

Biological Rationales and Clinical Applications of Temperature Controlled Hyperthermia - Implications for Multimodal Cancer Treatments

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Abstract

Hyperthermia (HT) - heating the tumor in the range of 40.0 - 44.0 °C - combined with radiation (RT) and/or chemotherapy (CT) is a well proven treatment for malignant tumors. The improvement of the techniques for monitoring and adapting of the desired temperatures even in deep seated tumors has led to a renaissance of, now quality-controlled, HT in multimodal tumor therapy approaches. Randomized clinical trials have shown improved disease-free survival and local tumor control without an increase in toxicity for the combined treatment. In this review, we will focus on biological rationales of HT comprising direct cytotoxicity, systemic effects, chemosensitization, radiosensitization, and immune modulation. The latter is a prerequisite for the control of recurrent tumors and micrometastases. Immunogenic tumor cell death forms induced by HT will be introduced. Modulations of the cytotoxic properties of chemotherapeutic agents by HT as well as synergistic effects of HT with RT will be presented in the context of the main aims of anti-tumor therapy. Furthermore, modern techniques for thermal mapping like magnet resonance imaging will be outlined. The effectiveness of HT will be demonstrated by reviewing recent clinical trials applying HT in addition to CT and/or RT. We conclude that hyperthermia is a very potent radio- as well as chemosensitizer, which fosters the induction of immunogenic dead tumor cells leading to local and in special cases also to systemic tumor control.

Keywords

Hyperthermia; radiotherapy; chemotherapeutics; immunogenic cell death; cancer, anti-tumor immunity; danger signals; magnetic resonance images

1. Effects of hyperthermia treatment on cells

Hyperthermia (HT) treatment describes the targeted and controlled heating of tumor tissues in the range of 40.0-44.0 °C. Various application techniques are used for treating cancer patients like local, interstitial, or regional hyperthermia and hyperthermic limb perfusion techniques. As a single treatment, the efficacy of hyperthermia alone is not enough to replace the conventional established cancer therapies (X-ray and chemotherapeutics), but it is known to induce thermal chemosensitization and thermal radiosensitization. The main aim of hyperthermia treatment is therefore the improvement of conventional therapies in multimodal cancer treatments. Further biological rationales of HT comprise direct cytotoxicity, systemic effects, and immune modulation, which will be elucidated in the following paragraphs.

1.1 Cytotoxic Effects of Hyperthermia

Since the early 70's, pre-clinical research with exponentially grown cells revealed the thermal dose, dependent on time and given temperature, being most critical for the induction of cell death and systemic effects. Temperatures ranging from 41 to 47 °C exhibited a direct cell killing effect in vitro and in animal hyperthermic experiments [2-4]. The survival curves after HT treatment show a two-step process of cell killing: in the beginning of heat exposure a linear growth arrest is

observed, followed by exponential cell death. A correlation between the thermal energy dose necessary to induce exponential cell death and the denaturation of cellular proteins was found *in vitro*. Therefore, the direct cytotoxicity of HT treatment seems to be based on the denaturation and aggregation of cytoplasmic, nuclear or membrane proteins, but similar relationships could not be detected for radio or chemosensitization phenomena.

1.2 HT Effects on Tumor Microenvironment

Malignant tumors are regarded as autonomous organs with specialized microenvironment, which is characterized by reduced blood flow and blood vessel density. Inside the tumor tissue this chaotic vasculature often leads to areas of acidosis, hypoxia and energy deprivation in form of ATP. These factors turn cells more sensitive to hyperthermia, especially in low perfused areas. Therefore, at temperatures between 40 and 44 °C hyperthermia induces an almost selective destruction of tumor cells in hypoxic and acidic parts of solid tumors *in vivo*, but leaves normal tissues intact.

1.3 HT Thermotolerance

When cells are exposed to various forms of stress, specific stress proteins are upregulated, which often fulfill functions as molecular chaperones and prevent lethal damage of the cells. In the case of hyperthermia, the proteins at least partly involved are heat-shock proteins (HSP), which might render cells transiently thermotolerant to further HT treatments, an undesirable side-effect in cancer therapy. However, following more intense or prolonged heat treatment, these compensatory mechanisms often fail to prevent tumor cell death.

2. Effects of radiotherapy on cells

Radiotherapy (RT) is one of the standard treatments anti-tumor therapy. RT can be given as an adjuvant or neoadjuvant treatment and its main function is the local control of tumors in cancer patients. Ionizing irradiation inflicts various types of DNA damage, but the subsequent production of DNA double-strand breaks (DSB) is thought to be the main damage after RT. Most of the DNA damage induced by RT occurs not in single DSB, but in clustered or bulky lesions with multiple DNA and base damages which exacerbate the proper repair for the cell.

The DNA damages induced by RT lead to a cell-cycle arrest in the G2/M phase, in which cells are highly susceptible to further irradiation, commonly utilized by fractionated RT. The fractionation scheme has been developed empirically over the last century and generally contains five daily treatments per week, mostly applying 1.8 -2 Gy per fraction.

Therefore, ionizing irradiation primarily leads to cell inactivation or to a proliferative stop rather than to direct cell killing, in contrast to chemotherapeutic agents. However, it has been shown that ionizing irradiation is capable of directly damaging mitochondria in cells, which may induce apoptosis. Furthermore, X-ray in a half-weekly or weekly cumulative dose of 5 or 10 Gy induces tumor cell death. We have just recently shown that necrosis is the prominent form of cell death in the days after irradiation of colorectal tumor cells.

Local tumor control, also termed radiocurability, is mainly achieved through the elimination of proliferating (clonogenic) tumor (stem) cells. Curability of a certain tumor cannot be predicted through local tumor control alone, because of each tumor's propensity for metastasis or recurrence. Radiotherapy as a single therapy is often not able to eradicate all clonogenic tumor cells. Therefore, radiotherapy is a local rather than systemic treatment modality which can improve patient survival but often needs additional treatments like chemotherapy and/or hyperthermia.

3. Effects of chemotherapeutics on cells

The primary cytotoxic mechanism of many conventional chemotherapeutic agents (including alkylating agents, platinum compounds, topoisomerase inhibitors and the antime-tabolites) is the emergence of DNA damage and the subsequent induction of cell death. All traditional cytostatic drugs lead to various side effects due to limited selectivity of the antitumor agents: leukopenia, mucositis, nausea, and vomiting.

The evolving field of chemotherapy in tumor treatment comprehends various clinical relevant classes of cytotoxic agents. In this review, we can only describe some chemotherapeutics exemplarily, which are also important in the combined use with hyperthermia.

3.1 Alkylating Agents

The alkylating agents (e.g. cyclophosphamide and ifosfamide) belong to the old-established anticancer drugs, which are still important for the treatment of various human cancers. Most of these agents are methylating (temozolomide) or chloroethylating (carmustine) active. In both cases 06-Guanine in DNA and RNA is an important cellular target, but also other sites are alkylated. Secondary effects of the alkylations are DNA-DNA cross-links, mismatches, and highly toxic DSB.

3.2 Platinum Compounds

For over 30 years, cisplatin has been a highly effective platinum-based anti-cancer drug that continues to play a central role in cancer chemotherapy. However, the use of cisplatin causes severe side-effects and various toxicities in patients. For this reason, new platinum compounds have been screened as potential anti-tumor drugs, less toxic than cisplatin but equally effective. Carboplatin and oxaliplatin have been approved for clinical use in 1989 and 2003, respectively. In general, the accepted cellular target for platinum complexes is the DNA. Cisplatin cytotoxicity was thought to result from the inhibition of DNA synthesis. However, recent evidence indicates that cisplatin can kill cells by apoptosis.

3.3 Anthracyclines

The first anthracyclines (including doxorubicin) were isolated from *Streptomyces* bacteria in the 1960's. Doxorubicin has a broad antitumor spectrum, with numerous solid tumors in addition to haematological malignancies. Anthracyclines are still frequently used in clinical practice and in particular doxorubicin remains an important cytotoxic component for the treatment of many human cancers. Current clinical practice often combines anthracyclines with novel agents to maximize the therapeutic effect, instead of replacing them. The main cellular target of anthracyclines is generally recognized to be topoisomerase-II. Inhibition of this enzyme blocks DNA replication as well as transcription. DNA strand breaks may trigger apoptosis of cancer cells via the p53 pathway.

4. Hyperthermia adds to radiotherapy

Ionizing irradiation and hyperthermia treatment act in a synergistic way, called thermal radiosensitization. Compared to HT alone, RT plus HT led to an increase in cell death even at lower temperatures. The thermal enhancement ratio (TER) defines the amount of thermal radiosensitization by the quotient of the survival fraction after X-ray alone and in combination with hyperthermia. The synergistic effects of HT and irradiation are mainly based on the complementary targets of both treatment modalities (Figure 1.).

Solid tumors may contain hypoxic areas because of diffusion- or reperfusion-limited oxygen supply. Hypoxic cells are two to three times more radioresistant than normoxic cells. Therefore, between fractionated doses of irradiation a certain time interval is needed to ensure reoxygenation of the tissue and to reduce the negative effect of tumor hypoxia on local control. Hypoxic areas in solid tumors represent a major therapeutic concern: the extent of hypoxic conditions in solid tumors has been shown to correlate with poor prognosis for the patient for different tumor types. However, hypoxic cells were shown to be highly sensitive to the combination of RT and HT. This may be due to increased vascularization and enhanced vessel permeability, with an increase in oxygen pressure levels in the tumor and the surrounding microenvironment after moderate HT treatment. To yield the highest synergistic effects between RT and HT, both treatments should be applied synchronously or after time intervals of 2-4 h. Hyperthermia alone may foster metastases but the combination with RT does not increase metastases and leads to systemic, immune activating effects.

During the cell cycle, the mitotic phase shows the highest heat sensitivity, but also S-phase cells are sensible to hyperthermia treatment. In contrast, cells in G2 phase are most sensitive to ionizing irradiation. The variations in heat sensitivity during the different cell cycle phases refer to the diversity of molecular mechanisms of cell death induction after HT. Additionally, hyperthermia affects the DNA repair, leading to increased radiation-induced chromosomal aberrations. The underlying mechanism of repair inhibition seems to be alterations in chromatin organization, due to aggregation of nuclear proteins. The major effects of heat on radiosensitivity are suggested to work via inhibition of the repolymerisation step in the repair of base damages (base excision repair), which leads to the formation of secondary, toxic DNA double strand breaks. Taken together, HT is one of the most potent sensitizers for ionizing irradiation.

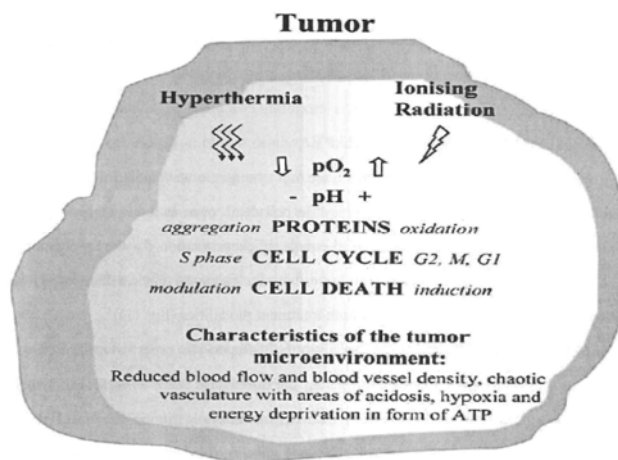


Figure 1. Synergistic mode of action of HT and RT. The tumor microenvironment is characterized by reduced blood flow and vessel density. This chaotic vasculature leads to areas of acidosis, hypoxia and energy deprivation in form of ATP. Radiotherapy (RT) and hyperthermia (HT) treatment act in a synergistic way, based on the complementary target functions of both modalities

5. Hyperthermia adds to chemotherapy

Hyperthermia is also capable of enhancing the cytotoxicity of chemotherapeutic drugs at multiple levels. The TER expresses the extent of a chemotherapeutics' thermal chemosensitization, as the quotient of cell survival at the elevated temperature and the normal temperature. The combination of HT and chemotherapy was shown to increase the inhibition of clonogenic cell growth both in vitro and in animal models. Chemotherapy and heat can interact in different ways. The platinum compounds (like cisplatin and oxaliplatin) and alkylating drugs (cyclophosphamide) show linear

enhanced cytotoxicity when temperatures are raised from 37 to 40.5 °C. Conversely, antimetabolites like 5-Fluorouracil have not been found to interact with heat. This lack of interaction may still lead to an improved therapeutic result in vivo because spatial cooperation and/or toxicity independency may nevertheless exist. In vivo studies have demonstrated that the thermal enhancement of cytotoxicity is maximized at temperatures between 40.5 and 43 °C for many chemotherapeutic agents.

Possible mechanisms for the thermal chemosensitization include an increased rate of alkylation, an increase in drug uptake, and the inhibition of drug-induced sublethal or lethal damage repair. The distribution of cytostatic drugs in the tumor tissue may be further affected by changes in tumor blood supply and variances in fluid and electrolyte balance, as well as pH-changes that may lead to altered drug solubility and volume distribution. In cancer patients, the drug heat interaction appears to be much more dependant on these environmental factors mentioned than those of irradiation and heat. Clinically achieved temperatures are rarely high enough (> 43 °C) to cause vascular damage and it was found that HT between 40 and 43 °C causes increased tumor blood supply. The critical factors for drug uptake are blood flow and vascular permeability, which are both increased by hyperthermia treatment.

In general, studies on drug-heat sequence show that, the administration of drugs immediately before HT is most effective. However, exceptions like the antimetabolite gemcitabine exist, where a time interval of 24 h between drug and heat application has been needed to yield a synergistic effect in vitro and in vivo.

One further benefit of combining chemotherapy with HT is that cells with acquired drug resistance (often multifactorial) can be made responsive to drugs again. In particular, this mechanism of reverting drug resistance could be shown for cisplatin. Moderate HT treatment itself is not able to induce directly chromosomal DNA strand breaks but can alter the chromatin structure, thus influencing DNA repair. When combined with heat, chemotherapy behaves similar to X-ray: heat appears to convert sublethal damage to lethal damage, which reduces the expression of malignant transformation.

The combination of chemotherapy and hyperthermia may not only be advantageous for the treatment of primary cancers, but may also result in a lower risk of treatment-induced secondary cancers. Table 1. displays exemplarily possible mechanisms how HT is capable of increasing the efficacy of certain classes of chemotherapeutic agents. How HT adds to many chemotherapeutics has been just recently overarchingly reviewed by Dr. Issels.

6. Technology for application and monitoring of hyperthermia

For heating of superficial and deep seated tumors many methods have been developed and studied in past. During the 80's of the last century, many home-made and commercially available hyperthermia systems were in clinical use.

Class of agent	Name	Cellular target	Mechanism
Platinum drug	Cisplatin	Membranes and DNA	Increased drug uptake, increased DNA-adducts and protein binding, increased cell death
Alkylating agents	Cyclophosphamide Mitomycin	DNA strand breaks DNA	Increased rate of alkylation Increased radical production
Antibiotics	Doxorubicin Mitoxantrone	Membranes and DNA	Increased drug uptake, increased drug half-life, increased oxygen radical production Increased drug uptake, increased inhibition of topoisomerase II

Table 1. Modulation of Cytotoxic Properties of Chemotherapeutics by HT

However, not all of them were capable of heating the target regions up to the desired temperatures. Electromagnetic energy (microwaves for superficial tumors and radiofrequency of deep heating) has been shown to be more suitable than other methods like ultrasound. Ultrasound used for thermal ablation (high intensity focused ultrasound) of small lesions has some disadvantages (e. g. reflections at bone structures, cavitation at air filled gaps) in heating larger volumes.

For the delivery of electromagnetic energy to the tumor tissues, two basic methods are used: radiative antennas and capacitor plates. Systems using capacitor plates are not able to steer the distribution of the energy. The energy absorption in the tissues between the plates depends on the characteristics of the tissues and is modified by blood perfusion. Therefore, overheating of poorly perfused fatty tissues is very common by using this technology and selectively heating of deep seated tumors is not possible. Hyperthermia systems with radiative antennas are used for superficial and deep heating. For superficial heating, waveguide or spiral antenna applicators are used. To increase the heating patterns for treatment of large lesions (e. g. breast wall recurrence of breast carcinoma) multiple antenna applicators have been developed. The penetration depth of systems for superficial heating is limited to 3 to 5 centimeters.

Selective deep heating of tumors is possible using radiative antenna arrays. Dipole antennas or waveguide applicators are placed around the body part containing the tumor. By steering amplitude and phase of the electromagnetic waves radiated from the antennas, a constructive interference is created at the target zone (tumor region). This means that high density electromagnetic fields are present in the tumor region. The energy of those fields is transformed into heat.

Monitoring of the temperature in the tumors and the surrounding tissues is mandatory. Because of the blood flow and its variations caused by the increased temperature, a homogeneous heating of the tumor region is not very likely. Therefore, the temperature of the tumor and the surrounding tissue cannot be predicted or calculated and must thus be measured. Temperature measurements can be performed by temperature probes or non-invasive methods. Temperature probes can be inserted into the human body either in natural orifices or percutaneously. In clinical practice, blindended plastic catheters are placed in and near the tumor region. Temperature probes are inserted in these catheters to measure the temperature. A continuous measurement is possible by using probes that are non-disturbed by electromagnetic fields. To get more temperature information, the probe can be mechanically moved along the catheter tracks. The use of multi-sensor probes is also possible. However, the temperature information obtained by those methods is rather limited. A lot of non-invasive methods for temperature mapping have been studied. Temperature maps derived from magnetic resonance images (MRI) have been shown to be a practical method in clinical routine and is used by a hybrid system for temperature-controlled hyperthermia (Figure 2.).

The method uses the temperature depended shift of the proton resonance frequency. This shift is included in the phase images acquired with a gradient echo sequence. By subtracting the images from a basal ("cold") image data set, the changes in proton resonance frequency can be recalculated into temperature changes. Part of the changes in proton resonance frequency is not caused by changes in temperatures but in perfusion. However, the comparison between MRI temperature measurements and temperature probe measurements showed a very good correlation. The resulting data set is color-coded (blue- cooler, green no change, orange, red and yellow-hotter) and superposed in the MR images. After some error corrections (e. g. drift of the static magnet field of the MRI system) the images are displayed as a temperature map in a three-dimensional data set. The temperature resolution is about 0.5 °C. Since this method has some disadvantages in clinical routine (e.g. movement of the patient creates artifacts) other methods like true T1 imaging or spectroscopy methods are under investigation.

7. Cancer and immune system – immune modulation by hyperthermia

Immune Evasion

Pre-malignant or mutated cells are normally removed efficiently by the immune system. It is well known that mice lacking essential components of the immune system get more susceptible to develop spontaneous tumors. Cancer cells have evolved manifold mechanisms to avoid the so called immunosurveillance, reviewed in. This seventh hallmark of cancer, to escape innate and adaptive immune responses, is conducted through immunoselection (selection of non-immunogenic tumor cell variants) and immunosubversion (active suppression of the immune response). Data from various human studies support the system of cancer immunosurveillance, which includes CD8+ T-cells, TH1 cells, NK cells, the local suppression by Tregs, and different tumor-cell products.

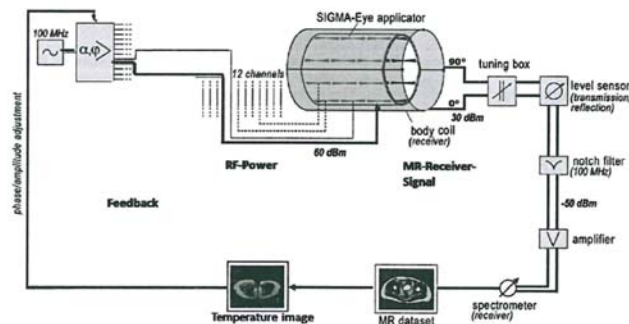


Figure 2. Schematic diagram of a hybrid system for temperature-controlled hyperthermia

The hybrid system consists of a hyperthermia system (BSD 2000/3D-MRI) and a magnetic resonance tomograph (Siemens Magnetom Symphony). This new technique provides the quality-assured heating of tumor tissues, even in deep seated malignancies. Modified according to Wust et al.

7.1 Ionizing Irradiation and Immune System

Besides the targeted effects of X-ray (DNA-damage) described in section 2, so called non-targeted effects exist, which influence "bystander" cells that received no irradiation themselves. This bystander effect may be conducted through genomic instability transmitted to the cell's progeny over generations or through various damage signals transmitted by irradiated cells to non-irradiated cells. Similar effects have been described for the abscopal effect, which is also called distant bystander effect. It describes the phenomenon that local irradiation of a specific body part results in a systemic outcome that is caused by the immune system. The involvement of the immune system could also be shown by in vivo experiments, in which irradiated immune competent mice showed tumor regression, whereas immune deficient nude mice showed tumor progression.

Immunogenic Cell Death

Because of the immune evasion of malignant cells, cancer therapy should not only stop the proliferation of cancer cells, and kill them but also restore a specific anti-tumor immunity against residual cancer (stem) cells and (micro-) metastases. This requires the induction of immunogenic cancer cell death forms, an increase in tumor antigen presentation, and a decrease in immune regulatory cells.

Cell death can be classified in two extreme forms: apoptosis and necrosis. Apoptosis is a physiologically controlled process, which is normally non-immunogenic or even anti-inflammatory, due to eat-me signals on the dying cells and the subsequent efficient clearance by macrophages. Primary and secondary necrosis, in contrast, both lead to inflammation, because of a

loss of the cellular membrane integrity and the successive release of danger signals or damage-associated molecular patterns (DAMP). These danger signals can be recognized by innate immune cells or dendritic cells (DC) subsequently, which together with antigenic peptides lead to DC maturation and the induction of an innate or adaptive immune response, respectively. Potent danger signals known are for example high-mobility group box 1 protein (HMGB1) and heat-shock proteins like HSP70.

7.2 Immune Functions of HMGB1

The HMGB1 protein is one prominent example of a danger signal being involved in inflammatory conditions. HMGB1 can be actively secreted or passively released during necrosis but not apoptosis, leading to immune activation. In the scope of cancer, extracellular HMGB1 can lead to chronic inflammatory responses that may lead to enhanced tumor cell survival, expansion and metastases. However, a therapy-induced pulsatile release of HMGB1 is capable of inducing specific and long-lasting anti-tumor immunity.

The amount of intracellular HMGB1 was shown to decrease after combined treatment with HT and ionizing irradiation, in comparison to single treatments. Moderate hyperthermia alone is capable of inducing necrotic tumor cell death forms, but only combined treatments (RT plus HT) led to high amounts of immunogenic necrotic cell death forms. We have further shown that combining RT with HT induces the release of HMGB1 by necrotic colorectal tumor cells, a process contributing to anti-tumor immunity.

7.3 Immune Functions of HSP70

HSP70 is a molecular chaperone, present in all cellular and subcellular compartments. It is often overexpressed in tumor cells and could further be found membrane-bound on the surface of tumor cells. Heat-stressed tumor cells release heat-shock proteins, which in turn.

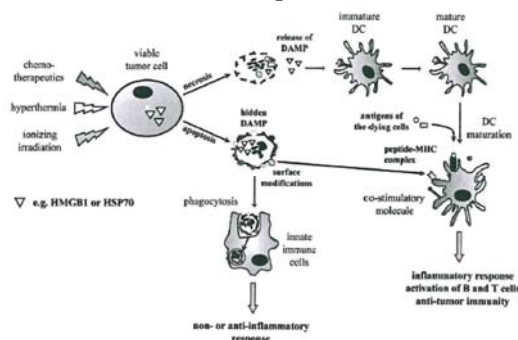


Figure 3. Immune modulation by anti-cancer therapy-induced immunogenic tumor cell death forms

Necrotic cell death forms lead to the release of various DAMP like HMGB1 or HSP70. Subsequent recognition by immature DC leads to DC maturation and together with uptake and presentation of peptides of the dying cells to activation of a specific anti-tumor immunity. Apoptotic cells are normally nonimmunogenic, but special surface modifications (like the exposure of calreticulin) also induce immunogenicity.

DC: dendritic cell; DAMP: damage-associated molecular pattern. activate tumor cells to produce chemokines for the attraction of cells of the adaptive immune system, like DC and T-cells. Once in the extracellular space, being e.g. released by necrotic tumor cells, HSP70 gains potent immune stimulatory functions by chaperoning peptides. These HSP70-peptide complexes can instruct DC to cross-present endogenously expressed, non-mutated tumor antigenic peptides. Simultaneously, HSP can also act as free soluble proteins and stimulate the innate immune system by inducing the maturation of DC, the secretion of pro-inflammatory cytokines, and by activating NK cells. Furthermore, HSP exposed membrane-bound on the cell surface render cells more susceptible to

NK cell lysis, which may represent an important cytotoxic mechanism induced by moderate HT treatment. Even hyperthermia in the fever range significantly increases NK cell cytotoxicity against tumor cells, improving the long-term efficacy of clinical HT. Combining HT with immune therapy with DC was also shown to increase the activity of CD8+ T-cells. These properties have made HSP attractive for the development of autologous tumor vaccines that are currently evaluated in clinical trials.

Further known danger signals are uric acid and ATP. Hyperthermia treatment often leads to the depletion of ATP, due to an increased metabolism in the tumor cells. Nevertheless, a reduced cellular ATP level is one initiating step of necrotic cell death, thereby leading to specific immune activation.

Current chemotherapeutics act mostly immunosuppressive, owing to the mainly non-specific cytostatic and cytotoxic effects. However, some anti-cancer agents can induce immune responses, e.g. cyclophosphamide which selectively depletes Tregs and restores normal CD8+ T-cell and NK-cell functions in patients. Kroemer's group could show recently, that some chemotherapeutic agents (mainly anthracyclines) are also able to induce immunogenic forms of apoptosis. Responsible is the very early membrane expression of the endoplasmatic reticulum protein calreticulin, which acts as eat-me signal for DC. Taken together, combinatory treatments (CT, RT and HT) may induce immunogenic necrotic and apoptotic tumor cell death forms finally leading to specific anti-tumor immunity (Figure 3.). HT in combination with standard treatments and HSP-based vaccination, like the autologous transfer of HSP-activated NK cells, may also offer a great potential as a new approach to directly activate the immune system of the patient at the tumor site. Future pre-clinical and clinical studies should focus on immune modulatory effects of HT, to gain more evidence based data supporting that HT in multimodal therapy settings leads to specific anti-tumor immunity.

8. Effectiveness of HT treatment in cancer therapy

Various clinical randomized studies have already proven the effectiveness of an additional hyperthermia treatment, with one or two sessions per week before or after the radiation fraction, for various human cancer entities: cervical, bladder, head & neck, anal canal, esophageal, malignant melanoma, and breast cancer. Clinical endpoints improved include response, local control or disease-free survival of patients without an increase in toxicity or late side effects, which make local or regional HT treatments attractive as radio- or chemosensitizer. Furthermore, a non-randomized clinical trial on bladder cancer revealed promising first results of integrating hyperthermia into the trimodality treatment of transurethral resection and radiochemotherapy (RCT) with enhanced response rates, local control rates, and overall survival.

In patients with poor risk malignancies of the childhood the introduction of HT into standard treatment protocols may be promising to improve tumor response and event-free survival. In addition to the direct interactions of HT with chemotherapy and/or radiotherapy, pharmacological targeted therapies are of great interest. In children and adolescents with unresectable malignant tumors thermochemotherapy resulted in substantial therapeutic efficacy and facilitated complete tumor resection in about 50% of the operated patients.

In general, the outcome of an additional hyperthermia treatment in multimodal therapies is strongly based on the quality of the applied heat treatment. Most of the published randomized data evaluated the effects of local and regional HT combined with RT.

8.1 Rectal Cancer

The Russian randomized trial published by Berdov et al. in 1990 compared RT alone (total dose 40 Gy in 10 fractions of 4 Gy) with RT plus HT treatment. If possible, the tumor was resected

afterwards or another RT (10x 4 Gy) session was applied. The results showed that the complete and partial response rate, 16 vs. 2% and 54 vs. 34%, respectively, were significantly better when RT was combined with HT. The overall survival rate (5 years) was likewise increased (36% vs. 7%) when HT treatment was added to RT. Another study randomized patients (n=143) with primary or recurrent rectal cancer. The radiation dose was 46-50 Gy in 1.8-2.3 Gy daily fractions, followed if possible by a boost of 10-24 Gy to the tumour mass. Regional hyperthermia was added once weekly with a total of five treatments. No significant differences in complete response and overall survival rates had been found, although there was a trend to better results for the combined arm. Late toxicity was not significantly enhanced by the addition of hyperthermia.

In a German trial, advanced rectal cancer was treated with neoadjuvant RCT or RCT plus deep regional hyperthermia (once a week before radiotherapy), followed by surgery and another CT session. The tumor response with complete and partial response rates could be significantly enhanced by the combined therapy (66% vs. 49%, $P<0.05$). In addition, the time to local recurrence (28 vs. 20 months, $P<0.05$) could be significantly delayed by RHT. Local control could not be significantly improved by the additional hyperthermia treatment. Overall survival probability (3years) was 89% vs. 80% in favor of the HT group, but also not statistically significant.

In a recent Cochrane review, the existing evidence for the possible beneficial effects of combined HT and RT treatment was summarized. A total of 520 patients from six randomized trials were analyzed. Overall survival after 2 years was significantly better in the hyperthermia group ($P=0.001$) compared to radiotherapy alone, but this difference disappeared after a longer period. A significantly higher pathologically complete remission rate (pCR) was observed in the hyperthermia group ($P=0.01$). However, the authors concluded that further studies are needed in well selected and quality controlled randomized trials.

A multi-institutional phase-II study for locally recurrent rectal cancer (HyRec Trial) is planned, where neoadjuvant chemoradiation with 5-fluorouracil/capecitabine and oxaliplatin and a total dose of 45 Gy will be combined with deep regional HT. Primary endpoints are feasibility rate and number of HT applications by patient.

8.2 Breast Cancer

For primary or recurrent breast cancer, several randomized trials (DHG (NL), MRC (UK), ESHO (EU), PMH (CDN)) were analyzed in a European meta-analysis which compared RT (biologically effective radiation dose between 40 and 70 Gy, with single irradiations from 1.8 to 4 Gy) with RT plus superficial HT. In this study, it was set value on a comparable temperature range (42.5-43 °C) whereas the number of hyperthermia treatments varied from 2 to 8. The primary endpoint of all trials was local complete response. A total of 306 patients were analyzed: 44% (135/306) received radiotherapy alone, and 56% (171/306) received combined treatment.

Compared to RT alone, RT plus hyperthermia was shown to improve the overall complete remission rate (59% vs. 41%, $P<0.001$) as well as the local control in patients. Despite a significantly enhanced local control, the overall survival was not improved by HT. The clinically relevant acute or long-term toxicity did not increase compared to irradiation alone, even in patients who had received RT before.

Another randomized trial was performed by the Duke University in USA. A total of 108 patients with superficial tumors of different origins were analyzed in detail, treated with radiation alone (n=52) or combined with superficial HT (n=56). Among patients in both arms, the median radiation dose was 41 Gy (range 18 to 66 Gy) if prior radiation was given and the median dose was 60 Gy (range 24 to 70 Gy) if no prior radiation was given. The complete remission rate in the HT arm was 66%, whereas the CR rate in the RT alone arm was only 42% ($P=0.02$). Previously irradiated patients showed the most improvement in local control; 15 of 22 patients in the HT arm (68%) had a complete remission versus 4 of 17 patients in the no-HT arm (24%). The overall survival rate was not found to be significantly different between the two groups.

8.3 Cervical Cancer

For locally advanced cervical cancer, several randomized trials were conducted comparing RT with RT plus HT treatment.

The Dutch Deep Hyperthermia Trial compared RT with combined RHT in 114 women (FIGO stages IIB-IVA). Radiotherapy was applied to a median total dose of 68 Gy and hyperthermia was administered once a week. Primary and secondary end points were local control and overall survival. Local control remained better in the combined group (37% vs. 56%; $P=0.01$) and in addition, overall survival was better (20% vs. 37%; $P=0.03$) after 12 years of follow-up. Pelvic-free-failure survival was similarly enhanced when hyperthermia was added, demonstrated by 61 vs. 41%. Late toxicities were not significantly different in both groups. Therefore, this combined treatment should at least be considered for patients who are unfit to receive chemotherapy.

A further randomized trial investigated the impact of hyperthermia in cervical carcinoma patients with FIGO Stage IIIB ($n=40$). The complete response rate was 80% in the combined group vs. 50% in the radiotherapy group ($P=0.048$). Overall survival rates (3 years), disease-free survival and relapse-free survival were better in the HT group than those of the patients treated with RT alone. Combined radiotherapy and hyperthermia was well tolerated and did not show significant changes in acute or longterm toxicity.

In 64 patients with cervical cancer (FIGO IIIB), Datta et al. reported on improved complete remission, pelvic control, and overall survival rates in the combined therapy group with radiation and hyperthermia.

In a randomized trial with a total of 50 patients (FIGO stages II-III) Sharma et al. reported on a better pelvic control (70% vs. 50%) in favour of the HT group. A further randomized study compared HT with RT in 120 patients with FIGO IIB-IIIB disease. Combined treatments showed significantly enhanced complete response rates.

8.4 Soft Tissue Sarcoma

For high-risk soft tissue sarcomas, a large phase-III randomized prospective trial compared neoadjuvant chemotherapy with or without additional RHT (EORTC 62961/ESHO RHT95 Intergroup Trial) in order to define the impact of RHT within the treatment strategy for patients with primary or recurrent high-risk soft tissue sarcoma. It was the biggest randomized study ever conducted on hyperthermia treatment, starting in 1997 and finished in 2006.

First results after median follow-up for all patients of 24.9 months (0-106.9 months) showed a significantly enhanced disease-free survival (31.7 vs. 16.2 months) for the patients who received CT (etoposide, ifosfamide and doxorubicin (adriamycin)) combined with RHT ($n=169$) compared to treatment with CT alone ($n=172$), respectively. Furthermore, tumor response and local progression free survival could also be significantly enhanced by the additional hyperthermia treatment, suggesting that postsurgical HT treatment may be crucial for local control. A further important observation was made that patients seem to improve most from the combined treatment regimen when chemotherapy is given combined with regional hyperthermia after inadequate surgery. A recent update on this phase-III randomized prospective trial after a median follow-up of 34 months could strengthen the previous results: median disease-free survival was 32 vs. 18 months, with an absolute difference at 2 years of 14%. Overall response was more than twice as high, 28.8 vs. 12.7% in favor of the hyperthermia group.

8.5 Further HT Techniques

Besides Regional Hyperthermia other treatment modalities are used in clinical concepts today. Cytoreductive surgery followed by hyperthermia intraperitoneal chemotherapy (HIPEC) was applied in various phase-II studies and one phase-III study (outlined in). One randomized trial for the prevention of peritoneal recurrence of gastric cancer and one multicentre clinical trial

comparing intravesical CT alone with microwave HT for prevention of recurrence in STCC of the bladder showed slight improvements in terms of local recurrence and recurrence-free survival, respectively. However, the HIPEC techniques still remain an experimental approach.

Another important technique is the hyperthermic isolated limb perfusion (ILP). Tumor necrosis factor (TNF) plus melphalan-based hyperthermic ILP has been proven to be highly effective in multicentre non-randomised trial settings, demonstrating response rates above 70% and limb salvage rates above 80% especially in locally advanced soft tissue sarcomas of the extremities. Similar to the HIPEC techniques, the relative effects of HT combined with TNF and melphalan are still not completely determined.

Conclusion

Taken together, hyperthermia in combination with radiotherapy and chemotherapy can be a useful multifunctional weapon to fight various tumor entities. There is no question of its efficacy in the treatment of cancer patients, provided that the achieved temperature in the tumor tissue is tightly quality controlled. Today, the important question remains for which tumor entity and clinical stage of disease patients are benefiting the most from an additional hyperthermia treatment. The technical improvements of the last ten years led to a quality assured heat delivery in the tumor tissue, even in deep seated malignancies, and therefore dose limiting "hot spots" in normal tissues can be avoided. The mechanisms of HT are complex and its pleiotropic effects are in favor of the combined use with CT and RT in the clinical situation. The current data from randomized phase-III studies clearly indicate the beneficial effects of HT. Future research and clinical trials should prove that in multimodal treatments hyperthermia may induce specific and long-lasting anti-tumor immunity.

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Abbreviations

ATP = Adenosintriphosphate
CT = Chemotherapy
DAMP = Damage-associated molecular pattern
DC = Dendritic cell
DSB= Double-strand break
HIPEC = Hyperthermic intraperitoneal chemotherapy
HSP =heat-shock protein
ILP = Isolated limb perfusion
HT = Hyperthermia
NK cell =Natural killer cell
RCT= Radiochemotherapy
RHT = Regional hyperthermia
RT = Radiotherapy
TER = Thermal enhancement ratio