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THERMAL DOSE REQUIREMENT FOR TISSUE EFFECT: EXPERIMENTAL AND CLINICAL FINDINGS

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Abstract

In this review we have summarized the basic principles that govern the relationships between thermal exposure (Temperature and time of exposure) and thermal damage, with an emphasis on normal tissue effects. We have also attempted to identify specific thermal dose information (for safety and injury) for a variety of tissues in a variety of species. We address the use, accuracy and difficulty of conversion of an individual time and temperature (thermal doses) to a standardized value (eg equivalent minutes at 43 degrees C) for comparison of thermal treatments. Although, the conversion algorithm appears to work well within a range of moderately elevated temperatures (2–15 deg C) above normal physiologic baseline (37–39 deg C) there is concern that conversion accuracy does not hold up for temperatures which are minimally or significantly above baseline. An extensive review of the literature suggests a comprehensive assessment of the “thermal does-to-tissue effect” has not previously been assembled for most individual tissues and never been viewed in a semi-comprehensive (tissues and species) manner.

Finally, we have addressed the relationship of thermal does-to-effect vs. baseline temperature.

This issues is important since much of the thermal dose-to-effect information has been accrued in animal models with baseline temperatures 1–2 deg higher than that of humans.

1.0. INTRODUCTION

The purpose of this review is to present basic concepts relating thermal dose (time at temperature) to cell killing and tissue damage.

This review summarizes the basic principles that govern the relationships between thermal exposure (temperature and time of exposure) and thermal damage, with an emphasis on normal tissue effects. Methods for converting one time-temperature combination to a time at a standardized temperature (cumulative minutes at 43° / CEM) are provided as well as some discussion about the underlying assumptions that go into these calculations. There are few *in vivo* papers, examining the type and extent of damage that occurs in the lower temperature range for hypothermic exposures (e.g. 39–42°C). Although not specifically calculated, the authors believe the CEM analysis for estimating an equivalent thermal does not retain a high degree of accuracy when temperatures above 55°C or so. Therefore it appears that estimation of thermal dose to effect at low (temperatures a few degree above baseline body temperature) and high temperatures are more difficult to assess and quantify. It is also apparent from this review that an extremely large variation in the type and the quality of tissue damage endpoint assessment significantly affects the ability to accurately compare study results.

The authors have assembled a detailed review of thermal thresholds for tissue damage in the majority of organs (based on what is detectable *in vivo*). The data are normalized using thermal dosimeter concepts. This database is available by request but not included in this manuscript for brevity. All of the studies reported are for single acute thermal exposures.

2.0 KINETICS OF CELL KILLING BY HYPERTHERMIA: THE ARRHENIUS RELATIONSHIP

2.1. *In vitro* studies

Numerous *in vitro* studies show that the rate of cell killing during exposure to heat is exponential and dependent on the temperature and length of exposure. The best review is on this topic is from Dewey (1).

A number of authors have used data such as that shown in Figure 2 to determine the heat of activation of cells, by using an Arrhenius analysis

This analysis is done by plotting the rate of cell killing (1/Do; where Do is defined as the number of minutes to reduce survival by 63% on the exponential portion of the survival curve) vs. 1/temperature (°K). Using equation 1, the heat of inactivation can be calculated.

$$K = Ae^{(-E/RT)} \quad \text{Equation 1}$$

Where E = heat of activation in kcal/mole, A is a constant that is assumed to be unchanged over the temperature range studied, R = molar gas constant (1.987×10^{-3} Kcal/mole-°K) and T is the absolute temperature in °K.

The slope of the Arrhenius plot is typically biphasic. The curves typically have a "break point" and the slope tends to be steeper below the break than above it. The activation energy for the temperature range above the "break point" is typically around 120–150 kcal/mole, which is consistent with the heat of inactivation of proteins and enzymes (3). The change in slope of the Arrhenius plot below the breakpoint is generally thought to be related to the

development of thermo tolerance (acquired thermal resistance) during heating (4). When heating is delivered at temperatures above the breakpoint, thermo tolerance does not occur during the heating period. It should be kept in mind, however, that thermo tolerance does develop after heating at temperatures above and below the breakpoint.

Arrhenius plots for human and rodent cells are different in two key aspects. For human cells, the breakpoint appears to be around 43.5°C whereas it is nearer 43°C for rodent cells. Second, the slopes curves for human cells tend to be shallower (smaller) than they are for rodent cells, at any defined temperature. This means that more time is needed to kill human cells at a defined temperature.

The higher breakpoint for human cells is interpreted to mean that human cells develop thermo tolerance at a higher temperature than rodent cells. This is verified by comparing the data in Figure 1a to that in Figure 1b. Note that CHO cells show no evidence for thermo tolerance at 43°C (i.e. change to shallower slope after several hours of heating), whereas there is clear evidence for thermo tolerance for human cells at that temperature. These characteristic differences in the Arrhenius plot between human and rodent cells indicate that human cells are more thermally resistant than rodent cells.

Above the breakpoint of the Arrhenius plot, the rate of cell killing essentially doubles for every degree increase in temperature. This means for a given isoeffect, such as a defined level of survival, the time at temperature needed to achieve that isoeffect is halved for each degree increase in temperature. At temperatures below the breakpoint the rate of cell killing decreases by a factor of 4–6 for every degree decrease in temperature (4). As will be discussed below, there are essentially no human data to establish whether these guidelines relating the rate of cell killing vs. temperature are valid for human tissues, particularly below the breakpoint. Acquisition of more definitive data may be important for establishing guidelines for human thermal exposure.

2.2 In vivo studies

Arrhenius data have also been derived from a large number of *in vivo* studies. In this case, the endpoint is usually the time to reach an isoeffect, at a defined temperature. One of the first attempts to do this type of study was performed by Henriques and Moritz in 1947 (5). A hot water applicator was used to create 2° and 3° burns in human and pig skin, over wide range of temperatures (44–70°C). In a subsequent paper, Stoll and Greene obtained very similar results using more precise thermometry, but over a more narrow temperature range (6). An important point from the Stoll/Green data, is that the threshold for pain is much lower than the threshold for grossly detectable physical injury. However, the isoeffect relationships appear to have the same slope, irregardless of the endpoint (pain, blister, full necrosis (Figure 3a). Note that there is near perfect agreement between the human and porcine data, indicating that the thermal sensitivities of human and pig skin are similar. However, the data for mouse ear necrosis are quite different. The specific activation emerges for skin/ear necrosis in these animals is shown in Table 1.

The most obvious difference is that the time-temperature relationships to achieve mouse ear necrosis are far lower than for the skin of humans / pigs. Second, determination of the

breakpoint for the human data is difficult because the curve appears to be continuously bending. However, good linear fits to the data can be obtained for temperatures between 44 and 47°C and for temperatures between 47 and 55°C. It is not known if there is a breakpoint near 43°C for human skin because there are no data in that temperature range. Moritz reported that heating of human skin to a temperature of 44°C for five hours (equivalent to 600 minutes at 43°C; see section 3.0 for explanation of equivalency) resulted only in mild hyperemia in two subjects. By comparison, heating at the same temperature for six hours (equivalent to 720 minutes at 43°C) resulted in complete epidermal necrosis. To test whether 43°C heating could cause thermal damage to human skin, they would have had to heat the subjects for periods between 600 and 720 min (10–12 hours), which probably explains why it was not done. This *in vivo* data tend to corroborate the *in vitro* studies which suggest that human tissues are more thermally resistant than rodent tissues. However, there is a fundamental weakness to the human and porcine data. The investigators only measured surface temperature, which is not an accurate estimate of what the temperature was at the level of the basal layer of the epithelium, where the stem cells reside. Buettner examined human skin surface and subsurface temperatures (0.2mm depth, using thermocouples) during heating with a radiant heating device (8). Depending upon the applied power, and the time of measurement after heating device was turned on, the temperature at 0.2 mm depth could be as much as 1–6°C lower than the surface temperature. Also, it takes a few seconds to reach thermal equilibrium. Thus for short heating times, the average temperature of the skin surface would have been much lower than the final target temperature, which is the temperature reported for the papers by Moritz and Stoll. Thus, heat up time and temperature difference at depth was not considered in the work of Moritz et al.(5) or Stoll and Greene (6). For temperatures > 50°C, heat up time could have been a large proportion of the total thermal exposure time to achieve a thermal injury. Additional theoretical and experimental studies have been performed to examine heat transfer in limbs. Under baseline conditions, room air exposure to rabbit hind limb, a 1°C temperature gradient was seen between the skin surface and the basal layer of the epithelium (9). A plexus of highly ordered vasculature, capable of very efficient heat transfer, is present immediately beneath the epidermis. Perfusion of the skin (including studies of human skin) has been measured under conditions of local thermal exposure and has been found to increase by a factor of 10–20 (10, 11). Therefore, when the epidermal surface is being heated, the heat transfer capability of the skin will increase, thereby effectively increasing the magnitude of the thermal gradient across the epidermal layer. Given these considerations, it is *likely that the temperatures to achieve injury in human skin were significantly lower* than what Moritz and Stoll reported and that the heating times to achieve isoeffect injuries would have been shorter than what was reported for temperatures in excess of 50°C, where the total heating times are relatively short (a few seconds). This means that the slopes of the Arrhenius plots as well as the breakpoint temperatures for both human and porcine skin that are derived from the data of Moritz and Stoll are very likely inaccurate. Another example of comparison of heat effects with the same organ in different species is shown in Figures 4a (spinal cord heating in rat, mouse and dog). Similar data related to the heating of cat, dog and rabbit brain is shown in Figure 4b. Both sets of data suggest thermal damage is generally comparable across species (for similar organs or tissues). From these data, it can be concluded that the thresholds for thermal damage to spinal cord and brain are seasonably similar across species.

There are a number of interesting and informative human thermal dose to tissue effect studies in the literature. Although these studies used varied, and probably relevant heating times and temperatures, the thermal dose information was not always obtained in verifiable or detailed manner. Therefore, the information is much less useful. In one such study, Lele and colleagues reported (1983) the thermal dose for brain damage in humans (12). Unfortunately, very little specific thermal dose – to – tissue effect information resulted from that study. Somewhat more thermal dose information resulted cat brain heating experiment that was performed by the same group (13). It should be noted that Lele found thermal thresholds for the human and the cat to be similar. It is unfortunate that what might be the most important data ever generated on thermal thresholds for damage to a vital human tissue is not available in a form that can be critically reviewed.

The relative resistance of human tissues to thermal damage is a significant issue when it comes to considering issues related to regulation of thermal exposure because direct data relating thermal exposure to injury in humans are virtually nonexistent aside from the skin data shown above. If all organs in animal models had the same thermal sensitivity, one could speculate that this might be true for humans also. This is, however, not the case.

Figure 5 compares thermal data for four thermal damage endpoints in murine tissue conditions; namely foot necrosis, ear skin necrosis, testis weight and intestinal damage. Note that while the curves tend to be parallel, the exact time-temperature combinations needed to achieve injury vary. Part of the difference could be due to the type of endpoint used. The ear skin and foot necrosis data are severe injuries, compared with stem cell survival in the intestine or testis weight (which is reflective of cytotoxicity to stem cells and sperm progenitors). Stem cell survival may recover after heating and it relates to treatment effects in a single cell type and thus may not be reflective of the overall thermal sensitivity of a complex tissue. Differences in tissue architecture and kinetics of repair and replacement are different between tissues, so it is also important to control the time of assessment after exposure to be more precise. There has not been consistency in the time of assessment of injuries between authors. It is also known that subtle differences in protein structure can dramatically alter thermal sensitivity to denaturation. For example, the inactivation energy for the protein kanamycin nucleotidyl transferase is around 140 Kcal/mole, which is similar to other proteins (14). When the protein is mutated with single and triple amino acid substitutions, the Arrhenius curve is shifted by 3–9°C without changing the slope of the curve. This means that the inactivation energy remains constant, but the entropy (cal/°C/mole) changes (15). Thus, there are biological and methodological explanations for why different tissues may have varying thermal sensitivities. We feel the amount, architecture, and type of stroma have a large effect in thermal sensitivity.

Careful examination of the detailed thermal dose to effect data, demonstrates remarkable concordance in the thermal sensitivities of individual tissues across species types. For example, the damage threshold for small bowel mucosa has been reported to be between 20 and 50 CEM 43°C for mouse and hamster (16–18). Data are not available for multiple species comparisons for every tissue at this time, but based on what we know about the thermal sensitivity of human cells in tissue culture and the similarity of tissue sensitivities

across species, we can conclude that it is very unlikely that human tissues are more thermally sensitive than what has been reported for other species.

3.0 THERMAL ISOEFFECTIVE DOSE

The recognition that the rate of cell killing is related to time and temperature has led to several different methods for normalizing time-at-temperature data to a common unit that would allow for comparison of different heating regimes. For clinical applications of hyperthermia, this is particularly important, since temperatures during heating are typically non-uniform and temporally unstable. Spatial variation in temperature is more of a problem typically than temporal variation. For a typical treatment during thermal steady state, temperatures can vary from 37 to 43°C within the same tumor. Temperatures can even be higher, particularly if there is a focus of necrosis in a tumor (4). The largest temporal variations occur typically during heat up and cool down. Depending on the tumor location and the efficiency of the heating device it can take from 5–20 min to reach an acceptable thermal steady state. Then if there are adjustments to applied power or changes in perfusion during heating, this can lead to further temporal variation. Sampling times for temperature measurement vary, depending upon whether the thermometers are fixed in one position or if they are mapped in indwelling catheters. A typical frequency might be one measurement per minute. Thus, a simple thermal prescription defining a desired temperature for a defined period of time is difficult if not impossible to achieve. In a classic paper, Sapareto and Dewey proposed a simple method for converting one time-temperature combination to another. This method is termed "thermal isoeffective dose". Typically, the time-temperature data are converted to an equivalent number of minutes at 43°C (19). There was no particular reason for choosing 43°C as the index temperature, aside from the fact that it is near the break point for CHO and several other cell lines. The equation for doing this conversion is shown below as Equation 2.

$$\text{CEM}_{43^{\circ}\text{C}} = tR^{(43-T)} \quad \text{Equation 2}$$

Where CEM 43°C = cumulative number of equivalent minutes at 43°C, t = time interval (min), T = average temperature during time interval t, R is the number of minutes needed to compensate for a one degree temperature change either above or below the breakpoint. When there is temporal variation in the temperature of a specific tissue, the time at each temperature must be determined and the CEM 43°C summed over contiguous intervals where temperature is relatively constant. The resultant CEM 43°C value represents the entire history of the exposure.

Temporal variation in temperature can have significant impact on CEM 43°C particularly when temperatures fall above and below the breakpoint. For example, consider a thermal exposure that lasts 30 minutes. The temperature is 41.5°C for 15 min, 44°C for 10 min and 46°C for 5 min. The numerical average temperature, normalized for number of minutes at each temperature is 43.08°C. However, the CEM 43°C for this exposure is actually 62 min (44°C = 20 ; 46°C = 40 ; 41.5°C = 2 CEM 43°C, respectively).

3.1 Relevance of the R-value for establishing thermal sensitivity of tissues

There is uncertainty about the slope of the Arrhenius plot below the breakpoint. The method of Sapareto and Dewey assumes that it is 0.25, but there are others who reported that it could be as low as 0.125 (20) indicating that the time to achieve an isoeffect at a defined temperature is increased by a factor of 8, as opposed to 4, for every degree drop below the breakpoint. Most rodent data, however, suggest that the R-value below the breakpoint is between 0.25 and 0.17 (21). For regulatory purposes, precise knowledge of the slope of the Arrhenius plot below the breakpoint would be advantageous, since it would allow for more detailed description of the thermal limits to achieve a specific tissue endpoint or to avoid tissue damage. With occupational exposure or accidents it is more likely that exposures will be in the region below the breakpoint as opposed to above it. The data in Figure 2 show similar R values for rodent and human cells (R above breakpoint = 0.43 vs. 0.45 and R below breakpoint = 0.23 vs. 0.25 for human vs. rodent cells, respectively). The breakpoint is slightly higher for human (43.5°C) than rodent cells (43°C). The thermal data for skin necrosis in humans, as derived from the work of Moritz (5) imply that the R-values (Figure 3) for human and rodent tissues are significantly different from the *in vitro* estimates. The R values for human skin above and below the breakpoint are 0.72 and 0.13, respectively. The rodent data are similar to the *in vitro* results, however, with R = 0.45 above the breakpoint and 0.25 below it.

As was discussed above, there is uncertainty about the accuracy of the human skin data. This leads to lack of precision of the threshold temperature for damage, the breakpoint temperature, and the slopes of the Arrhenius plot above and below the breakpoint. In rodent studies, by comparison, the breakpoint derived from *in vivo* and *in vitro* data has been consistently between 42.5 and 43°C.

An example of how Thermal Isoeffective Dose could be used is provided in Figure 6.

The conversion to number of minutes at other temperatures was done using Equation 2. Input variables were as described in Table 2. The analysis suggests that input variables provided from human *in vivo* studies would predict greater thermal resistance than if parameters from rodent studies or human cells from tissue culture are used. NOTE: There is considerable uncertainty in the data derived from the human skin data, as described in considerable detail in the text. These data are included to point out the importance of obtaining accurate thermometry. The object of the simulation in Figure 5 is to predict the times required to achieve muscle fibrosis over the temperature range from 37–50°C. For comparison three sets of assumptions about the slopes of the Arrhenius plot above and below the breakpoint and the location of the breakpoint are compared. The assumptions consider the parameters derived from: (1) Figure 1 for rodent cells (which are typical for most rodent cell lines and tissues), (2) the human cell lines shown in Figure 1 and (3) the *in vivo* human skin data. A summary of the R values and breakpoints used for calculations of time to reach isoeffect and used for the simulations described in Figure 5 are shown in Table 2 below.

Based on the published work of Meshorer et al., the threshold for significant damage to pig muscle, leading to fibrosis at one month post treatment, is >43°C for 30 min (22). From

these data we derived damage threshold curves for the isoeffect of muscle necrosis covering the temperature range from 37–50°C. Using equation 2, the number of minutes of exposure at set temperatures was calculated to reach the same isoeffect. The differences in the slopes of the Arrhenius plots affect the resultant isoeffect curves significantly, particularly at the extremes of the temperature range. The analysis shows that over most of the simulated temperature range, that data derived from tissue culture experiments yield similar predicted heating times when based on parameters derived from either rodent or human cell lines. The apparent increase in thermal sensitivity using human *in vitro* parameters between 43 and 45°C is the result of small differences in the slopes of the Arrhenius plot and the temperature of the breakpoint for the two species over that temperature range. If the *in vivo* human skin data are used, however, then the prediction for the predicted threshold for muscle damage is much higher than either of the other two predictions. The uncertainties about the human skin data, as explained above, cast great doubt about the voracity of that prediction. The prediction is left in the simulation set to serve as an example of how important it is to obtain accurate thermal data when doing studies to assess thresholds for thermal damage.

There are other cautions to be aware of in interpreting these simulations. The data in Figure 6 have been plotted to cover a large temperature range, but the data from which these predictions were derived do not span that range. Most murine studies have been conducted in the range from 41.5–46°C, while the only human data available, which are based on skin tolerance and not muscle, span the range from 44–70°C (5). The slopes of the Arrhenius plots for skin and muscle are likely parallel, as has been shown for several species and tissues. Therefore, the main concerns with respect to interpretation of such predictions are: 1) uncertainty about where the breakpoint is for human tissue 2) whether or not the threshold for thermal damage in animal studies is similar to humans (if the studies were conducted identically). Given that there is a paucity of human data and uncertainty about the accuracy of the data that exist for human skin, the most conservative approach is to use the isoeffect dose parameters derived from rodent tissues. This was done to set boundaries for thermal damage to tissues, as described below.

There may be a temperature boundary for most tissues, below which no clinically detectable injury occurs within the practical time-limits to test them. To illustrate how important this is, the time predicted to cause thermal damage to muscle, assuming a temperature of 37°C, and using the R factors for human skin from Moritz, as shown in Table 3, is 6.2×10^6 minutes, or approximately 12 years. Obviously, one would not expect to see damage to muscle, even if it was at 37°C continuously and this prediction is consistent with that expectation. Because thermal isoeffect dosimetry is an exponential relationship with temperature, increasing temperature leads to a rapid drop in heating time. At 41°C, the predicted heating time is down to 1800 minutes, or 1.23 days and at 42°C the predicted time of heating to approach the threshold for damage in muscle has dropped to 230 min. Using the rodent data for input, the predicted times to reach damage are much shorter. For example at 41°C, the threshold would be 0.3 days as opposed to 1.23 days using the human data.

It is important to note that thermal isoeffect "dose" is not a physical quantity such as energy absorbed, as would be used for quantitating radiation dose, for example. Nor is it directly analogous to pharmacokinetic data that are commonly used to measure drug "dose". In both

of these cases, the dose is based on a physical measurement of the quantity of agent administered. Thermal isoeffect dose is based empirically on the tolerance of specific cell components, the inherent cell tolerance, and the in situ conditions encompassing the cells/tissue being heated. Some authors have been justifiably critical of this approach, because it does not represent a physical dosimetric quantity and more importantly the value of it is entirely dependent upon the cell or tissue being studied (23). This means that a thermal threshold for damage assessed from one tissue cannot be extrapolated to another, as was discussed above.

For specific tissues, at temperatures up to 50°C, the CEM 43°C isoeffect dose method works well to predict defined types of damage from a range of defined time-temperature combinations. This is best illustrated in the Appendix Database Tables, which were too lengthy to include here. The tables show data for several studies in which the times at a range of temperatures to reach a 50% probability for an isoeffect have been determined. A typical example is the work of Morris, et al., who examined times to reach a 50% probability of losing 10 or more vertebral bodies in baby rat tail at twelve different temperature steps between 41.8 and 46°C (24). When these data were converted to CEM 43°C, there was remarkable consistency, averaging 83 ± 14 CEM 43°C to achieve the same level of damage. Put in real terms, this means that 41.8°C for 500 min caused the same amount of damage as 43°C for 85 min or 46°C for 12 min. If these were the only data of this type, one could perhaps dismiss it as being coincidence. However, this type of analysis has been done for a range of tissues, including bowel, skin, ear necrosis, foot loss and testis weight. In addition, a wide range of tumors has been studied (21). The CEM 43°C for a defined level of damage yields a consistent value over a broad range of temperature-time combinations.

The human skin data from Moritz also yield a consistent prediction of CEM43°C to achieve thermal burns, at least over the lower range of temperature-time combinations (Appendix Database). At higher temperatures, the value of the CEM 43°C threshold for burns increases, which may be due to non-linearities in the relationship between surface and deeper layer skin temperatures or uncertainties in what the actual surface temperature was, as was discussed above. The CEM 43°C thermal isoeffective dose concept has been successfully tested in several clinical trials as a predictor of tumor response to the combination of radiation and hyperthermia. Collectively, these data provide some assurance that the parameter has biological validity for determining thresholds for thermal damage to tissues in humans (25–28).

4.0. THRESHOLDS FOR THERMAL DAMAGE IN TISSUE

Some studies have carefully documented levels of tissue damage over a wide range of temperatures and times of exposure. From these data it is possible to determine thresholds for thermal damage. In Figure 7, the data for onset of burns vs. highest time-temperature combinations at which burns were not seen in human skin are plotted (5).

According to most of the current literature, at any defined temperature, the difference in time to achieve thermal injury and avoid it is very small (this appears especially true for acute effects, whereas the differences are less well defined for chronic effects). These data

are typical of most of the rodent data seen in the Appendix Database and suggest that the CEM 43°C - effect relationships for damage are very steep. As a further demonstration of the steepness of the dose effect curves, the fraction of mice developing ear skin necrosis following water bath heating at 43.5°C is shown in Figure 8 (Data taken from Law et al (20)). Although clinically apparent thermal injury is avoided for treatment times up to 40 min, heating for 60 min leads to 100% incidence of injury.

Most of the data that have been acquired to establish thresholds for thermal damage to tissues have been in the context of using hyperthermia to treat tumors. The data largely relate to local or regional heating of the body and nearly all of the data deal with single thermal exposures. As mentioned, the authors have assembled an appendix database, which contains virtually all published thermal dose – tissue injury formula, if all heating and evaluation criteria are met. The appendix is available from the two senior authors (MWD, PJH) upon request (dewhirst@radonc.duke.edu or p.jack.hoopes@dartmouth.EDU).

There are a number of issues that are important to consider when examining and using the tissue thermal threshold data.

1. Thresholds are endpoint dependent. In the Appendix Database the endpoints are indicated. The sensitivity of the endpoints varies considerably. For example, histologic assessment of tissues can reveal damage that may be subclinical. Alternatively, functional endpoints may miss subtle changes that, if cumulative over repeated thermal exposures, could lead to significant consequences.
2. Most of the studies listed in the table are for acute one-time exposures. There is virtually no information on whether repeated subclinical exposures can cause cumulative damage. One exception to this is an epidemiological study that was done to establish the risk of developing cataracts in steel workers (29). This study demonstrated that the temperature of the working environment was strongly related to the likelihood of developing cataracts. The risk was highest for those persons working in the regions of the steel mills that had the highest ambient temperature. The temperature of the eye or the lens was not measured under these circumstances, however; so one cannot completely rule out that some other environmental factor that existed in the regions of highest ambient temperatures may have contributed to the development of these lesions.
3. The amount of damage observed would depend upon the time post exposure that the assessment is made. For example, we show data indicating that the threshold for acute damage to muscle is lower than for chronic damage. Transient acute damage to this organ that does not progress to chronic damage might be relatively unimportant. In other organs, such as brain, the acute physiological changes resulting from heat exposure could be lethal (brain edema) even when few cells actually die from direct thermal injury.
4. The importance of damage is often judged by the criticality of the tissue being evaluated, but this makes the various degrees of heat injury difficult to weigh and assess since the judgment of severity of injury is subjective. For example, scattered loss of hepatocytes is probably much less important for the overall health of a

patient than scattered loss of neurons. Judgments such as this are included in the summary data, which is why brain is listed as being one of the two most thermally sensitive tissues. We assume that any loss of neurons should be considered significant. It should also be noted that a number of brain - assessment endpoints were designed to identify relatively minor losses in brain cellularity. The relative clinical importance of such changes remains unclear.

5. There is a range of thresholds of thermal sensitivity for different tissues, but thresholds for damage within a defined tissue type are remarkably similar, when multiple species have been evaluated for the same endpoint. By extrapolation, those organs most thermally sensitive in rodents are likely to be the most thermally sensitive in humans. However, additional data over a wider temperature range in species with tissues more like the human would add considerable confidence to this statement.
6. The accuracy of the thermal threshold for damage is highly dependent upon the accuracy of the temperature measurement. This is a particular issue for the only data that exist for human skin, as was discussed in detail above.
7. The Appendix Database tables include thresholds for thermal damage to some organs following whole body hyperthermia. The thresholds for damage when hyperthermia is administered this way are typically higher than what is observed following local hyperthermia, which may be due to effects of thermo tolerance development occurring during induction of whole body hyperthermia. Thermo tolerance has been shown to develop following whole body hyperthermia exposure. Kapp found that the LD50 (time of heating at 42.5°C to result in 50% lethality) for rats exposed to a conditioning whole body hyperthermia treatments (41.8°C for 60 min at 30hr prior to the test hyperthermia treatment) was two fold longer than the LD50 for animals that did not receive the conditioning treatment (30). However, thresholds for tissue damage from accidental whole body exposure are dependent upon other variables, such as the age and cardiovascular condition of the subject as well as degree of hydration. More detail on whole body exposures to hyperthermia are provided in a separate report in this issue (Keatinge et al).
8. There are physiological responses that occur in response to heat stress that may or may not be related to thermal damage, per se. For example, perfusion in skin of both rodents and humans increases at temperatures near 40°C (10, 31). The increase in perfusion may not reflect damage; it is a normal physiologic response to increased temperature. Some of these types of responses are listed in the Appendix Database tables, but are not considered to be indicative of toxicity by themselves. Some authors have examined time-temperature relationships for vascular damage, however. Two examples related to perfusion reduction in mouse muscle and rat subcutis are provided in the Appendix Database (32, 33). The slope of the Arrhenius plot for vascular damage is the same as that seen for other types of damage, as reported above.
9. The ranking of thermal sensitivities by tissue type does not follow the general guidelines that one would associate with tissue sensitivity to other cytotoxic entities

such as radiation or chemotherapeutic drugs. For those entities, the proliferative status of target (most sensitive) cells necessary for tissue function is a dominant feature in determining sensitivity. For example, tissues most sensitive to chemotherapeutic drugs include testis, bone marrow and gut epithelium. Clearly this general rule does not seem to follow for hyperthermia, since brain tissue seems to be meaningfully affected by relatively low thermal doses. Most types of brain cells have low or almost no proliferative potential whereas testis, which has high proliferative potential, is also demonstrated to have high functional and morphologic sensitivity to heat. Thus, there is no clear ranking by tissue type. For the nervous system for example, peripheral nerves are very heat resistant; spinal cord is intermediate and brain falls into the very sensitive category. Similarly, different components of the eye have widely varying thermal sensitivities. Although some tissues of relatively similar makeup show large variations in thermal sensitivity, we believe the heat sensitivity differences shown in similar organs (even in different species) would be smaller if all studies had been performed under the same conditions using the same endpoints and parameters. For example, in the studies that have examined thermal sensitivity of gut, some investigators have reported LD50 values, whereas others have reported histologic endpoints. In some cases, histologic endpoints may identify subclinical lesions that may not have significant clinical consequences. In other cases, such endpoints may identify lesions that may be of potential importance that would not be seen clinically. The most pertinent example in this latter case is the neuronal apoptosis observed following local heating of the brain that would not be found if the tissue had not been assessed quantitatively using histomorphometry. Additionally, the time at which the damage is assessed can make a large difference in the damage measurement, as was discussed above for muscle damage in the pig. When the assessment is made acutely, the tissue appears more sensitive than when it is assessed 30 days after exposure.

5.0 FACTORS THAT INFLUENCE THERMAL SENSITIVITY OF TISSUES

There are several factors that influence the thermal sensitivity of tissues. Some of these could occur from occupational or accidental exposure to RF fields and therefore should be discussed. The factors that will be outlined include thermo tolerance, pH and pressure effects and a phenomenon referred to as "step down" heating. The effect of these factors on thermal sensitivity is well characterized and can be described quantitatively based on Arrhenius analysis.

5.1 Thermotolerance

Thermotolerance is defined as an acquired resistance to thermal cytotoxicity. It occurs when tissues or cells are exposed to thermal stress. It is regulated by a special class of proteins, known as heat shock proteins. During and for some time following heat stress, the production of most proteins is downregulated. The exception to this rule is the heat shock proteins, which are up-regulated following heat stress. The purpose of these proteins in the context of heat shock is to act as "chaperones" to either target proteins for degradation or refolding (34).

Thermo tolerance is a transient phenomenon. The degree of protection afforded to cells from thermal stress is dependent upon the severity of the initial thermal damage and the amount of time elapsed from the time that the initial injury occurred. Many papers have been published examining these kinetics. Three examples will be provided here for discussion. Law and colleagues examined the resistance of mouse ear to skin necrosis using fractionated thermal exposures, following an initial thermal injury at 43.5°C for varying lengths of time, ranging from 2 to 40 min (20). A second heat treatment at 43.5°C for varying lengths of time was administered at time intervals between 1 and 160 hours after the first treatment. The time to achieve 50% incidence of ear skin necrosis was used as the endpoint. For a single heat treatment, 50 min at 43.5°C was sufficient to achieve this effect. The degree of thermo tolerance achieved was dependent upon the length of the initial exposure, but maximized at a value of 2. Thus, when thermo tolerance was at its maximum, it took twice as long to achieve 50% incidence of ear skin necrosis than when the treatment was administered without prior treatment. The time required to reach maximum thermo tolerance was also dependent upon the initial exposure. The time required to reach maximal thermo tolerance increased by 0.7 hr for each minute of initial heating (Figure 9). The time required for complete decay of thermo tolerance was not reported in this study, but based on limited data presented it was over 80 hr for the most severe treatment. A similar study was done by Nielson and Overgaard, using a mouse tumor model. The temperature of the test heating was also 43.5°C (35). In this case, the endpoint was tumor growth delay time. The time to reach maximum thermo tolerance increased by 0.8 hr for each additional minute of heating, similar to the data by Law for mouse ear. Better data were available for time to decay of thermo tolerance in the Nielson and Overgaard data. The fit for these data were linear and predicted a time to thermo tolerance decay to increase by 2hr for each additional minute of heating (Figure 10).

Other investigators have studied the effect of thermo tolerance by examining the temperatures required to achieve an isoeffect either alone or after a priming heat dose to induce thermo tolerance. When such results have been examined on an Arrhenius plot, it has become clear that the effect of thermo tolerance is to shift the curve to the right, but in parallel with the original curve. The shift can be between 1–2°C. Thus, when tissues are maximally thermotolerant, the temperature required to achieve an isoeffect increases by about 1–2°C, as compared with a single heat treatment (36, 37). The one degree shift is consistent with the requirement to double the heating time at a temperature above the breakpoint for rodents as discussed above.

Thermotolerance is a ubiquitous phenomenon, occurring in nearly all living organisms, ranging upward from algae and bacteria to mammalian cells. It has been demonstrated following fractionated hyperthermia applications to a variety of tumor and normal tissues (34) and in experiments testing fractionated exposures to whole body hyperthermia (30). Thermo tolerance also affects vascular response to thermal injury. Normally tissue perfusion increases in response to thermal injury. However, it has been demonstrated that this response is reduced if the tissues were heated previously. Presumably, this type of physiologic thermo tolerance is still based on development of tolerance at the cellular level (38). For example, we have shown that gaps between endothelial cells open up upon thermal stress. However, these gaps close again after 4–6 hr and if heating is repeated eight hours after the first

exposure, the gaps do not open again (39). These results suggest that cellular thermo tolerance can be expressed physiologically.

There are no data published regarding thermo tolerance in humans. Moritz and Henriques performed a limited study using the pig skin model, however (5). Using a test temperature of 49°C, they found that nine minutes of continuous heating was sufficient to induce a full thickness burn. If the treatment was broken into 3 minute segments and spread evenly over a 48–72 min time interval, the damage was less severe. When the three treatments were spread over 4, 24 or 48 hr only mild erythema was seen. These results are consistent with the induction of thermo tolerance. They did not follow the animals long enough to determine the kinetics of thermo tolerance decay.

5.2 Vascular occlusion and acidosis

If the blood supply to a region of the body is partly or completely shut down, the thermal sensitivity of the tissue increases. This difference in tissue sensitivity could occur if the reduction in perfusion leads to a decrease in thermoregulatory capability. However, even in studies where this has been controlled, the tissue sensitivity increases. Using rat baby tail stunting as an endpoint it has been determined that above the breakpoint, the primary effect is to shorten the overall time to reach an isoeffective level of damage. The Arrhenius plot slope, however, is parallel to that for heating applied without clamping (24). Below the breakpoint, there is a dramatic effect. Under control conditions, dropping the temperature by one degree below the breakpoint required an 8-fold increase in heating time. If the tail was clamped for 20 min prior to heating, this ratio dropped from 8 to 1.3. Since the slope above the breakpoint is relatively unaffected, the primary effect of clamping is to eliminate the breakpoint. In addition, clamping of the tail lowered the temperature threshold for thermal damage. A significant percentage of animals lost their tails after several hours of heating at 37°C with clamping whereas this was not seen without clamping. Normal skin temperature in the rat is 34°C, so 37°C does represent an elevation above normal for this tissue. The biological implication of this is that abrupt loss of perfusion blocks the onset of thermo tolerance during heating. It is known from *in vitro* studies that acute acidification of cells prior to heating will accomplish the same effect. Thus, the underlying mechanism leading to increased thermal sensitivity as a result of clamping may be due to conversion to anaerobic metabolism and acidosis. During an RF exposure, thermal burns could occur at lower than predicted temperature if perfusion to a portion of the heated volume is reduced. Moritz and Henriques examined this question in one series of experiments, using pig skin (5). They applied extra pressure to the skin at a pressure of 90mmHg at the time of the burn application. This degree of pressure did not affect the degree of thermal injury achieved. In clinical applications of hyperthermia, it has been reported that burn injuries over pressure points can develop during whole body hyperthermia procedures using water blankets where the skin temperature does not exceed 43°C (40).

5.3 Step Down Heating

This phenomenon occurs when there is fluctuation in temperature from being above to below the breakpoint during exposure. If the initial heat stress is at a level above the breakpoint, thermo tolerance induction will be inhibited, even if the temperatures drop back

below the threshold. Thus, the slope of the Arrhenius plot will be identical above and below the normal breakpoint. The net result will be that the amount of thermal damage resulting from the thermal exposure will be greater than predicted (41).

5.4 Rate of Heating

The rate that cells or tissues are heated at can have a profound effect on the degree of cytotoxicity. Most tissue culture experiments have been done by heating cells in a water bath and the contents reach target temperature within 1–2 min. Even in the clinical setting where hyperthermia is used to treat tumors, target temperatures are reached within 10–20 min. If the heating period occurs over an hour to reach the same target temperature, there will be less killing (23, 42). We also investigated the effects of high vs. low heating rates on vascular damage in normal tissues (43). The threshold for arteriolar stasis was between 45 and 46°C for heating rates ranging from 0.1 to 0.7 °C/min. At a heating rate of 1.0 °C/min the stasis temperature dropped to 42°C. Venular stasis temperature dropped from 43.4 to 42.1 °C when comparing 0.1 to 1.0 °C/min heating rates, respectively. The difference in temperature for damage onset occurs because thermo tolerance develops to a greater degree during slow vs. fast heating rates.

5.5 Differences between resting temperature and final temperature

The degree of cytotoxicity that occurs from heating depends upon what the resting temperature is. It is known that the resting temperature of peripheral tissues is lower than core temperature, as would be measured in a major vessel or internal organ (44). Normal skin temperature in humans is about 34°C. The amount of damage from a thermal exposure would be greater for the temperature differential between 34 and 42°C than for the differential between 37 and 42°C (45–47). Even though this phenomenon has been demonstrated in tissue culture, there is no clear evidence to suggest that skin is more thermally sensitive than other tissues because of it.

5.6 Assessment of thermal dose-to-tissue effect

One of the most difficult aspects of determining the specific effects of tissue heating is the accurate coregistration of tissue temperature (thermal dose) and pathological effect. Unless the co-registration methodologies are worked out prior to the delivery of heat and tissue collection, the accurate matching of morphologic change and thermal dose can be difficult, if not impossible. It is often useful to use in vivo imaging to accurately determine the spatial position of heat delivery and the volume and heat doses of various regions of heated tissue. It is well known that tumor tissues respond very heterogeneously to heat and that the most successful tumor heating protocols have relied on the use of “minimal thermal dose” when documenting the level of heating. Although less dramatic, normal tissues can also respond heterogeneous to heat delivery. Even relatively homogenous normal tissues have numerous alterations in tissues architecture and vascular supply. Therefore, the delivery of accurate thermal doses requires the placement of sufficient and strategically placed thermometry. Although sometimes impractical, continuous thermal mapping throughout the heated tissues, for the duration of heat exposure, is the preferred method.

The following figures demonstrate how thermal mapping can be effectively used with *in vivo* imaging and pathologic assessment to accurately determine the effect of a specific thermal dose in a specific organ or tissue. In the thermal dose-to-effect study detailed below, canine brain was heated locally using a single 915 MHz antenna. The ultimate goal was to combine interstitial brain heating with interstitial radiation to improve the therapeutic ratio for brain tumor treatment. Figure 11a demonstrates the parallel and perpendicular positioning of temperature catheters, with respect to the antenna. The temperature catheters housed 0.5 mm fiberoptic temperature assessment probes which were continuously moved (during the heat episode) to gain a complete linear temperature map across the heated field. The use of 3 temperature and the continuous pullback technique allowed for the generation of a reasonably complete temperature map over the entire heated region and over the entire duration of heating. The temperature graph in Figure 11a shows the highest temperature (adjacent to the antenna) and the temperature fall off at distance from the antenna. Figure 11b demonstrates a contrast enhanced MRI image of the heated dog brain 7 days following a 60 minute heat treatment (a target temperature of 41.5 °C @ 1.5 cm from the antenna was used). The tissue within the contrast ring was histologically shown to be necrotic, the tissue immediately outside of the contrast ring was congested and mildly inflamed but survived. Figure 11c is a complete coronal histologic section (slide) of the exact region of brain seen in the MRI (Figure 11b). This type of histology - *in vivo* imaging (MRI) - thermometry co-registration allows accurate determination of the thermal dose necessary to produce a specific tissue effect. Figure 11d demonstrates the method used to study the tissue effect – thermal dose relationship in a detailed manner. The thermal isodose curves created from the temperature maps were computer positioned on the histologic preps to allow accurate co-registration of histologic change and temperature. A histologic assessment at each of the 5 temperature isodose lines/regions was assessed for cellular alteration. The level of cellular change was then compared to the same region in the contralateral hemisphere (data not shown). In this manner one could assess the exact cellular changes which occurred at a number of different thermal doses. The results of these experiments (Figure 11e) support the CEM concept; that temperature and heating time (to achieve a specific tissue effect) have an Arrhenius relationship that can be accurately determined with a moderate temperature zone.

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References

1. Dewey WC. Arrhenius relationships from the molecule and cell to the clinic. *Int J Hyperthermia*. 1994; 10(4):457–483. [PubMed: 7963805]
2. Roizin-Towle L, Pirro JP. The response of human and rodent cells to hyperthermia. *Int J Radiat Oncol Biol Phys*. 1991; 20(4):751–756. [PubMed: 2004951]
3. Morris CC, Myers R, Field SB. The response of the rat tail to hyperthermia. *Br J Radiol*. 1977; 50(596):576–580. [PubMed: 890233]

4. Dewhirst, M. Thermal Dosimetry. In: Seegenscluniedt, MH.; Fessenden, P.; Vernon, C., editors. Thermo-radiotherapy and thermochemotherapy. Berlin: Springer-Verlag; 1995. p. 123-136.
5. Moritz A, Henriques F. Studies of thermal injury 11. The relative importance of time and surface temperature in the causation of thermal burns. *Am J Pathol.* 1947; 23:695–720. [PubMed: 19970955]
6. Stoll A, Greene L. Relationship between pain and tissue damage due to thermal radiation. *J Appl Physiol.* 1959; 14:373–382. [PubMed: 13654166]
7. Law MP. Induced thermal resistance in the mouse ear: the relationship between heating time and temperature. *Int J Radiat Biol.* 1979; 35(5):481–485.
8. Buettner K. Effects of extreme heat and cold on human skin. 11. Surface temperature, pain and heat conductivity in experiments with radiant heat. *J. Applied Physiol.* 1951; 3:703–713. [PubMed: 14850401]
9. Weinbaum S, Jiji LM, Lemons DE. Theory and experiment for the effect of vascular microstructure on surface tissue heat transfer--Part 1: Anatomical foundation and model conceptualization. *J Biomech Eng.* 1984; 106(4):321–330. [PubMed: 6513527]
10. Song CW, Chelstrom LM, Haurnschild DJ. Changes in human skin blood flow by hyperthermia. *Int J Radiat Oncol Biol Phys.* 1990; 18(4):903–907. [PubMed: 2139018]
11. Song CW, Kang MS, Rhee JG, Levitt SH. Effect of hyperthermia on vascular function in normal and neoplastic tissues. *Ann N Y Acad Sci.* 1980; 335:35–47. [PubMed: 6931527]
12. Lele, P. Physical aspects and clinical studies with ultrasonic hyperthermia. In: Storm, F., editor. *Hyperthermia in Cancer Therapy.* Boston: G.K. Hall; 1983. p. 333-367.
13. Lele, P. Thresholds and mechanisms of ultrasonic damage to "organized" animal tissues. In: Hazzard, D.; Litz, M., editors. *Symposium of Biological Effects and Characterization of Ultrasound Sources.* Rockville, MD: DHEW (Pub) FDA; 1977. p. 78-8048.1977
14. Dewey WC, Hopwood LE, Sapareto SA, Gerweck LE. Cellular responses to combinations of hyperthermia and radiation. *Radiology.* 1977; 123(2):463–474. [PubMed: 322205]
15. Matsumura M, Yasumura S, Aiba S. Cumulative effect of intragenic amino-acid replacements on the thermostability of a protein. *Nature.* 1986; 323(6086):356–358. [PubMed: 3020429]
16. Milligan AJ, Metz JA, Leeper DB. Effect of intestinal hyperthermia in the Chinese hamster. *Int J Radiat Oncol Biol Phys.* 1984; 10(2):259–263. [PubMed: 6706722]
17. Hume SP, Marigold JC, Field SB. The effect of local hyperthermia on the small intestine of the mouse. *Br J Radiol.* 1979; 52(620):657–662. [PubMed: 486896]
18. Merino OR, Peters LJ, Mason KA, Withers HR. Effect of hyperthermia on the radiation response of the mouse jejunum. *Int J Radiat Oncol Biol Phys.* 1978; 4(5–6):40714.
19. Sapareto SA, Dewey WC. Thermal dose determination in cancer therapy. *Int J Radiat Oncol Biol Phys.* 1984; 10(6):787–800. [PubMed: 6547421]
20. Law MP, Coultas PG, Field SB. Induced thermal resistance in the mouse ear. *Br J Radiol.* 1979; 52(616):308–314. [PubMed: 444822]
21. Field SB, Morris CC. The relationship between heating time and temperature: Its relevance to clinical hyperthermia. *Radiother Oncol.* 1983; 1:179–186. [PubMed: 6680222]
22. Meshorer A, Prionas SD, Fajardo LF, Meyer JL, Hahn GM, Martinez AA. The effects of hyperthermia on normal mesenchymal tissues. Application of a histologic grading system. *Arch Pathol Lab Med.* 1983; 107(6):328–334. [PubMed: 6687797]
23. Gerner EW. Thermal dose and time-temperature factors for biological responses to heat shock. *Int J Hyperthermia.* 1987; 3(4):319–327. [PubMed: 3668313]
24. Morris CC, Field SB. The relationship between heating time and temperature for rat tail necrosis with and without occlusion of the blood supply. *Int J Radiat Biol.* 1985; 47:41–48.
25. Kapp DS, Cox RS. Thermal treatment parameters are most predictive of outcome in patients with single tumor nodules per treatment field in recurrent adenocarcinoma of the breast [see comments]. *Int J Radiat Oncol Biol Phys.* 1995; 33(4):887–899. [PubMed: 7591899]
26. Dewhirst MW, Sim DA. The utility of thermal dose as a predictor of tumor and normal tissue responses to combined radiation and hyperthermia. *Cancer Res.* 1984; 44(10 Suppl):4772s–4780s. [PubMed: 6380715]

27. Oleson JR, Samulski TV, Leopold KA, Clegg ST, Dewhirst MW, Dodge RK, et al. Sensitivity of hyperthermia trial outcomes to temperature and time: implications for thermal goals of treatment. *Int J Radiat Oncol Biol Phys*. 1993; 25(2):289–297. [PubMed: 8420877]
28. Seegenschmiedt MH, Brady LW, Rossmeissl G. External microwave hyperthermia combined with radiation therapy for extensive superficial chest wall recurrences. *Recent Results Cancer Res*. 1988; 107:147–151. [PubMed: 3375547]
29. Wallace J, Sweetnam P, Warner C, Graham P, Cochrane A. An epidemiological study of lens opacities among steel workers. *Brit. J. Industr. Med*. 1971; 28:265–271.
30. Kapp DS, Lord PF. Thermal tolerance to whole body hyperthermia. *Int J Radiat Oncol Biol Phys*. 1983; 9(6):917–921. [PubMed: 6863064]
31. Dewhirst M, Gross JF, Sim D, Arnold P, Boyer D. The effect of rate of heating or cooling prior to heating on tumor and normal tissue microcirculatory blood flow. *Biorheology*. 1984; 21(4):539–558. [PubMed: 6487766]
32. Brown SL, Hunt JW, Hill RP. Differential thermal sensitivity of tumor and normal tissue microvascular response during hyperthermia. *Int J Hyperthermia*. 1992; 8(4):501–514. [PubMed: 1402130]
33. van den Berg-Block AE, Reinhold HS. Time-temperature relationship for hyperthermia induced stoppage of the microcirculation in tumors. *Int J Radiat Oncol Biol Phys*. 1984; 10(5):737–740. [PubMed: 6735759]
34. Burgman, P.; Nussenzweig, A.; Li, C. Thermo tolerance. In: Seegenschmiedt, M.; Fessenden, P.; Vernon, C., editors. *Thermo-radiotherapy and Thermo-chemotherapy*. Berlin: Springer-Verlag; 1995. p. 75-87.
35. Nielsen OS, Overgaard J. Importance of preheating temperature and time for the induction of thermo tolerance in a solid tumour in vivo. *Br J Cancer*. 1982; 46(6):894–903. [PubMed: 7150483]
36. Suit HD. Hyperthermic effects on animal tissues. *Radiology*. 1977; 123(2):483–487. [PubMed: 322206]
37. Henle KJ. Thermo tolerance in the murine jejunum. *J Natl Cancer Inst*. 1982; 68(6):1033–1036. [PubMed: 6953268]
38. Nah B, Choi I, Oh W, Osborn J, Song C. Vascular thermal adaptation in tumors and normal tissue in rats. *Int J Radiat Oncol Biol Phys*. 1996; 35:95–101. [PubMed: 8641932]
39. Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res*. 2001; 61(7):3027–3032. [PubMed: 11306483]
40. Bull, JM. Systemic Hyperthermia. In: Storm, F., editor. *Hyperthermia in Cancer Therapy*. Boston: G.K. Hall Medical Publishers; 1983. p. 401-405.
41. Henle KJ, Dethlefsen LA. Time-temperature relationships for heat-induced killing of mammalian cells. *Ann N Y Acad Sci*. 1980; 335:234–253. [PubMed: 6931521]
42. Herman TS, Gerner EW, Magun BE, Stickney D, Sweets CC, White DM. Rate of heating as a determinant of hyperthermic cytotoxicity. *Cancer Res*. 1981; 41(9 Pt 1):351923.
43. Dewhirst, M.; Gross, JF.; Sim, D. Effects of heating rate on normal and tumor microcirculation. In: Diller, K.; Roemer, R., editors. *Heat and Mass Transfer in the Microcirculation of Thermally Significant Vessels*. New York: American Society of Mechanical Engineers; 1986. p. 75-80.
44. Thrall DE, Page RL, Dewhirst MW, Meyer RE, Hoopes PJ, Kornegay JN. Temperature measurements in normal and tumor tissue of dogs undergoing whole body hyperthermia. *Cancer Res*. 1986; 46(12 Pt 1):6229–6235. [PubMed: 3779643]
45. Culver P, Gerner EW. Temperature acclimation and specific cellular components in the regulation of thermal sensitivity of mammalian cells. *National Cancer Institute Monograph*. 1982; 61:99.
46. Herman TS, Henle KJ, Nagle WA, Moss AJ, Monson TP. Exposure to pretreatment hypothermia as a determinant of heat killing. *Radiat Res*. 1984; 98(2):345–53. [PubMed: 6729042]
47. Hill SA, Denekamp J. Site dependent response of tumours to combined heat and radiation. *Br J Radiol*. 1982; 55(660):905–912. [PubMed: 7171934]
48. Goffinet DR, Choi KY, Brown JM. The combined effects of hyperthermia and ionizing radiation on the adult mouse spinal cord. *Radiat Res*. 1977; 72(2):238–245. [PubMed: 918258]

49. Sasaki M, Ide C. Demyelination and remyelination in the dorsal fimbiculus of the rat spinal cord after heat injury. *J Neurocytol.* 1989; 18(2):225–239. [PubMed: 2732760]
50. Sminia P, Haveman J, Wondergem J, van Dijk JD, Lebesque JV. Effects of 434 MHz microwave hyperthermia applied to the rat in the region of the cervical spinal cord. *Int J Hyperthermia.* 1987; 3(5):441–452. [PubMed: 3681044]
51. Sminia P, Troost D, Haveman J. Histopathological changes in the spinal cord after 434 MHz microwave hyperthermia in the cervical region of the rat. *Int J Hyperthermia.* 1989; 5(1):85–98. [PubMed: 2921537]
52. Britt RH, Lyons BE, Pounds DW, Prionas SD. Feasibility of ultrasound hyperthermia in the treatment of malignant brain tumors. *Med Instrum.* 1983; 17(2):172–177.
53. Fike JR, Gobbel GT, Satoh T, Stauffer PR. Normal brain response after interstitial microwave hyperthermia. *Int J Hyperthermia.* 1991; 7(5):795–808. [PubMed: 1719106]
54. Harris A, Erickson L, Kendig J, Mingrino S, Goldring S. Observations on selective brain heating in dogs. *J Neurosurg.* 1962; 19:514–521. [PubMed: 13904819]
55. Lyons BE, Obana WG, Borcich JK, Kleinman R, Singh D, Britt RH. Chronic histological effects of ultrasonic hyperthermia on normal feline brain tissue. *Radiat Res.* 1986; 106(2):234–251. [PubMed: 3704114]
56. Sminia P, Haveman J, Ongerboer de Visser BW. What is a safe heat dose which can be applied to nonnal brain tissue? *Int J Hyperthermia.* 1989; 5(1):115–117. [PubMed: 2921531]

Figure 1.

a shows a family of survival curves for Chinese hamster ovary (CHO) cells covering the range from 42–45°C with heating times up to five hours (2). These survival curves typically have a shoulder. The width of the shoulder region varies with cell line, and is also dependent upon temperature. The shoulder region shows that there is a threshold for thermal damage to cells. Once cytotoxicity starts to occur, the rate of cell killing, which is exponential with time of heating, is dependent on temperature. For the example provided, there is very little cytotoxicity for up to five hours of heating at 42°C. At 42.5°C, however, three logs of cell

kill are achieved after five hours of heating. For comparison purposes, a similar family of survival curves is plotted for a human tumor cell line (HTB-66; **Figure 1b**). The CHO and HTB-66 cell lines show a reduction in slope of the cytotoxicity curve after four hours of heating at 42.5°C and three hours of heating at either 42.5 or 43°C, respectively. This reduction in slope is due to acquired resistance to heating, or thermo tolerance. (Roizin-Towle (2) reproduced with permission of the publisher)

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Figure 2.

Comparison of Arrhenius plots for a series of rodent and human cell lines, derived from cell survival curve data, as shown in Figure 1. The Arrhenius plots consider the rate of cell killing on the exponential portion of the curves. The size of the shoulder of the survival curve is not included in this analysis. The rodent cell lines included in this figure are CHO, AD-%, C3H 10T-1/2: Human cell lines are KB7, MIA-PACA2, glioblastoma (not otherwise defined), WiDR, AG-1522, HTB66, HTB72, KB8 and A549. Data from a paper by (Roizin-Towle (2) reproduced with permission of the publisher)

Figure 3.

a. Time - temperature combinations to achieve varying thresholds of thermal damage to human skin. Data obtained from Moritz (5) and Stoll (6). Note that the isoeffect curves are parallel for the various forms of injury. Pain threshold is significantly lower than the threshold for significant injury, indicating that pain avoidance effectively minimizes significant injury to skin. The human data are compared with porcine studies of skin tolerance, which had the same endpoint as the human results Data derived for mouse ear skin necrosis are also included (7).

b. Time to reach epidermal necrosis as a function of temperature for mouse, human and pigskin. The data for mouse ear skin necrosis were derived from a paper by Law (7). The human and porcine data come from a paper published by Moritz and Henriques (5). Note that the thermal sensitivities of pig and human skin are virtually identical and both appear to be more resistant to thermal damage than mouse skin.

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Figure 4.

a. Time-temperature relationships for thermal damage to spinal cord across three species. □ = no detectable damage, ⊠ = histologic damage, but subclinical, ■ = severe histologic damage and/or clinical symptoms or death. ▲ = rat, ■ = mouse, ▼ = dog. Data obtained from the following references (48–51).

b. Time-temperature relationships for thermal damage to brain across three species. □ = no detectable damage, ⊠ = histologic damage, but subclinical, ■ = severe histologic damage

and/or clinical symptoms or death. ▲ = cat, ■ = dog, ▼ = rabbit. Data obtained from the following references (12, 13, 52–56).

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Figure 5.

Time-temperature relationships to achieve isoeffective thermal damage in several mouse tissues. Note that the slopes for these isoeffects are parallel, but some tissues appear more sensitive than others (i.e., the thresholds for thermal damage vary from one tissue to the next). There are multiple reasons for this, some of which may not relate to actual differences in tissue sensitivity (see text for details).

Figure 6.

Predicted times to reach muscle necrosis over the temperature range from 37–50°C, based on data by Meshorer indicating that 30 min at 43°C is sufficient to cause damage to muscle (22).

Figure 7.

Thresholds for thermal damage. The open circles indicate the highest time-temperature combination studied for which there was no full thickness burn. The solid circles indicate the lowest time-temperature combinations that caused full thickness burns. Data obtained from (5). This figure demonstrates the very small temperature-time transition between survival and necrosis of tissues subjected to heating.

Figure 8.

Relationship between time of heating at 43.5°C and incidence of ear skin necrosis. Note the steepness of this dose-effect curve. Assuming 60 min of heating is necessary to achieve 100% incidence of ear skin necrosis, a 30% reduction in heating time will completely avoid the injury. Data replotted from (7).

Figure 9.

Time for development of a maximum amount thermo tolerance as a function of length of heating time at 43.5°C. Data are plotted for a mouse tumor line (open symbols) (35) and for mouse ear necrosis (closed symbols) (20). The lines are the best linear fits to the data.

Figure 10.

Time for decay of thermo tolerance, as a function of length of heating time at 43.5°C. Data abstracted from (35)

Figure 11.

Table 1

Activation energies calculated from double exponential fits (data from Figure 3). The breakpoints were 47°C for man and pig and 42.5°C for mouse

Species	Temperature Range (°C)	Activation energy (kcal/mole)
Man	44 – 47 / 47 – 60	182.2 / 95.78
Pig	44 – 47 / 48 – 56	150.75 / 106.38
Mouse	41.5 – 42.5	273.89

Table 2

		R value	R values
Species	Breakpoint	< Breakpoint	> Breakpoint
Mouse	43.0° C	0.25	0.5
Man (in vitro)	43.5° C	0.233	0.428
Man (in vivo)	47.0° C	0.13	0.72