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The cellular and molecular basis of hyperthermia

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Abstract

In oncology, the term 'hyperthermia' refers to the treatment of malignant diseases by administering heat in various ways. Hyperthermia is usually applied as an adjunct to an already established treatment modality (especially radiotherapy and chemotherapy), where tumor temperatures in the range of 40–43 °C are aspired. In several clinical phase-III trials, an improvement of both local control and survival rates have been demonstrated by adding local/regional hyperthermia to radiotherapy in patients with locally advanced or recurrent superficial and pelvic tumors. In addition, interstitial hyperthermia, hyperthermic chemoperfusion, and whole-body hyperthermia (WBH) are under clinical investigation, and some positive comparative trials have already been completed. In parallel to clinical research, several aspects of heat action have been examined in numerous pre-clinical studies since the 1970s. However, an unequivocal identification of the mechanisms leading to favorable clinical results of hyperthermia have not yet been identified for various reasons. This manuscript deals with discussions concerning the direct cytotoxic effect of heat, heat-induced alterations of the tumor microenvironment, synergism of heat in conjunction with radiation and drugs, as well as, the presumed cellular effects of hyperthermia including the expression of heat-shock proteins (HSP), induction and regulation of apoptosis, signal transduction, and modulation of drug resistance by hyperthermia.

Keywords: Hyperthermia-induced; Clinical trials; Radiotherapy; Cytostatic therapy; Thermal radiosensitization; Thermal chemosensitization; Tumor vascularization; Heat-shock proteins; Apoptosis; Drug resistance

1. Introduction

The term 'hyperthermia' refers to various techniques of heat application administered as an adjunct to already established strategies (especially radiotherapy and chemotherapy) in the treatment of cancer patients. Regarding the different hyperthermia approaches, therapeutical potentials, expenditure of treatment, technical problems and evidence of effectiveness are diverse. Local/interstitial, and regional hyperthermia are distinguished from whole-body hyperthermia (WBH), and hyperthermic perfusion techniques (e.g. hyperthermic isolated limb perfusion (HILP), hyperthermic peritoneal perfusion (HPP)). All hyperthermia modalities have in common that their efficacy is not enough to replace any one of the established therapy modalities when applied alone, but, undoubtedly, they are suitable enough to enhance the cell-killing effect of cytotoxic drugs and/or radiation ('thermal chemosensitization', 'thermal radiosensitization'). Therefore, hyperthermia aims at improving the results of the conventional treatment strategies within the framework of multimodal treatment concepts.

One major argument for utilizing local and regional hyperthermia, results from the convincing clinical results obtained from trials on patients with locally ad-vanced malignancies. Improved response and survival rates were observed in patients treated with hyperthermia and radiotherapy compared with radiotherapy alone in several comparative phase-III trials, with a very distinct response benefit in the hyperthermia group in some of these studies (Table 1, [1–19]).

In spite of the inhomogeneous temperatures acquired (due to inhomogenous temperature deposition and physiological reasons), locoregional techniques of

Table 1
Randomized trials on hyperthermia

Refs.	Author	Year	Type of hyperthermia	Tumor site	Control arm	Experimental arm	Number of patients	Primary objective	HT better? ($P = 0.05$)?	Survival benefit?
[1]	Datta	1990	LHT	Head and neck (primary)	Rad	Rad+LHT	65	Response (at 8 weeks)	×	
[2]	Overgaard	1996	LHT	Melanoma (metastatic/recurrent)	Rad	Rad+LHT	68 (128 Lesions)	CR (at 3 months)	×	×
[3]	Perez	1991	LHT	Superficial (h & n, breast, misc.)	Rad	Rad+LHT	245	Initial response	(×)	
[4]	Valdagni	1993	LHT	Head and neck (N3 primary)	Rad	Rad+LHT (2–6)	44	Response (3 months)	×	×
[5]	Vernon	1996	LHT	Breast (advanced primary/recurrent)	Rad	Rad+LHT	307 (317 Lesions)	Initial response	×	
[6]	Emami	1996	IHT	Superficial (h & n, breast, melanoma, others)	IRT	IRT+IHT	184	Best response		
[7]	Sneed	1998	IHT	Glioblastoma	brachyRad	brachyRad+IHT	79	Two-year survival	×	×
[8]	Berdov	1990	EHT	Rectum (T4, locally advanced)	Rad	Rad +EHT	115	Initial response	×	×
[9]	Kitamura	1995	EHT	Oesophagus (stages I–IV, neoadjuvant)	Rad+ZTX	Rad+ZTX+EHT	66	Histological CR	×	×
[10]	Sugimachi	1998	EHT	Oesophagus (stage I–IV, neoadjuvant)	ZTX	ZTX+EHT	40	Initial response	×	
[11]	Harima	2001	Regional pelvic HT	Cervix uteri (primary, stage III)	Rad	Rad+RHT	40	Initial CR	×	
[12]	Zee	2000	Regional pelvic HT	Primary/recurrent pelvic (cervix, rectum, bladder)	Rad	Rad+RHT	361	CR rate	×	×
[13]	Hamazoe	1993	Peritoneal perfusion	Stomach ($\geq$T3, locally advanced)	OP	OP+IPP	82	Five-year survival	×	×
[14]	Ghussen	1989	Limb perfusion	Melanoma (stages I–III)	OP	OP+ILP	107	Disease-free survival	×	×
[15]	Hafstrom	1991	Limb perfusion	Melanoma (recurrent)	OP	OP+ILP	69	Disease-free survival	×	
[16]	Koops	1998	Limb perfusion	Melanoma (stages I–III)	OP	OP+ILP	832	Disease-free survival		

Abbreviations: CR, complete response; EHT, endocavitary hyperthermia; HT, hyperthermia; IHT, interstitial hyperthermia; ILP, isolated limb perfusion; IPP, isolated peritoneal perfusion; IRT, interstitial radiotherapy; LHT, local hyperthermia; Rad, radiotherapy; RHT, regional hyperthermia; ZTX, cytostatic therapy.

Table 2
Interactions between heat and drugs

Pharmocodynamics
Acceleration of primary mode of action (alkylating reaction, protein damage, oxygen-radicals; DNA-strand breaks)
Increased intracellular drug concentration (drug uptake, membrane damage, protein damage, pH changes)
Pharmacokinetics
Drug uptake (decreased gastrointestinal or transdermal absorption)
Distribution (pH changes, fluid sequestration, increased tumor blood flow)
Metabolism and excretion (changes in hepatic and renal blood flow)

Table 4
Molecular effectors of hyperthermia

Cell membrane, cytoskeleton
Changes in fluidity/stability of cell membrane
Changes in cell shape
Impaired transmembranal transport
Changes in membrane potential
Modulation of transmembranal efflux pumps (MDR)
Apoptosis induction
Intracellular proteins
Impairment of protein synthesis
Protein denaturation
Aggregation of proteins at the nuclear matrix
Induction of HSP-synthesis
Nucleic acids
Impairment of RNA/DNA synthesis
Inhibition of repair enzymes
Altered DNA conformation
Other alterations of cell function
Intracellular metabolism of other substrates
Gene expression, signal transduction

hyperthermia revealed a significant correlation between thermal dose and clinical outcome in these trials. This also holds true for further investigations, where local and regional hyperthermia were combined with chemotherapy and radio-chemotherapy in the scope of phase-II trials. These findings strongly suggest, on principle, the clinical effectiveness of hyperthermia [20–25].

Contrarily to hyperthermic radiotherapy, only few comparative trials have been completed to date where hyperthermia was applied as an adjunct to chemotherapy [16–19]. All those trials refer to the comparison of surgery alone with surgery followed by adjuvant hyperthermic chemoperfusion, applied either as HILP with melphalan in patients with melanomas, or as HPP with mitomycin in patients with gastric cancer. A benefit for hyperthermic chemoperfusion was observed in two of these 'adjuvant' trials (Table 1). Regarding HILP induction therapy in patients with melanomas or sarcomas limited to one limb, it is remarkable that no phase-III trials have been performed yet, due to the very high response rates have been observed in some non-randomized series (reviewed in [26,27]; Tables 2–4).

Regarding regional hyperthermia of the pelvis and extremities in conjunction with chemotherapy, encouraging results of phase-II trials gave raise to the initiation of various comparative trials during the last years. Special attention is attracted to one multicenter EORTC/ESHO-trial subjecting the evaluation of neoadjuvant chemotherapy $\pm$RHT in high-risk soft tissue sarcomas of the limb or pelvis followed by surgery, adjuvant radiotherapy, and subsequent chemotherapy again ($\pm$RHT). Further ongoing phase-III trials are addressing the effect of chemotherapy with Cisplatin $\pm$RHT in pre-irradiated patients with recurrent cervical cancer, as well as, preoperative neoadju-

Table 3
Thermal enhancement ratios (TER) of selected drugs in various animal tumors

Drug	TER at 40–42 °C (range)	TER at 42.5–44 °C (range)	Tumor entity	Authors
Cyclophosphamide	1.52–2.28	1.27–2.74	RIF-1, Mammary-Ca, Fsa-II, Lewis lung-Ca	Honess, 1982; Monge, 1988; Hazen, 1981; Urano, 1985
BCNU	1.5–2.96	2.71	RIF-1, KHT, Fsa-II	Honess, 1982; Honess, 1985; Urano, 1991
Melphalan	1.5–3.9	n.d.	RIF-1, KHT, Fsa-II	Honess, 1985; Urano, 1995
Cisplatin	1.48–3.9	1.39–4.96	BT4A, Mammary-Ca, Lewis lung-Ca, SCC VII, R1-RMS	Mella, 1985; Douple, 1982; Herman, 1988; Nishimura, 1990; Lindegaard, 1992; van Bree, 1996
Bleomycin	1.24	1.65–2.90	Adeno-Ca 284, SCC, Fsa-II	von Sazazepauski, 1981; Hassanzadeh, 1982; Urano, 1990
Mitomycin C	1.0	2.8	Mammary-Ca, Fsa-I1	Monge, 1989; Urano, 1994
5-Fluorouracil	1.0	1.0	Human leukemia, Colon-Ca, Fsa-II	Mini, 1986; Rose, 1979; Urano, 1991
Doxorubicin	1.0	1.0	Mammary-Ca, Fsa-II	Monge, 1988; Urano, 1994

Data given as calculated by Urano et al., 1999; [27].

vant radiochemotherapy ± RHT in patients with primary non-metastatic, locally advanced rectal cancer [28–30].

Available data on WBH in conjunction with chemotherapy merely demonstrate feasibility and acceptable toxicity of this approach when radiant heat applicators are employed to induce homogenous body-care temperatures up to 42 °C for 1 h (Fig. 1). The application of WBH is relatively invasive and accompanied with a broader spectrum of toxic effects than local and regional hyperthermia. On the other hand, WBH is part of a systemic therapy with the potential to treat metastatic disease. This is the reason for the commencement of the first phase-III trials in Germany on WBH with adjunctive chemotherapy in patients with metastatic cancer [31–34].

Recent reviews on clinical hyperthermia are given in [27,28,35].

Parallel to these encouraging clinical results, a large number of pre-clinical investigations have been performed on various aspects of heat effects since the early-70s. In vitro and animal hyperthermic experiments exhibited a direct cell killing effect at temperatures ranging from 41 to 47 °C [27,36,37]. Further studies have revealed a large variability of hyperthermia effects regarding cell death (especially with respect to tumor entity, cell line, growth fraction). In general, hyperthermic cell death has been shown to be markedly enhanced at temperatures above 43 °C, as well as, in combination with radiation and various cytostatic drugs by sensitization. More recent publications have focused on the effect of hyperthermia on distinct cellular signalling pathways, particularly of those involved in 'heat shock response', cell cycle regulation, and apoptosis. Furthermore, hyperthermia influences tumor blood flow, oxygen and nutrient supply, as well as, the cellular immune response under in vivo conditions [38–46].

Despite a large number of publications on the pre-clinical aspects of hyperthermia, little certainty exists about the extent of transferring these results into clinical practice. This is mostly due to difficulties in achieving a direct correlation between clinical and molecular effects for practical and ethical reasons (as discussed in [47,48]). Here, we outline the major topics on the cellular and molecular targets of hyperthermia. However, since a medline-search for the term 'hyperthermia, induced' produces a total number of 11 233 citations (March 2001), we cannot give a complete summary of the facts here.

2. Basic features of hyperthermic cell death

2.1. Cytotoxic effect of hyperthermia

When exponentially growing cultured cells (e.g. Chinese hamster ovarian (CHO) cells) are exposed to a defined temperature between 41 and 47 °C, a dose–effect curve can be defined by plotting the rate of cell death against the duration of hyperthermia. The corresponding survival curves show a typical 'shoulder' that reflects a two-step process of cell killing. This is marked by a linear growth arrest in the beginning of heat exposure (reflecting a reversible, non-lethal heat damage), that is followed by exponential cell death (Fig. 2). One fundamental observation is, that the capability to induce cell death at lower temperatures < 42–43 °C

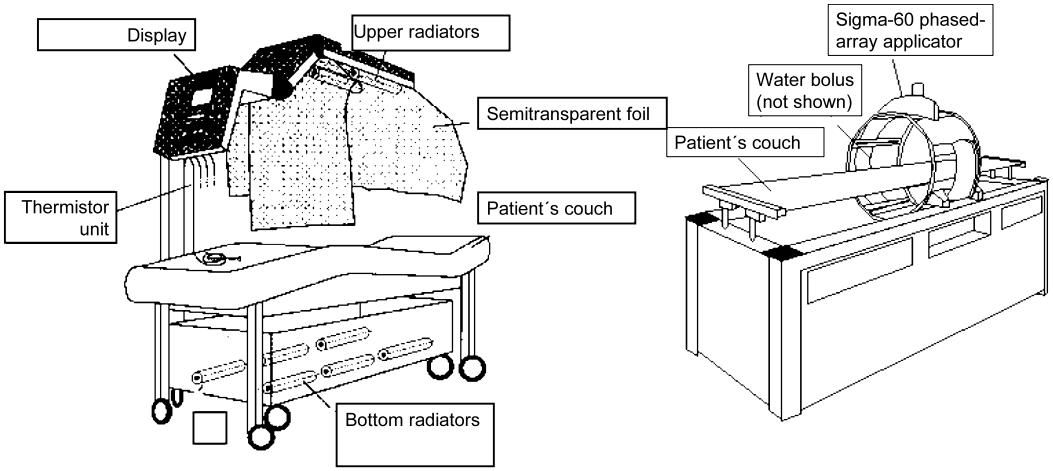

Fig. 1. Examples for commercially available hyperthermia devices. 'IRATHERM®-2000' radiant heat applicator (WBH) and 'BSD®-2000' regional hyperthermia system.

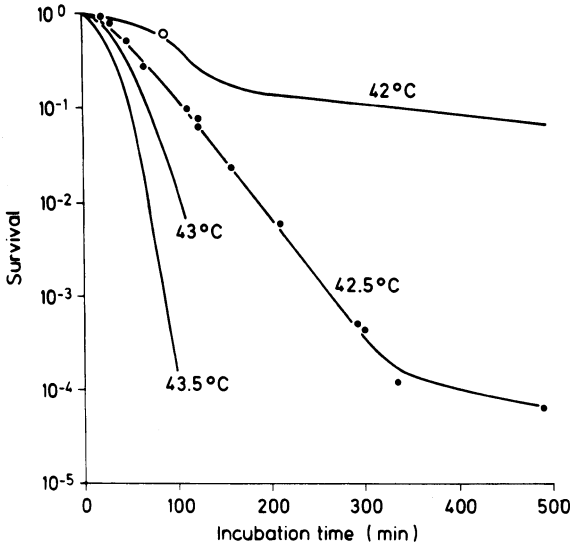

Fig. 2. Dose–response relationship of thermal cell killing. Schematic drawing of the surviving fraction of asynchronous CHO cells heated at different temperatures, emphasizing the typical 'shoulder' ([40,205], with permission).

(below a certain 'breakpoint'), is markedly lower than above 43 °C. Therefore, one common definition of the thermal dose (D) derived from exposure time (t) and given temperature (T), is $D = tR^{T=43}$ with $R = 2$ for temperatures ≥ 43 °C, and $R = 4$ for temperatures < 43 °C, or, in the case of varying T, a summation of dose fractions and duration ($t + T$).

The thermal dose required to induce hyperthermic cell death varies with factor 10 between different cell types (Fig. 3). However, there is a particular thermal dose variation in the survival curves where the transition into the exponential phase starts ('activation energy'). The thermal energy dose required to induce exponential cell death is closely correlated to that required for cellular protein denaturation and amounts to 140 kcal/mol in vitro, as well as, in experimental tumors. This led to the hypothesis that the cytotoxic effect of hyperthermia is mainly based on denaturation of cytoplasmatic and membrane proteins, although corresponding relationships have not been elaborated for sensitizing phenomena with respect to radiation and/or cytostatic drugs [40,46,49–52].

2.2. Thermal isoeffect dose

Calculation of the thermal dose applied in hyperthermia, as it can be derived from the above-mentioned formula, has also been integrated into the concept of the 'thermal isoeffect dose' (TID) that is commonly used in clinical practice in order to compare different hyperthermia exposures with each other. The TID was introduced to convert a given thermal dose into so-called 'equivalent heating minutes at 43 °C' (EM43). Calculation of the TID is using the fact that—as it known from cell death curves—a temperature decline of 1 °C in the temperature range between 42.5 and 47 °C can be compensated by doubling the exposition time ($R = 2$, see above), whereas below 42.5 °C the heat exposure has to be prolonged even more ($R = 4$, see above). Although the TID is influenced by a number of environmental factors in clinical hyperthermia (such as the time-behavior of temperature during the heating phase, temperature distribution, thermal tolerance, intracellular pH and other milieu factors), it seems to be a valuable approach to describe the effectiveness of a clinical heat treatment. This has been demonstrated in various trials on local and regional hyperthermia, where significant correlations between EM43 and response parameters have been observed [21,22,24,49,52,53].

2.3. Hyperthermic cell death in different phases of the cell cycle

Synchronized cell cultures exhibit variations in their susceptibility to heat in accordance to their phase in the cell cycle. In general, highest heat sensitivity can be observed during the mitotic phase. Microscopic examinations of M-phase cells subjected to hyperthermia show damage of their mitotic apparatus leading to inefficient mitosis and consecutive polyploidy. S-phase cells are also sensitive to hyperthermia, where chromosomal damage is observed. Both S- and M-phase cells undergo a 'slow mode of cell death' after hyperthermia, whereas those exposed to heat during G1-phase are relatively heat resistant and do not show any microscopic damage. Cells during G1-phase may follow a 'rapid mode of death' immediately after hyperthermia. These variations existing between the different cell cycle phases indicate the possible diversity of molecular mechanisms of cell death following hyperthermia [46,54–56].

2.4. Thermotolerance as an antagonist of hyperthermic cell death

Malignant cells exposed to temperatures < 43 °C or cooled down to 37 °C between two heat shock treatments > 43 °C, show an impairment in their susceptibity to heat-induced cytotoxicity that results in a flattened inactivation curve (Fig. 4). This phenomenon, the so called 'thermotolerance', is principally reversible. Thermotolerance of multifactorial origin as it is not inherited in cell cultures. It is at least partially based on the induction of heat-shock proteins (HSP) and other post-transitional adaptation processes (e.g. cell cycle arrest in the G2-phase, changes in cell metabolism). The ability to express thermal tolerance might be attenuated under some environmental conditions such as (sud-

denly) lowered intracellular pH, and may also occur together with some forms of acquired or inherited drug resistance (see below) [57–61].

3. Special features of in vivo hyperthermia

3.1. Hyperthermia > 42 °C induces alterations of tumor blood flow and microenvironment

The microenvironment of malignant tumors is characterized by a reduction of blood flow and blood vessel density that favors hypoxia, acidosis and energy deprivation. Hyperthermia at temperatures above 42 °C, besides its cytotoxic effect, has been shown to decrease tumor blood flow in a number of fundamental studies in the 70s and 80s, thereby impairing oxygen and nutrient supply, and inducing acidosis (although some exceptions have been reported). The thermal dose required to alter tumor blood supply varies between individual tumors and different tumor types. It seems to mostly depend on the percentage of responsive vessels that have maintained their ability of thermal regulation. In addition, heat induced damage of tumor

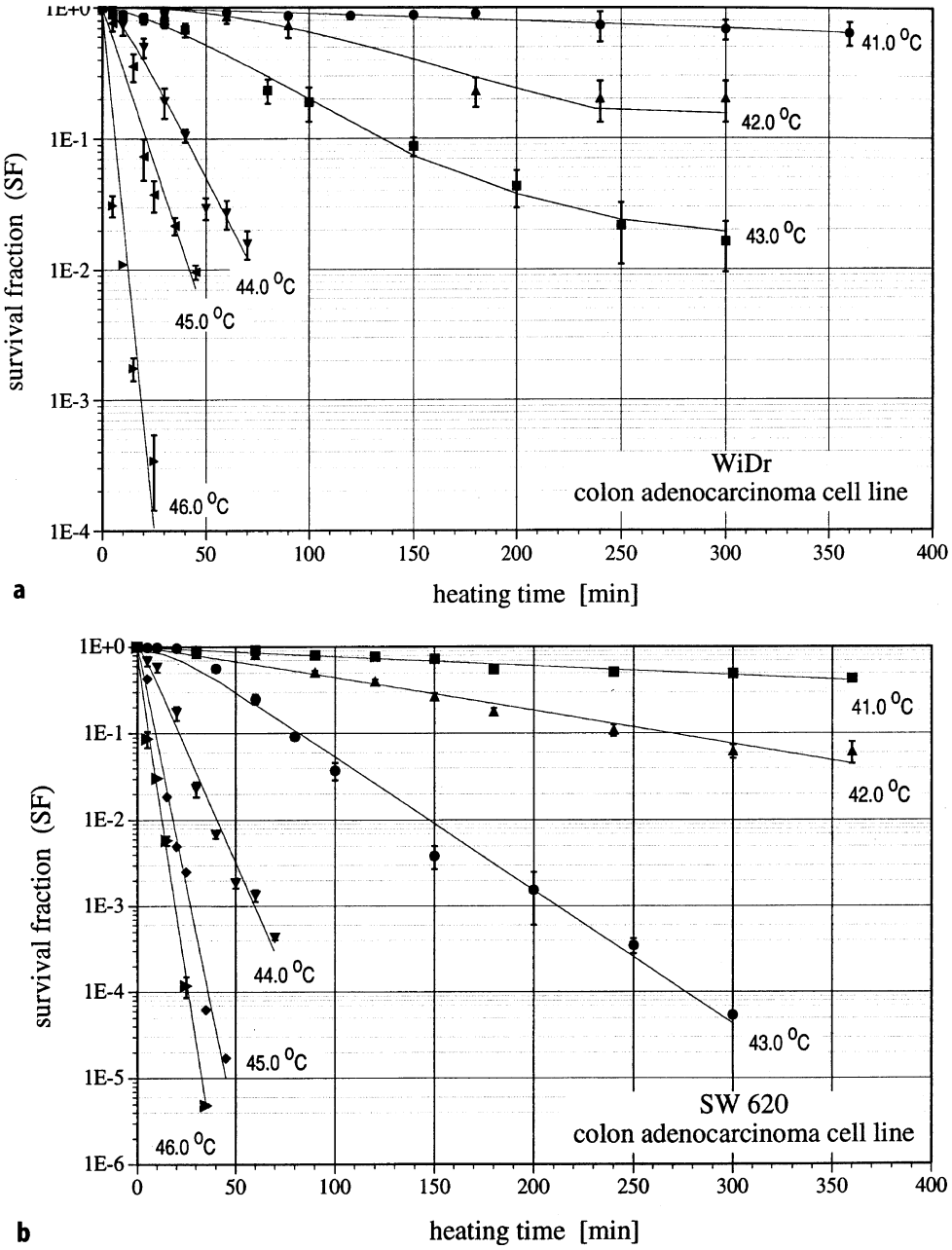

Fig. 3. Differences of the dose–response relationship in different cell lines. Survival curves of two different colon carcinoma cell lines indicate differences in susceptibility to heat ([206], with permission).

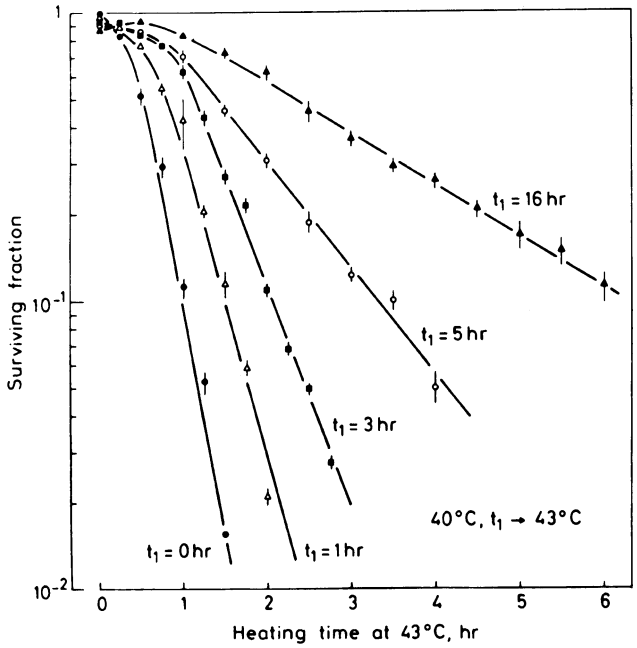

Fig. 4. Thermotolerance. Surviving fraction of CHO-cells at 43 °C that were preheated at 40 °C for various periods of time ([207], with permission).

vasculature may occur at temperatures which may alter, but not damage those of normal tissue. In some cases, hyperthermia-induced changes of microcirculation were reported to be irreversible, and in others a breakdown of circulation even occurred after termination of heat exposure. Remarkably, the considerable inhomogenicity of blood supply within the same tumor persists after heat treatment >42 °C. The morphological changes associated with hyperthermia include endothelial swelling, shift of plasma fluid into the interstitium, microthrombosis due to activation of hemostasis, and changes of the viscosity of blood cell membranes. All of these factors also promote a reduction of oxygen and nutrient supply, as well as, intratumoral acidosis [62–68] (Fig. 5).

3.2. Whole-body hyperthermia and von Ardenne's 'systemic Cancer Multistep Therapy'

Decrease of tumor blood flow in experimental tumors at temperatures >42 °C may be further enhanced by reactive hyperaemia of surrounding (healthy) tissues that have maintained their ability of thermoregulation. In animal models, this 'steal-phenomena' can be intensified by additional application of vasodilatating agents, or by inducing intratumoral lactic acidosis with highly-concentrated glucose-infusion [63,65,69–73].

The idea that hyperthermia might be able to starve out the tumor represents the basis for the concept of sCMT. sCMT has been concipated and propagated as a pre-clinical construct by M. von Ardenne in Germany since the late 1960s, consisting of WBH, induced hyperglycemia, and hyperoxaemia. Here, WBH is thought to induce a decrease in tumor blood and nutrition supply that is further enhanced by (hyperglycaemia-induced) intratumoral lactate acidosis and the above-mentioned vascular steal-effect. In parallel, hyperoxemia was induced to ensure oxygenation of healthy tissues. It has also been hypothesized that sCMT sensitizes tumor cells for cytostatic therapy and radiation. Even if the sCMT-theory has never been proven, it gained worldwide interest in the early-1970s.

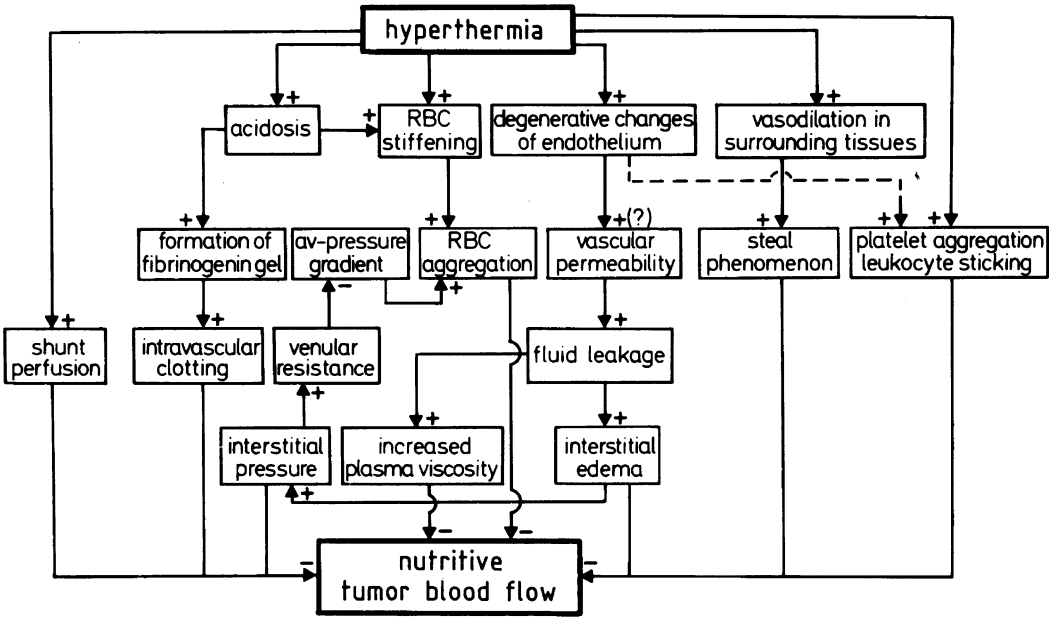

Fig. 5. Possible mechanisms involved in hyperthermia-induced breakdown of blood flow ([65], with permission).

The lack of systematic clinical trials on sCMT or WBH alone is mostly due to the fact that no appropriate devices were available up to the mid-1980s. All previously used WBH-applicators have been either not effective enough to induce body core temperatures of 41.8–42 °C, or treatment was too toxic. Today, at least two applicators are available at which temperatures in the range of 41.8–42.2 °C can be induced and maintained for 1 h by using radiant heat with acceptable toxicity. This enables the evaluation of WBH in conjunction with cytostatic therapy in larger clinical trials in the future. Interestingly, all recent WBH-investigators—most of them disapproving the concept of sCMT—generally recommend the application of hyperglycaemia and hyperoxaemia during therapy. Anyway, this recommendation is not based on the assumed antineoplastic properties of these applications, but on the consideration that supportive oxygen and nutrient supply may be helpful to prevent severe organ toxicity [34,74–79].

3.3. Does 'moderate' hyperthermia increase tumor blood flow?

Contrary to those earlier studies on microenvironmental changes in tumors treated with hyperthermia >42.0 °C, 'moderate' hyperthermia (<42.0 °C)—that is much more easy to apply in vivo and offers a better comparison to the clinical situation—has been demonstrated to rather improve tumor blood flow and thereby oxygen content. It, thereby, may increase the effectiveness of radiotherapy (which is more effective in tumors with higher oxygen supply) and chemotherapy (the delivery of which may be favored by increased tumor blood flow). Indeed, this hypothesis would give a good explanation for the correlation of applied thermal dose and clinical outcome, as was observed in clinical trials on local/regional hyperthermia where intratumoral temperatures do not exceed 42 °C during most of the treatment time. However, the situation in cancer patients appears to be much more complex than in experimental systems, and behavior of tumor vascularity under hyperthermic conditions may also depend on the method of hyperthermia applied. Provided the clinical effectiveness of certain hyperthermia applications is actually based on the breakdown of tumor vascularization, it may be useful to combine this effect with other antiangionetic agents in the future (some of which have been recently introduced into anticancer therapy). If, on the other hand, hyperthermia acts by increasing tumor blood supply, one possible consequence could be to combine hyperthermia with antineoplastic drugs ($\pm$ radiation) in tumors with low blood supply that can not be treated adequately by conventional modalities (e.g. local recurrences of pre-irradiated cervical, rectal, or breast cancer). At least, there are a number uncertainties concerning the effect on hyperthermia on blood flow, and this is why further investigations are desirable [62,66,80,81].

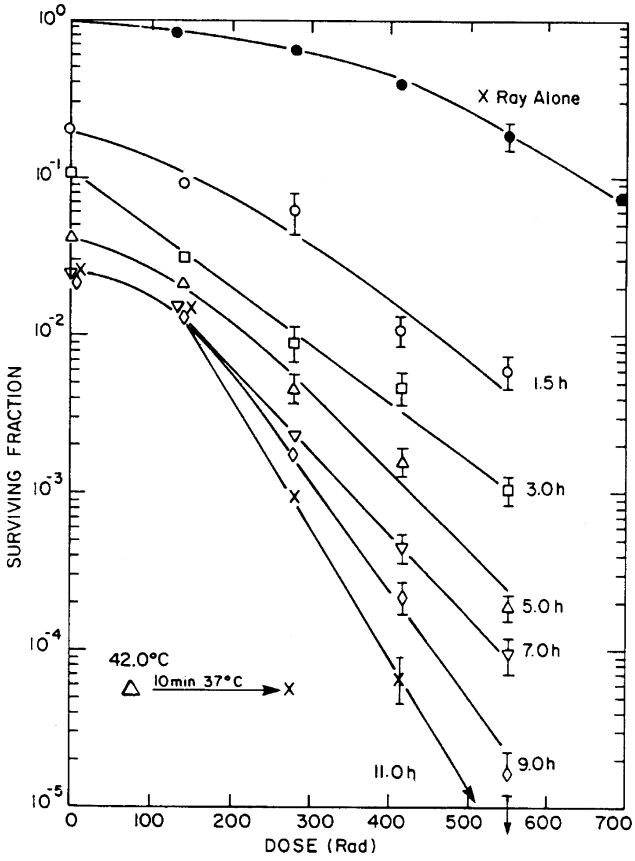

Fig. 6. Thermal radiosensitization. The effect of heating at 42 °C on the thermosensitivity of V 79 cells. Heating was completed 10 min before acute X-irradiation ([208], with permission).

4. Synergistic effect of hyperthermia and radiation

4.1. Thermal radiosensitization

One of the most important observations from in vitro studies on heat action was that hyperthermia and radiation act in a synergistic way. This synergism induces an increase in cell killing even at lower temperatures, which is not the case when hyperthermia is implemented alone. This so-called 'thermal radiosensitization' results in a reduction of the shoulder of the dose–effect curve (Fig. 6). It appears most pronounced in S-phase cells that are usually resistant to radiation alone. The extent of thermal radiosensitization can be quantified by the quotient of survival fraction of cells treated with radiation alone and those treated with radiation and heat ('thermal enhancement ratio', TER) [36,40,42,46,52,82–84].

4.2. Radiation-heat sequence

The extent of synergism between heat and radiation depends on the temperature applied, time interval between heat and radiation, and treatment sequence. It is most distinct when both modalities are applied synchronously. In vitro, a supraadditive effect of heat and radiation may occur for 8 h or longer when CHO-cells are heated at >43 °C before radiation. Using an inverse sequence, a similar effect is often observed for a shorter period of time (2–4 h), but also appears at temperatures below 42 °C. A synergistic effect of heat and radiation can also be observed in thermotolerant cells when single radiation doses of 2–4 Gy are applied, but this effect may be altered depending upon the cell type and degree of tolerance. Hypoxic cells, as well as, those with impaired nutrient supply and/or acidic pH have been shown to react very sensitively to the combined treatment of heat and radiation [36,41,46,82, 83,85] (Fig. 7).

Synchronous application of heat and radiotherapy is not possible in clinical practice yet, even if respective devices have been designed and are under evaluation. As clear instructions on the optimal radiation-heat sequence can not be derived from experimental data, the general recommendation and practice today is due to this reason, where heat and radiation are to be applied within a short period of time. Some investigators prefer a time interval of 2–4 h between radiation and heat to increase the therapeutic ratio. Others apply heat prior to irradiation as it may be advantageous due to the prevention of vascular stasis, which probably promotes radioresistance. Another aspect is that some radiotherapy fractions before the first course of hyperthermia may be beneficial for radiobiological reasons. However, all of these strategies are not proven by clinical data and, at least, the optimal radiation-heat sequence in clinical hyperthermia remains to be clarified.

5. Interactions between hyperthermia and drugs

5.1. Thermal chemosensitization

Analogous to thermal radiosensitization, hyperthermia also enhances the cytotoxicity of various antineoplastic agents ('thermal chemosensitization'). Additional application of selected chemotherapeutic drugs has been shown to enhance the inhibition of clonogenic cell growth at elevated temperatures both in vitro and in animal experiments. The extent of a drug's 'thermal chemosensitization' can also be expressed by the TER that basically is the ratio of cell survival at the elevated temperature to normal temperature for a certain drug level. It mainly represents the pharmacodynamic features of drug–heat interaction (e.g. changes of the kinetics of the primary mode of drug action), but further attention should be attracted to clinical pharmacokinetics and modulation of side effects. At hyperthermia, additional interactions between drugs and hyperthermia have been described. For example as some drugs lose their chemical stability at higher temperatures, or get impaired at contact with glass or

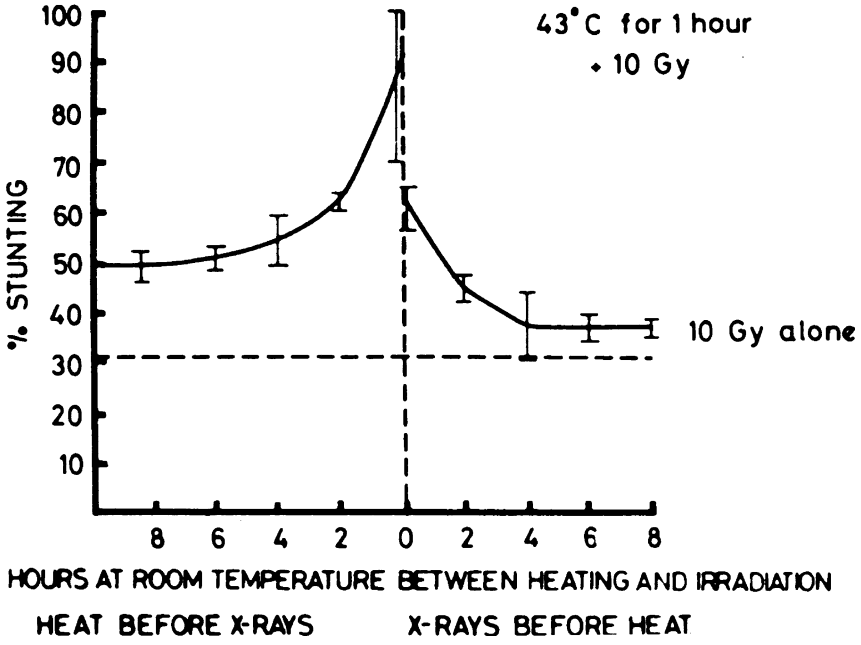

Fig. 7. Radiation–heat sequence. Effect of treatment intervals and sequences for combined radiation and hyperthermia—hyperthermia at 43 °C/60 min is applied before, respectively, after 10 Gy radiation in growing rat cartilage ([209,210], with permission).

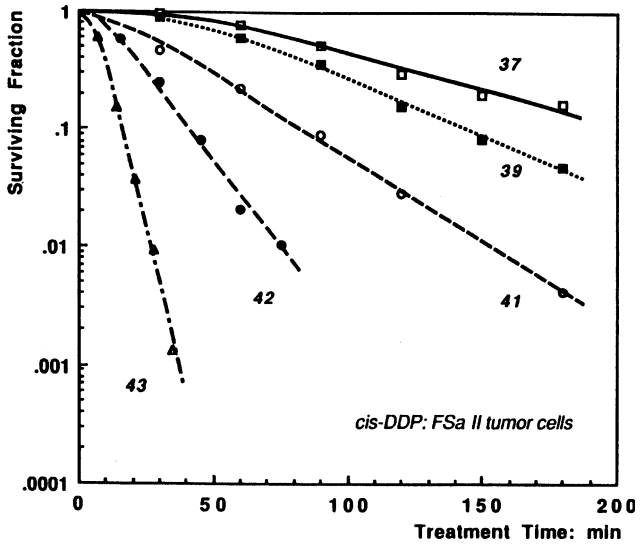

Fig. 8. Thermal chemosensitization. Survival fractions of FsaII cells treated with a fixed dose of cisplatin, heated at various temperatures ([211] with permission).

plastic. It has also to be considered that solvents or additives may interact with heat and thus may induce either thermotolerance or thermosensitization [39,86–88] (Fig. 8).

5.2. Different modes of drug–heat interaction

The interaction of heat with chemotherapeutic drugs has been classified by terms like 'additive' or 'superadditive' (linear increase with increasing temperature), 'threshold-behavior' (little or no increase of cytotoxicity at lower temperatures, marked increase above a distinct threshold temperature), or 'independent' (no dependency at all) [39,88]. It is generally accepted that most alkylating agents (e.g. cyclophosphamide and ifosfamide) and the platinum compounds, are linearly enhanced in their cytotoxic effect when temperatures are raised from 37 to above 40.5 °C. Conversely, doxorubicin has a temperature threshold, whilst most antimetabolites (e.g. 5-fluorouracil) as well as vinca alkaloids and taxanes show no dependency to hyperthermia. A further group of drugs, the so called 'thermosensitizers', act in a cytotoxic way only at elevated temperatures. Some well-known drugs like the local anesthetic drug lidocaine or the antifungal amphotericin B have been demonstrated to act as thermosensitizers [39,86–89].

While reviewing the published data on heat–drug interactions, attention is attracted to the controversial discussions about the findings on the mode of interaction of various drugs and heat, mainly for the following reasons. For example, data on thermal chemosensitization often refer to in vitro studies in which temperatures >43 °C were applied, and thus cannot be transferred to clinical hyperthermia. In addition, different animal tumors vary in their susceptibility to a certain cytostatic agent (at room temperature, respectively, 37 °C as well as at elevated temperatures). Moreover, it is known fact that drugs most effective at normal temperature do not necessarily have a higher sensitization capacity at higher temperatures. Further, the extent of thermal chemosensitization of a drug in a given experimental system varies among different modes of drug administration and dosage. Please note that certain modes of heating may promote thermotolerance and/or drug resistance, and that most pharmacokinetic aspects cannot be simulated in animal models. At least, the lack of demonstrating thermal chemosensitization may be due to an inadequate interval between drug administration and heat exposure for various agents (see below) [27,86–88].

5.3. Drug-heat sequence

Available data suggest that thermal chemosensitization can be yielded best by synchronous application or administration within a short interval for most drugs, but there might be some exceptions. For example, the oxacephasporines cyclophosphamide and ifosfamide undergo an extensive hepatic metabolism, and, therefore, should best be applied some hours before hyperthermia. In opposite, favorable clinical results have also been achieved by applying regional hyperthermia in conjunction with ifosfamide within a shorter interval of time [21,27,90]. Another example is the antimetabolite gemcitabine, where a time interval of ca. 24 h between drug application and heat was necessary to achieve a synergistic effect in vitro and in a rat model. A correlation with clinical data has not been drawn yet [91]. On the contrary, it was shown that simultaneous application of etoposide (VP-16) and heat leads to a decrease of cytostatic activity in vitro, whereas application of this drug led to persuading results when applied in combination with other agents and regional as well as WBH [92–95]. As a conclusion, further pharmacological studies are necessary to optimize the application of cytostatic agents in conjunction with different hyperthermia approaches in clinical practice. Continuing preclinical research may have setbacks regarding important factors concerning the interaction of heat and drugs, and will be helpful in the evaluation of novel drugs for hyperthermic chemotherapy.

5.4. Pharmacokinetics of drugs applied synchronously with hyperthermia

As already discussed above, 'thermal chemosensitization' preferentially reflects pharmakodynamic aspects of drug action during hyperthermia, but does not consider the assumed complex changes in drug pharmakokinet-

ics under hyperthermic conditions. Unfortunately, data available on this topic are not very detailed so that the basis of cytostatic therapy under hyperthermic conditions is still poorly understood. In general, one would expect that changes in tumor blood supply occur which affect the distribution of cytostatic drugs in the neoplastic tissue. It is also presumable that changes in fluid and electrolyte balance as well as pH-changes may result in changes of solubility and volume distribution of drugs. By influencing the effective dosage of a drug this could be applied, especially, for WBH. Gastric hyperchlorhydria and/or gastrointestinal fluid sequestration may represent further sources of distribution problems, but at least antineoplastic drugs will be mostly administered intravenously during hyperthermia. At least, hepatic and renal metabolism and excretion may show relevant changes under various hyperthermic treatment modalities [96].

In a clinical phase-I study on WBH, a slight decrease in the renal elimination of carboplatin (CBCDA) was detected, and it was argued that this might be the reason for an increase in nephrotoxicity of CBCDA while combined with hyperthermia [97,98]. In another investigation on CBCDA—pharmacology in WBH, occurrence of excess nephrotoxicity was mainly due to the use of an extracorporeal hemodialysis—system to induce WBH, a method that is thought to produce a relevant rate of nephrotoxicity by itself [97,99]. In regional hyperthermia of the pelvis, a trend towards a higher peritoneal clearance after interperitoneal carboplatin application was detected in patients with ovarian cancer [100].

Altogether, only very few studies on clinical drug pharmacology under hyperthermic conditions have been published. Data suggest that at least long lasting systemic heat exposure of larger parts of the body, e.g. in WBH, may influence especially the pharmacokinetics of synchronously administered cytotoxic drugs, due to changes in the organ circulation (e.g. of the liver or kidney), to temperature-depended metabolism rates, or to fluid shifts. In general, drug–heat interactions in cancer patients appear to be much more dependant on environmental factors (e.g. blood supply, fluid balance or pH-value) than those of radiation and heat. As those factors usually cannot be simulated by pre-clinical experiments in a convincing manner, corresponding data on drug–heat interaction and thermal chemosensitization should be interpreted with great caution. Further concomitant research in the scope of clinical trials is required.

6. Cellular effectors of hyperthermia

6.1. History

A large number of investigations concerning the cellular effects of hyperthermia exist, dating back to the 1970s and early-80s. Here, different aspects of hyperthermic action were described that were conducted with the research techniques available at that time. As the interest in basic research of hyperthermia decreased markedly since the mid-80s, information is insufficient to translate all the described phenomena into our more recent understanding of biological changes, which are dominated by molecular biological and genetical aspects.

6.2. Cell membrane and cytoskeleton

Hyperthermia affects fluidity and stability of cellular membranes and impedes the function of transmembranal transport proteins and cell surface receptors in vitro. An increased fluidity of cell membranes was observed in thermosensitive, but not thermotolerant cells. Suggesting that membrane alterations represent an important target in hyperthermic cell death [101–105], these observation gave raise to numerous reports on changes in membrane potential, elevated intracellular sodium and calcium content, as well as an elevation of potassium-efflux under hyperthermia. However, none of these phenomenas seems to correlate with the rate of cell death in vitro, even if one may assume an effect of hyperthermia on transmembrane transport of ions (Na^+/H^+—respectively, HCO_3^-/Cl^-ATP—transport) and intracellular pH from these data [60,101,106–111].

Besides, hyperthermia has been demonstrated to induce various changes of cytoskeletal organization (cell shape, mitotic apparatus, intracytoplasmatic membranes such as endoplasmatic reticulum and lysosomes), but again there was no clear correlation found between these phenomenological changes and thermosensitivity of various cell lines [54,88,112–114]. In this context, the appearance of membrane blebbing in cultured cells exposed to heat was described first, and it was noted that cells that exhibited this phenomenon underwent cell death after a single heat dose [115]. From a more recent point of view, membrane blebbing does not represent a primary damage of the cell membrane, but is a typical feature of programmed cell death (apoptosis). Until today, various authors have demonstrated that hyperthermia is able to induce apoptosis in in vitro and in animal experiments (see below).

6.3. Cellular proteins and nucleic acids

Intracellular de novo synthesis and polymerization of both RNA- and DNA-molecules during protein synthesis is decreased in vitro at temperatures between 42 and 45 °C in a dose-dependant manner. Whereas RNA- and protein-synthesis recover rapidly after termination of heat exposure, DNA-synthesis is inhibited for a longer period [64,88,116].

Heat shock induces an aggregation of denaturated

proteins at the nuclear matrix. This is mainly due to insolubility of cellular proteins after heat-induced protein unfolding, entailing an enhancement of the nuclear protein concentration. Recently, elevated binding affinity and redistribution towards nuclear structures has been described for more than 100 different cellular proteins, including the so called heat shock proteins (see below) [64,88,116].

Increasal of the nuclear protein content by heat consecutively affects several molecular functions (including DNA-synthesis and -repair) when a certain thermal dose is exceeded. This threshold dose is diverse between distinct cell lines. HeLa-cells that enter the S-phase of the cell cycle at 41.5 °C despite an impairment of nuclear enzymes, consecutively undergo M-phase death after completion of DNA-synthesis. On the other hand, CHO-cells that exhibit a markedly impaired DNA-replication at the same conditions experience G1-arrest, and become thermotolerant. Both the threshold character and the distinct susceptibility to heat between different cell lines can be explained best by differences in recovery from heat shock, and not by the extend of heat-induced cell damage itself [117–121].

In the 1960s, hyperthermia was supposed to act similar as radiation by inducing direct DNA-damage and double-strand breaks. Later, it became evident that heat is not able to cause severe DNA-damage by itself, but instead hinders the repair of radiation-induced, sublethal cell damage, and thus boosts radiation-induced DNA-fragmentation. From a recent point of view, this may be caused by a temperature-dependant inhibition of DNA-repair enzymes. Indeed, an inhibition of the DNA-polymerases-α and -β by hyperthermia has been demonstrated. Similarly, it was assumed that hyperthermia may specifically inhibit the enzymes of the 'mismatch repair' or the 'nuclear excision repair'-system, but this has not been proven yet. However, even if a temperature-dependent inhibition of DNA-repair enzymes would be an intriguing explanation, an impairment of DNA-enzymes may be caused by unspecific protein inactivation or interaction at nuclear structures as well [122–127].

6.4. Heat-shock proteins

Whereas synthesis of most cellular proteins is impaired under hyperthermic conditions, this cannot be applied for the so called heat shock proteins. Heat shock proteins represent a heterogeneous group of molecular chaperones consisting of at least five subgroups with different molecular mass and partially varying biologic function. HSP are usually divided into small HSP (molecular mass < 40 kDa), and the HSP 60, HSP 70, HSP 90 and HSP 100 proteins families. All HSP families share their chaperoning function, i.e. they unselectively bind to hydrophobic protein sequences liberated by denaturation. Thus they prevent irreversible interaction with neighbor proteins (e.g. at the nuclear matrix, see above) that would result in a loss of function. However, elevated expression and chaperoning function of HSPs are not restricted to elevated temperatures. They can also be observed under various other stress conditions, and some HSPs even carry out similar functions during regular protein synthesis as long as some amino acids have not developed complex structures. HSP-synthesis can be induced within minutes by activation of so called 'heat shock factors' (HSF). Those HSFs rapidly bind to and activate the promoter regions of various heat shock genes after trimerization, in particular those involved in the synthesis of HSP 70. Today, at least HSP 27 and HSP 70 are supposed to represent 'general survival proteins' that are able to defend cells against a variety of potentially lethal (proapoptotic) stimuli [128–131] (Figs. 9–11).

In hyperthermia, HSPs are thought to be involved in the protection of cells against heat damage. The connections between HSPs and thermotolerance on the one, and the interesting immunogenic features of the HSP on the other hand are discussed in a separate chapters of this article.

Intracellular HSP-synthesis increases when cultured cells are exposed to moderate heat application. At higher temperatures, inhibition of HSP-synthesis occurs above a distinct threshold temperature. In general, the temperature, respectively, thermal dose at which HSP-synthesis is inhibited in a given experimental system varies between different cell types, but the respective threshold can be lowered when further (proapoptotic) stimuli are added. As lack of HSP-synthesis is associated with exponential cell death, it is generally accepted that HSPs prevent cells from lethal thermal damage. Indeed, an increase of apoptosis can also be observed when HSPs are inactivated at normothermia (e.g. by antisense technology). Vice versa, induction of apopto-

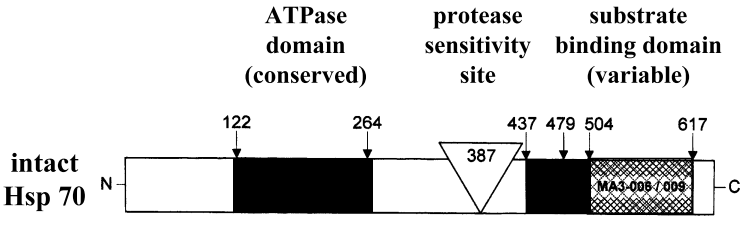

Fig. 9. Schematic illustration of heat shock protein 70 and its different epitopes ([150] with permission).

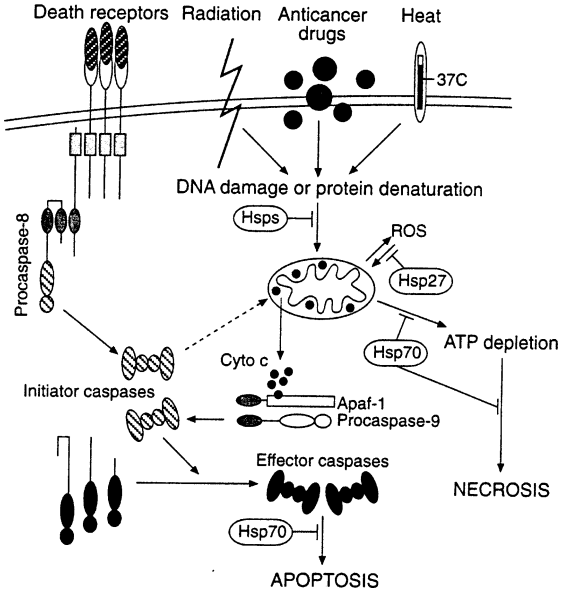

Fig. 10. Different ways by which heat shock proteins may act as inhibitors of cell death ([130] with permission).

sis by direct stimulation of the fas-ligand or by natural killer (NK)-mediated lysis cells cannot be prevented by elevated HSP-expression in experimental systems. Therefore, induction of programmed cell death may occur at normal intracellular HSP-levels using these pathways. Taken together, data suggest a close connection between HSP-expression and inhibition of hyperthermic cell death, especially apoptosis, under certain conditions, but further investigations are necessary to further understand the underlying mechanisms in the future [129,130,132–135].

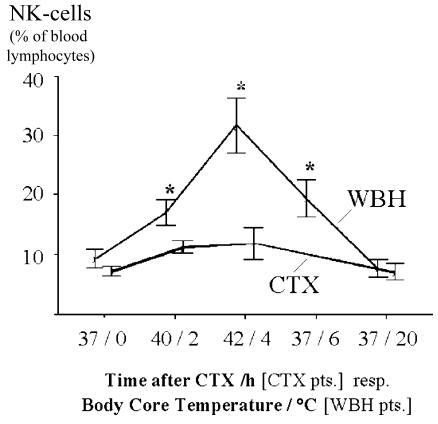

Fig. 11. Course of NK-cells in patients treated with chemotherapy and WBH compared with patients treated with chemotherapy (CTX) alone (Ahlers et al., previously unpublished). *y*-Axis: NK-cells ($CD3^-/CD16^+/CD56^+$) calculated as percent of total blood lymphocytes; *x*-axis: 'WBH', measurements at 37, 40, 41.8 and 39 °C body-core temperature; 'CTX', measurements before, immediately after, and 2 and 4 h after the application of chemotherapy.

7. Characteristics of hyperthermic cell death

7.1. Different modes of cell death

Today it seems to be proven that the antineoplastic properties of drugs and radiation are mainly based on their ability to (directly or in an indirect way) induce either apoptotic (synonym programmed) or necrotic cell death. Whereas necrosis is marked by a passive pathological cell damage followed by an inflammatory response originating from the surrounding tissue, apoptosis represents a genetically controlled, active death program. It may be activated by cell damage or physiologically, hereby contributing to maintain tissue hemostasis as well as preventing severe cell damage that may lead to sustained viral infection or cancer. On the other hand, excess of programmed cell death may lead to impaired development and degenerative disease in a given tissue or organism. Interestingly, most of the stimuli activating apoptosis are also able to induce necrosis at prolonged or intensified exposure [136].

In apoptosis, potential lethal stimuli like cytotoxic drugs, radiation, viruses, or starvation, activate cascades of specific cysteine proteases, the so-called caspases, in different ways. Each of these signaling pathways can be boosted in the mitochondriae. Regulation of programmed cell death is controlled through the expression of a number of genes with activating (e.g. bax gene family) or inhibiting (e.g. bcl gene-family, p53) properties. As genetic alteration of these genes were found to contribute to malignant transformation and disease progression in various hematological and solid malignancies, basic research on apoptosis was propelled during the last decade [130,136–140].

7.2. Hyperthermia-induced apoptosis

Hyperthermia is suitable to induce both necrotic and programmed cell death in vitro in a temperature-depending manner [141]. The susceptibility of cultured cells to apoptosis could be demonstrated, in particular, in a number of experiments using hematological cell lines [141–144]. On the other hand, Yonezawa and coworkers explicitly reported that out of various soft tissue and osteosarcoma cell lines apoptosis could only be induced by heating up to 43 °C in a single cultured cell-line of malignant fibrous histiocytoma. It seems that at least some cell types exhibit different susceptibility to apoptotic cell death induced by heat. Above a distinct temperature, it is more likely to induce necrosis instead.

Considering in vivo experiments, a significant tumor growth delay due to apoptosis was observed in a xeno-transplanted Ward colon carcinoma, but not fibrosarcoma, in rats exposed to long-term moderate hyperthermia. Analyses of the host tissues moreover

revealed the occurrence of programmed cell death in various lymphatic tissues (especially thymus). Moderately increased apoptosis rates were also established within the small intestine, but not in any of the remaining organs [45,145].

Taken together, programmed cell death seems to represent an important effector of heat action. Anyway, it has to be taken into consideration that most of the pre-clinical data refer to higher temperatures than those that can be applied in the treatment of cancer patients with hyperthermia. As hyperthermia is always applied in combination with radiation and/or antineoplastic drugs in clinical practice, it is conceivable that at least an additive proapoptotic effect may become relevant in the scope of multimodal strategies. This might further be supported by additional vascular, nutritive, and immunologic changes.

7.3. Immunogenic effects of heat-shock proteins

HSP isolated from cancer cells, but not those from normal tissues, are able to induce a cytotoxic T-cell-activation against the tumor derived from. Interestingly, it was found that this reaction is not caused by the HSP-molecule itself, but by complexes of HSP with tumor specific peptides. These HSP/peptide complexes are thought to be internalized into antigen presenting cells by specific receptors in order to get presented together with major histocompatibility complex (MHC) class I molecules. This unique property makes the HSP an attractive target for anticancer vaccination strategies-regardless of an additional hyperthermia treatment [146–148].

A connection between HSP-expression and hyperthermia that goes beyond the finding of elevated HSP-synthesis during heat exposure was recently demonstrated by the group of Multhoff and Issels [149–154]. They reported about a stress-inducible form of HSP-70/72 that is expressed on the surface of distinct cell cultures. It was also found that this presentation of HSP 70/72 on the cell-surface may appear either constitutively or heat-induced, and, is able to induce a MHC-independent tumor cell-lysis, too. However, the mechanism by which cytoplasmatic HSP reaches the cell surface and induces cell death have not been clarified in detail yet. It was speculated that HSP 70/72 cell surface expression modulates immune response against certain tumor cells (e.g. if it functions as a foreign antigen by itself or when complexed with other molecules). A further suggestion is that HSP mimikris another antigen when expressed on the cell surface thus inducing immunostimulation against specific tumor cells. Whereas, cell surface expression of HSP 70/72 on tumor cells was not confirmed by other groups yet, Roigas and coworkers recently detected a HSP cell surface expression by FACS analysis using an antibody differing from the one used by Multhoff and coworkers. Work is going on to further confirm these results and to clear up how and why HSP 70/72 gets onto the cell surface of some tumor cells [149–154].

7.4. Signal transduction

The antitumor efficacy of antineoplastic drugs depends on dosage, as well as, on tissue-specific or/and cellular characteristics. The way a potential lethal stimuli finally results in cell death, is mediated by a number of specific genes, the gene products of which play important roles in different signal transduction pathways (e.g. regulation of the cell cycle, programmed cell death, and DNA-repair). With regard to hyperthermia, heat-shock response represents a typical, but by no means specific reaction. All together, there is only little knowledge about the signaling pathways that mediate the cytotoxic effects of heat, and it also is not known whether these possess any specificity [43,57,130,131, 136,155–157].

In this context, the p53 gene product represents an important cellular protein that exhibits the features of a transcription factor. p53 protein expression becomes elevated when cells are exposed to potentially lethal stimuli, and activation of the p53-pathway may result in either G1-arrest or apoptosis. In case, this pathway is disturbed, e.g. by genetic alterations, predominance of proliferative signals may occur, leading to malignant transformation under certain circumstances. Vice versa, p53-mutations resulting in an absent protein synthesis or synthesis of a mutant protein with oncogenic potential represent the most common genetic alterations observed in human solid tumors [140,158–160].

First evidence for a connection between hyperthermia and p53-function came from the finding that HSP 70/72 interacts with certain mutants but not wild-type (wt)-p53 protein. Furthermore, a heat-induced accumulation of wt-p53 was reported to result in an increased affinity of p53 to HSP 70/72. Cultured fibroblast and colon carcinoma cells containing wt-p53 exhibit a higher thermosensitivity than those with p53 null-mutations, and the rate of cultured colon carcinoma cells undergoing spontaneous apoptosis was shown to be significantly lower in cells when either the p53- or retinoblastoma (Rb) protein was inactivated [44,91,161–163]. From these findings, it was proposed that tumors containing distinct p53- or Rb-mutations might be less thermosensitive than those with wild-type alleles, and that thermosensitivity in wt-p53 cells might be due to a suppression of the cell saving properties of HSP 70.

The few clinical data available on this topic did not confirm correlations of p53-alterations, HSP-expression

and outcome of patients treated with hyperthermia. In particular, members of our group did not find a correlation between HSP70 expression and outcome in patients with rectal cancer treated with either neoadjuvant radiochemotherapy or radiochemotherapy plus regional hyperthermia. Others described the occurrence of tumor cell apoptosis in 28 patients with rectal cancer who received a multimodal therapy consisting of 5-fluorouracil, radiation, and intraluminal hyperthermia. Apoptosis rate was significantly correlated with therapeutic effect (but not p53 status), while the effect of hyperthermia could not be studied because of the lack of an adequate control group [164,165].

8. Hyperthermia-induced changes in cellular immune response

8.1. Pre-clinical effects of heat on lymphocytes and experimental tumors

There is no doubt about a close connection between cancer and the immune system today, and different immunotherapeutic strategies are already under clinical evaluation. In addition, knowledge on mechanisms contributing to malignant transformation in patients with comprised immune system has markedly increased during the past years [166–169]. The consideration that hyperthermia seems to imitate fever, an apparent immunologic reaction, prompted various in vitro studies on the effect of heat on human lymphocytes since the early 1980s, mostly focussing on immunologic functions of non-migratory lymphocytes [170–176].

In this context, most investigators have observed impairments of lymphocyte function after applying non-physiologically high temperatures (>42 °C) in vitro, and especially NK-lymphocytes have been found to react very sensitive to heat. From a recent point of view, it is very difficult to come to a conclusion by comparing these studies with each other, as different tests and calculations were used to measure lytic NK-cell activity, sometimes without regard to the total NK-lymphocyte count. However, Shen and coworkers demonstrated in a persuading way that NK-cell function was increased at temperatures of about 40 °C, but impaired at temperatures above 42 °C. More recent findings referring to in vitro temperatures below 41 °C actually revealed an increased NK-cell proliferation, that was accompanied with heat-shock response, as well as secretion of selectin. The clinical relevance of these findings remains to be clarified [171,173,177,178].

Additional clues on the influence of heat on cellular immune response came from a recent animal study by Burd and coworkers. Here, a growth delay of breast cancer xenografts in SCID- und Balb/c mice was observed after long-lasting moderate hyperthermia. Thermal doses applied were too low to induce changes in any host tissue here, but at the tumor site accumulation of both host lymphocytes and adoptively transferred NK-cells was found to be responsible for the marked rate of tumor cell apoptosis observed in both animal models after hyperthermia. This effect was inhibited by selectively blocking the NK-cell function. Therefore, NK-cell mediated cell lysis may represent an important cytotoxic mechanism induced by (moderate) hyperthermia [38].

8.2. Immunologic changes in cancer patients treated with whole-body hyperthermia

Most data published on immunologic changes in humans exposed to systemic heat, refer to investigations of lymphocyte subpopulations and/or serum cytokines in healthy persons whose body core temperature was moderately raised to temperatures of 39–39.5 °C in a water bath [177,179,180]. Others reported about immunologic changes in patients after a heat-stroke [181]. In addition, few publications are available on short-term changes in serum cytokine levels in patients treated with either radiant heat or extracorporal WBH at 42 °C [182].

Regarding *lymphocyte subpopulations*, a significant reduction of both T4-cell count and T4/T8 ratio was observed in both healthy persons that underwent moderate water bath-hyperthermia, as well as patients suffering from heat-stroke. On the other hand, T8- and NK-cells were significantly elevated, thus resulting in a slight increase of the total lymphocyte count despite the decrease of T4-cells mentioned [180,183]. As we recently demonstrated, a decrease of T4-cells also takes place in patients treated with WBH, but a reduction of T8-cells was not detected in this context [184–186].

Changes of cellular immune response observed in subjects exposed to systemic heat are relatively unspecific and may be interpreted as part of a general response to major physiological stress, the presence of which is clearly reflected by significant elevations of heart rate and cardiac output in WBH patients [187,188]. Similar changes, including an elevation of NK-cells, respectively, activity, can also be induced by adrenaline-infusion or moderate exercise, whereas heavy exercise may entail an impairment of NK-cell-activity [180,189]. Today, it is generally believed that the immunologic response to different kinds of stress is relatively uniform [180,189]. On the one hand, lymphocyte migration into the bone marrow has been demonstrated to be adrenaline-dependant [180,189].

On the other hand, β-2-receptor-mediated mobilization of endothelium-bound lymphocytes into the blood would give a favorable explanation for the differing course of lymphocyte subpopulations during WBH [180,189]. Madden and coworkers demonstrated a differential β-2-receptor expression on the cell surface of different lymphocyte subpopulations. β2-receptor expression on NK-cells was higher than expression on T8- and on T4-lymphocytes. Moderate plasma catecholamine-levels resulted in a stimulation of NK-cell function, whereas impaired NK-cell activity was found in the presence of high catecholamine levels. Catecholamines may exert their influence on blood lymphocytes by direct stimulation on the one, and sympathetic innervation of lymphatic tissues on the other hand. [190–194].

Investigation of *serum cytokine levels* in patients undergoing WBH has been performed by Robins and coworkers. They reported about alterations in patients treated with WBH, comprising an elevation of the antiinflammatory interleukins (IL) 6 and 10, whereas IL-2 and interferon (IFN)-γ remained unchanged. These findings have recently been confirmed by others, including our group. Remarkably, both changes in cytokine levels in WBH-patients as well as those in lymphocyte subpopulations were reversible spontaneously. Moreover, an increase of antiinflammatory cytokines can also be induced by physiological stress in animal models, sometimes even inducing decreasing IL-2 and IFN-gamma serum levels [182,185,195].

Regarding the above mentioned facts, WBH and other forms of systemic heat exposure >41 °C have been found to induce severe alterations in circulating blood lymphocytes, resulting in a suppression rather than a stimulation of the immune system. However, future research will reveal if these findings just represent para-phenomenas or if there is a connection between changes in blood lymphocytes and increased lymphocyte migration and NK-cell activity at the tumor site as it was demonstrated in experimental tumors.

9. Modulation of drug resistance by hyperthermia

9.1. Reversal of drug resistance induced by hyperthermia

Drug resistance represents the major cause of treatment failure in human malignancies, and can be induced by different mechanisms, of which the pleitrope 'multidrug resistance (MDR)', mediated by the transmembranal 'glycoprotein p170' efflux pump, has gained particular interest [196–198]. Pre-clinical data suggest that hyperthermia is a good candidate to overcome various modes of drug resistance, and this has been demonstrated in particular for the platinum derivate cisplatin (DDP). Overcoming DDP-resistance by hyperthermia is exemplary, because its causes are multifactorial (e.g. changes in transmembrane conductivity, activity of sodium/potassium-ATPase, glutathion metabolism, DNA repair). It, therefore, gives the possibility to investigate the effect of hyperthermia on drug resistance at different cellular levels (but please note that the MDR-phenotype is not involved in DDP-resistance). However, one has to keep in mind that temperatures and thermal doses were significantly higher in these in vitro studies, than those usually achieved in clinical practice [199]. On the contrary, the course of individual patients previously refractory to platinum compounds who responded to therapy after addition of hyperthermia are strongly suggestive of a reversal of drug resistance induced by hyperthermia at lower temperatures [99,200].

9.2. Thermotolerance is often associated with drug resistance

On the other hand, moderate heat exposure has been demonstrated to induce HSP-expression in cultured cells, and elevated levels of intracellular HSP-70 have been shown to be associated with thermotolerance. Vice versa, transfection of HSP-70 into cultured fibroblasts resulted in a marked decrease of thermosensitivity, with similar findings obtained for other members of HSP-families. Thermotolerance may be associated with different forms of drug resistance (e.g. MDR), and hyperthermia has been shown to induce various forms of drug resistance, too (including MDR or the heat-dependant inactivation of the enzyme topoisomerase II). Occurrence of a heat-inducible, simultaneous resistance to heat and drugs (thermotolerance + MDR) has been reported to occur under certain conditions [59,93,132,157,201–203].

9.3. Does hyperthermia overcome or induce drug resistance?

It seems curious that hyperthermia has been shown to both overcome and induce drug resistance in vitro. Reversal of drug resistance has been particularly shown for platinum compounds at temperatures >42 °C, whereas induction of drug resistance (alone or in conjunction with thermotolerance and HSP-accumulation) may appear when lower temperatures are applied. Despite of this pre-clinical observations, a favorable effect of different hyperthermia approaches on drug sensitivity has been reported in the scope of clinical trials. Here, patients with principally chemosensitive, but individually refractory diseases (e.g. germ cell tumors and sarcomas), were successfully

treated with additional hyperthermia to a given chemotherapeutic schedule [30,92,94,95]. In addition, there is one study available in which tumor specimens from patients participating in a clinical hyperthermia trial were investigated with regard to the modulation of drug resistance by hyperthermia on a molecular level. Here, an induction of MDR-drug resistance by regional hyperthermia of the pelvis in conjunction with radiotherapy and chemotherapy was excluded [204]. As a conclusion, available data on hyperthermia and drug resistance suggest that the positive effect (reversal of drug resistance) overweighs the disadvantages (induction of drug resistance) in clinical practice. However, one should keep in mind, that drug resistance could be induced by hyperthermia on principle, particularly at moderate temperatures.

10. Summary and discussion

Local and regional hyperthermia have been demonstrated to improve treatment results in conjunction with radio- and/or chemotherapy for several indications until now, thus encouraging further evaluation of various hyperthermia approaches. However, the cellular and molecular pathways underlying this beneficial outcome of patients are still poorly understood, although a large number of pre-clinical studies are available on different aspects of hyperthermia action. It is well-known from these data that heat develops an independent cytotoxic effect on cultured cells in vitro at temperatures ≥ 43 °C. In addition, hyperthermia acts in a synergistic way when combined with radiation or cytostatic drugs at lower temperatures (40.5–43 °C) in vitro and in vivo. In cancer patients, complex changes were found during and after the application of the various hyperthermia modalities, concerning blood, nutrient and oxygen supply of the tumor, metabolic changes, signal transduction, immunology, as well as, pharmacological effects. Reports on these pre-clinical effects of hyperthermia rather raise a cascade of further questions (for example, the details of the immunological processes caused by hyperthermia), but definitely do not answer one of the various questions on heat action in the treatment of cancer patients. Indeed, it is extremely difficult to extract reliable information about molecular and immunological effects of hyperthermia from clinical investigations, since among other reasons, the application of hyperthermia is usually only a part of multimodal treatment schemes.

Many of the pre-clinical investigations concerning heat action have been performed in the seventies and early-1980s, and were followed by a comparatively low number of systematic molecular investigations in the scope of concomitant research to clinical studies. Unfortunately, it has been an unproven dogma in hyperthermia research for many years, that antineoplastic heat action requires temperatures >43 °C. This is why many of those pre-clinical studies refer to thermal doses above values that cannot be realistically achieved in clinical approaches, such as 43.5–45 °C for 60 min or longer. Another point to consider, hyperthermia under experimental conditions ensures a more homogeneous heating than under most clinical conditions, especially than in local and regional hyperthermia. Therefore, in vitro hyperthermia at >43 °C may lead to an overestimation of the cytotoxic potency of heat. This may, vice versa, lead to an underestimation of undesired effects that may occur in certain situations, e.g. an impairment in the availability of drugs and drug action, induction of drug resistance, genetic alterations, clonal selection of resistant tumor cell clones, or dissemination of tumor cells. However, clinical data strongly support the dominance of the beneficial effects of hyperthermia over its possible negative features.

It is curious that one of the fundamental questions in hyperthermia—the temperatures and thermal doses applied at the tumor site necessary to achieve a clinical benefit—is still unresolved and thus discussed controversially. Recent data, however, suggest that optimal thermal parameters may differ between the various modalities of heat application. Clinical trials on local and regional hyperthermia in combination with radiotherapy and chemotherapy have revealed a clinical benefit at temperatures significantly lower than postulated in former in vitro data. Remarkably, a dose–response relationship could be established even at this comparatively low thermal dose level. In contrast, a similar correlation cannot be drawn for patients treated with WBH and chemotherapy, where a body-core temperature of 41.8–42 °C can be maintained for 60 min or even longer in almost every patient, but a clear benefit is not evident (even if the different treatment indication for both approaches are taken into consideration). On the other hand, animal studies on 'mild' WBH (e.g. 40.5–42 °C, for 60–360 min) and some clinical phase-I and -II studies suggest an antineoplastic activity of clinical hyperthermia even at these low temperatures.

Regarding future research, efforts should be made to conduct modern randomized phase-III trials to further establish local and regional hyperthermia, where hyperthermia should be applied as an adjunct to a generally accepted standard treatment (e.g. hyperthermic vs. normothermic radiochemotherapy in primary cancer of the uterine cervix). In addition, ongoing comparative trials on regional and WBH should be completed as soon as possible in a multicenter setting, performing concomitant laboratory investigations on

different aspects of heat action. Regarding technical aspects of hyperthermia, clinical experience with presently existing loco-regional applicators demonstrate that improvements are still necessary. Temperature distributions achieved to date are far from satisfactory, regarding both the absolute values and homogeneity. A better temperature control may be provided by introducing novel thermometry units with magnetic resonance tomography (MRT) support, coupled with hyperthermia control systems. Development of adapted antenna arrays for the different anatomical localizations may further improve effectiveness of local and regional hyperthermia. In addition, basic research produced some very exciting novel applications for hyperthermia, e.g. in gene therapy, where the targeting of therapeutic genes was performed by heat via the HSP promoter, or in pharmacology, where temperature-dependant liposomes may be triggered by a temperature increase.

According to our opinion, it would be beneficial in hyperthermia research, to abandon the rather mechanistic idea of a dose-dependent antineoplastic effect at temperatures with a breakpoint above 43 °C. As discussed earlier, the antineoplastic effect of hyperthermia cannot be explained in clinical studies from these dose–effect curves alone, because administered thermal doses were markedly lower than necessary for in vitro 'hyperthermic cell death'. This strongly supports the hypothesis that the mechanisms responsible for hyperthermia action in the clinical situation are multifactorial and complex. Undoubtedly, extensive preclinical data on thermal effects are important to understand the basic mechanisms of hyperthermia and to highlight some of these heat effects in a very detailed way, but most of the available data cannot be transposed into clinical schemes. It is very probable from current clinical data that the benefit of hyperthermia clearly overweighs its possible disadvantages when applied in conjunction with radiation or cytotoxic drugs for certain neoplastic diseases. In this context, one convincing issue is a repeatedly demonstrated correlation between various thermal parameters and clinical outcome, and this also holds true for the temperature range below 43 °C. This is one of the major argument justifying further evaluation of hyperthermia in the laboratory and clinic. In addition, clinical trials of hyperthermia have to be accompanied by laboratory investigations on different aspects of heat action in order to further understand the cellular and molecular targets of hyperthermia.

Reviewers

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Biographies

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