actual thermal data and see what types of temperatures were actually achieved in these two trials (Table I). Note that the T_{90} values achieved in both trials, irregardless of treatment arm, were not expected to be very cytotoxic. However, there are other effects that are temperature dependent. For example, it has been shown that thermal radiosensitization is temperature dependent and that the slope of the Arrhenius plot for thermal radiosensitization is similar to that for thermal cell killing [66]. It is possible that temperatures in this range may have altered oxygen consumption rates, thereby reducing oxygen demand and increasing oxygenation. Data from Vujaskovic and Song [11] suggest that the degree of reoxygenation achieved increases with increasing temperature, up to the point where vascular damage is created.

In the canine trial, duration of heating was inversely related to outcome, independent of treatment arm in multi-variate analysis. In other words, higher thermal dose was better than lower thermal dose, but within either dose arm increasing duration of heating was inversely related to outcome. It was surprising to find this, since longer durations of heating generally deliver higher thermal doses. It is believed that this effect was created by situations

where there were large thermal gradients. In these cases, maximum temperature was near the upper limit allowed by protocol, whereas the T_{90} temperature was relatively low. When T_{90} values were relatively low, longer periods of heating were needed to achieve the prescribed T_{90} dose. Because the T_{max} values were high in these particular sub-sets of subjects, thermal damage resulted, thereby leading to vascular damage, hypoxia and radio-resistance. Clearly, more work needs to be done to understand this potentially complex scenario that may involve thermally induced physiologic changes that can favour or disfavour improved treatment outcome, depending upon the nature of the temperature distribution.

Heat radiosensitization may have played a role in the results of these trials; typically >30 min elapsed between radiation (which was given first) and delivery of heat. The heat radiosensitization effect tends to fall toward baseline within 60–120 min, based on pre-clinical murine data [67].

A look toward the future

Even though this study has achieved perhaps a more realistic set of biologic goals for hyperthermia when combined with radiotherapy, there is more work to do to identify how to optimize this therapy and to take further advantage of known biologic effects and those that are as yet unidentified.

The two positive thermal dose escalation trials are an important milestone, but they fall far short of identifying the ideal method for achieving a desired thermal dose. The thermometry needed to prove the merits of increasing dose was extensive and invasive—a scenario that is far from being economically and clinically acceptable. As non-invasive thermometry is used to visualize full three dimensional temperature distributions it is likely that thermal goals will be redefined again and may very well be different for different types of applications. It will be important to maintain open lines of communication between the biologists and those who manufacture equipment as well as those who use it. It is time that realistic thermal goals are defined for future clinical investigations.

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