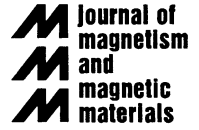




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Invited Paper

Magnetic fluid hyperthermia (MFH): Cancer treatment with AC magnetic field induced excitation of biocompatible superparamagnetic nanoparticles

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Abstract

The story of hyperthermia with small particles in AC magnetic fields started in the late 1950s, but most of the studies were unfortunately conducted with inadequate animal systems, inexact thermometry and poor AC magnetic field parameters, so that any clinical implication was far behind the horizon.

More than three decades later, it was found, that colloidal dispersions of superparamagnetic (subdomain) iron oxide nanoparticles exhibit an extraordinary specific absorption rate (SAR [W/g]), which is much higher at clinically tolerable H_0f combinations in comparison to hysteresis heating of larger multidomain particles. This was the renaissance of a cancer treatment method, which has gained more and more attention in the last few years. Due to the increasing number of randomized clinical trials preferentially in Europe with conventional E -field hyperthermia systems, the general medical and physical experience in hyperthermia application is also rapidly growing. Taking this increasing clinical experience carefully into account together with the huge amount of new biological data on heat response of cells and tissues, the approach of magnetic fluid hyperthermia (MFH) is nowadays more promising than ever before. The present contribution reviews the current state of the art and some of the future perspectives supported by advanced methods of the so-called nanotechnology. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Magnetic fluids; Hyperthermia; SAR; Nanoparticles; Cancer; Biomedical applications

1. Basic principles of hyperthermia

Heating of certain organs or tissues to temperatures between 41°C and 46°C preferentially for

cancer therapy is called ‘Hyperthermia’. Higher temperatures up to 56°C, which yield widespread necrosis, coagulation or carbonization (depending on temperature) is called ‘thermo-ablation’. Both mechanisms act completely different concerning biological response and application technique. The ‘classical’ hyperthermia induces almost reversible damage to cells and tissues, but as an adjunct it

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enhances radiation injury of tumor cells and chemotherapeutic efficacy. Modern clinical hyperthermia trials focus mainly on the optimization of thermal homogeneity at moderate temperatures (42–43°C) in the target volume, a problem which requires extensive technical efforts and advanced therapy and thermometry systems.

Heating to the target temperatures causes moderate cellular inactivation in a dose-dependent manner. Although thermal dose–response curves look quite similar to radiation or drug dose response curves, the critical target of thermal inactivation in the cell is not known yet. The most probable reason for this situation is that there is no individual cellular target of hyperthermia, in contrast to the well known DNA damage after irradiation [1,2]. Most of the biomolecules, especially regulatory proteins involved in cell growth and differentiation and the expression of certain receptor molecules (involved in signal transduction pathways) are therefore largely influenced by hyperthermia.

Today, many cellular effects are known to be important for thermal inactivation. New insights from molecular biology have shown that a few minutes after hyperthermia, a special class of proteins is expressed in the cell, the so-called heat shock proteins (hsp). They protect the cell from further heating or subsequent thermal treatments and lead to an increase of cell survival after pre-heating, an effect called thermotolerance [3]. Additionally, the activity of certain regulatory proteins, kinases or cyclins is influenced by hyperthermia, causes alterations in the cell cycle and can even induce apoptosis, the cell death driven by the cell regulatory system itself [4–7]. Current research is also trying to characterize the interactions of thermal tolerance and multidrug resistance [8]. The combined effect of radiation and hyperthermia takes place at the cellular level and is mainly due to the heat-induced malfunction of repair processes after radiation-induced DNA damage. They are less effective when heat is given either before or after irradiation, and a well-defined time interval has been described for the two modalities [9]. Further effects were observed on the tissue level such as changes of microvasculature, blood flow, energy and oxygen status [10]. Interestingly, heat treated

cancer cells may be better recognized by the host immune system due to alterations of some cell surface receptor molecules which are then recognized by natural killer (NK) cells and inactivate the cancer cells, as has been recently demonstrated in vitro [11]. The joint action of all the molecular mechanisms involved in hyperthermia is still under investigation.

2. Clinical hyperthermia

State-of-the-art radiofrequency (RF-) hyperthermia systems, e.g. annular phased array systems (APAS) for regional hyperthermia of deep seated tumors, are still limited by the known heterogeneity of tissue electrical conductivities or high perfused tissues, which makes selective heating of those regions with such *E*-field dominant systems very difficult. Further application techniques are whole body hyperthermia (WBH, with water-filtered infra-red irradiation), local hyperthermia (e.g. with current sheet applicators) and interstitial hyperthermia (requires implantation of microwave- or RF-antennas or self-regulating thermoseeds). A nearly unsolved problem are the bone of the pelvis or the skull, which ‘shield’ deep tissues in the cavity of bones, which often result in ‘hot spot’ phenomena, which are difficult to predict in certain different locations of the body. The overall effect is thermal underdosage in the target region, which often yields recurrent tumor growth. Despite these uncertainties, several (preferentially European) randomized trials demonstrated therapeutic benefit of ‘state-of-the-art’ (RF-) hyperthermia, so far: Van der Zee and his co-worker [12] reported higher local control after three years follow-up with the combination of hyperthermia and radiation (RHT) with advanced rectal carcinoma, bladder and cervix carcinoma. With the ladder tumor entity even a survival benefit was observed. Our group in Berlin (Rau et al., [13]) has found higher response rates with RHT of advanced rectal carcinoma in a pre-operative approach. Valdagni and his co-worker in Italy [14] found higher local control of advanced lymph node metastases after RHT. The Danish group of Jens Overgaard [15] had success with malignant melanoma, i.e. increase of local

control (2 years follow-up). Claire Vernon with her group in London [16] obtained higher response rates of recurrent mammary carcinoma when irradiation was combined with local hyperthermia. Penny Sneed and her group (USA) had success with glioblastoma multiformae in a randomized trial of brachytherapy boost plus interstitial hyperthermia which yielded an overall survival benefit in the hyperthermia arm [17].

Summarizing all these clinical studies, it is mainly accepted that preferentially the problems of physical power deposition still limit the clinical outcome. This includes not only thermal underdosage of critical regions, there are also large limitations on the body target sites, which are too difficult to treat, like brain tumors. Conclusively there is a large demand on alternative physical concepts, which may offer deep seated power deposition in almost every region of the body.

3. Much more than simple particle heating: the biological concept of magnetic fluid hyperthermia (MFH)

In the early 1960s, a few US groups were the first, who tried to perform hyperthermia with magnetizable microparticles, which were heated by an externally applied AC magnetic field. The use of H -field dominant systems together with power absorbing material instead of power steering of E -field dominant systems is therefore an old idea. However, before the early nineties, the status of this research was diffuse and clinical application was unthinkable. Poor defined animal systems [18] or ex vivo tissue samples were used to test an intratissue heating up to the present [19]. In contrast, solid and comparable in vivo tumor growth data are needed including all the important controls, precise on-line temperature monitoring and pathological tissue inspection. Those studies have been rarely performed, so far.

For the first time, our group could demonstrate in 1993 [20], which H -field amplitudes and frequencies (i.e. specific absorption rate, SAR) are tolerable in humans. Based on the Brown and Néel relaxivity, it was shown, that subdomain particles (nanometer in size) absorb much more power at

tolerable AC magnetic fields than is obtained by well known hysteresis heating of multidomain (microns in size) particles. The SAR of magnetic fluids is $\kappa H_0^2 f$, where κ is a material constant for a given $H_0 f$ combination.

This was the renaissance of a cancer treatment method, which has gained more and more attention in the last ten years.

Systematic in vitro studies were presented by Chan and co-worker (1993) [21] and in a more extended study by our group in 1996 [22], which both show consistently, that inactivation of cancer cells with AC magnetic field excited nanoparticles is equal to the best homogeneous heating, i.e. water bath heating, for a given time temperature schedule. This result was not self-evident, because a large number of single particles, but each acting in principle as a hot source surprisingly yield a temperature homogeneity, which was comparable to water, containing much more excited molecules than particles existing in a magnetic fluid. According to these encouraging in vitro results, a homogeneous cell or tissue inactivation was expected in vivo, too, if the fluid could be administered almost homogeneously throughout the target region.

On the basis of this extended physical and biological knowledge, advanced AC magnetic field applicators were constructed for animal experiments. New studies were started with the isogenic C3H mammary carcinoma of the mouse [23], which was transplanted into the right hind leg. The animals were treated for 30 min at 47°C (intratumoral steady state temperature) including several controls (untreated, coating substance and magnetic fluid alone). In this approach, the temperature was incredibly high in order to test unconventionally (for a hyperthermia study) the potential of MFH as a mono-therapy in order to reach local tumor control. Surprisingly, this was obtained in 44% of the animals (Fig. 1). The magnetic fluid was given intralesionally without anaesthesia. Before AC magnetic field treatment, magnetic fluid depots in the target region were observed as expected, but after the first MFH session, this distribution had been homogenized, which has been termed now as 'thermal bystander effect'. This effect offers broad perspectives not only in hyperthermia, but also in drug targeting, gene and immune therapy. Moreover, the

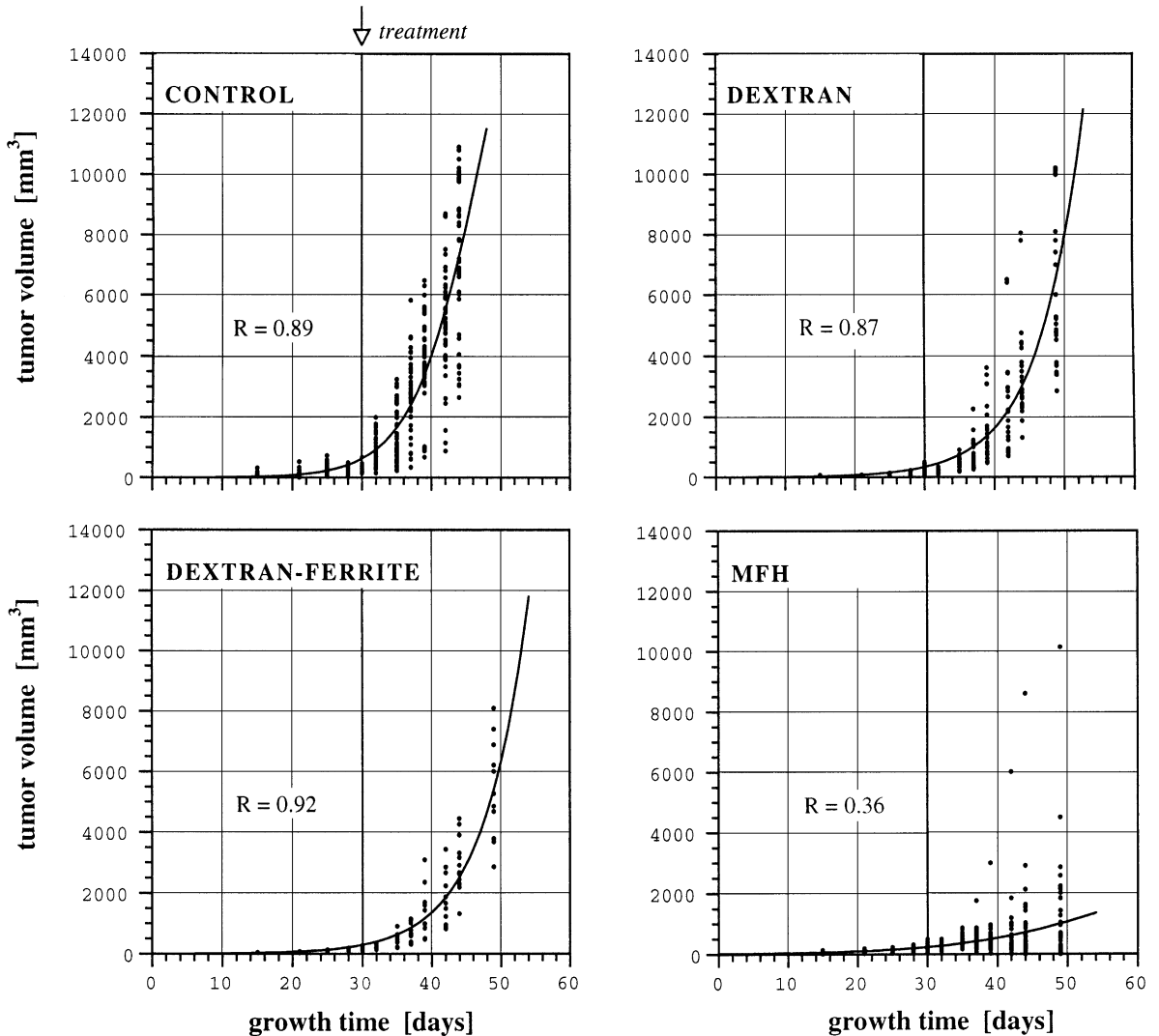


Fig. 1. C3H mouse mammary carcinoma growth curves of untreated (control), dextran, dextran-ferrite and MFH treated animals. Note, that tumor growth remains nearly unaffected until AC magnetic field-induced heating yields 44% tumor control 30 days after treatment.

results suggest, that MFH might become a new minimal invasive modality for regional selective heat treatment on the microscopic level, which is not possible by any other method, so far.

4. Recent results

In order to proceed from the encouraging results with the mammary carcinoma of the mouse, more

animal experiments are required to fix the current state of MFH as an almost site-specific modality, which allows regional heating in different locations of the body. As a precondition, the technology of AC magnetic field application is currently under development [24]. If an almost regional AC magnetic field application could be realized, migration of any ferrofluid to distant locations could be neglected. Many aspects have to be considered, like *E*-field shielding of the 'patient', heat loss of the



Fig. 2. Prototype applicator for regional AC magnetic field administration at a frequency of 500 kHz and field amplitudes of up to 10 kA/m. Depending on body cross-section and tissue conductivity lower frequencies should be used with humans, i.e. 50–100 kHz. The applicator site has a gap height of 20 mm, which is suitable for treating mice or other small animals.

core material, mechanical stress of the overall construction and more. A prototype AC magnetic field applicator for regional MFH of small animals is shown in Fig. 2.

The second component of MFH –, the magnetic fluid is currently optimized, too. Since we know from theoretical estimations, that for a given excitation frequency ν^* an ‘ideal’ core size d^* exists [25,26], which yields maximum SAR, an almost sharp core size distribution is required in order to minimize the therapeutic metal oxide mass required for a given target volume.

The second aspect is the shell of the nanoparticles in a magnetic fluid. It supports colloidal stabilization, but it has also a contribution to the power absorption due to the Brownian relaxation process. From the theoretical point of view, it is largely interesting how the core interacts exactly within the shell in an AC magnetic field. If the core exhibits an oscillation within the shell depending on excitation frequency and core magnetization, some optimization parameters related to the viscosity and structure of the shell material are expected. Alternatively, if the whole particle is oscillating in the field, far less optimization would be possible, because the necessity of aqueous non-toxic dispersion media for medical purposes would not allow any hard changes to e.g. hydrocarbon-

related substitutes. Therefore, more physical studies are required to exactly describe nanoparticle oscillation with respect to power absorption in AC magnetic fields around 50–100 kHz, using different shells of different hydrodynamic behaviour. Additionally, modifications of core magnetization could further enhance the specific power absorption, but again, biocompatibility rules out many of the promising elements, e.g. a cobalt dotation.

Besides this potential of SAR optimization, another large field of research has been recently opened: the transition from site-selectivity to tissue and cell specificity. The shell is able to support functionalized surfaces which can be coupled to a wide variety of biomolecules.

Two different ferrofluids were used in our recent study: magnetite particles with aminosilan type shell (#BU48, core diameter 10 nm/hydrodyn. 30 nm) with largely positive surface charges and of a magnetite with dextran type shell (#P6, core diameter 3 nm/hydrodyn. 70 nm) with a neutral to negative surface charge.

Primary human glioblastoma cells (staining positive for GFAP/S100) originated from intraoperative material were maintained in the 0 to 1st passage and nanoparticle uptake in vitro was compared to the uptake of established normal neuronal cells of the cerebral cortex (HCN-2, CRL-10742, GFAP negative) and a fibroblast (EBF) line. The cells grew in presence of 0.6 mg ferrite per ml medium for 2–8 days and uptake of each cell type was compared to similar cells in normal medium (controls). The cells were investigated by light and electron microscopy and the iron content of the cells was determined quantitatively.

In a preliminary set of in vitro experiments, largely higher uptake of the positive charged aminosilan particles in comparison to the dextran magnetite (up to 1000-fold) was observed with the primary glioblastoma cells. Also larger uptake of both particle types was observed into glioblastoma cells compared with normal neuronal cells and fibroblasts (about 500–2000-fold). If this observed differential particle endocytosis could be validated by further systematic series of experiments, this would be a new strategy of particle targeting, unlike conventional methods, e.g. with monoclonal antibodies. Obviously, both particle species induce

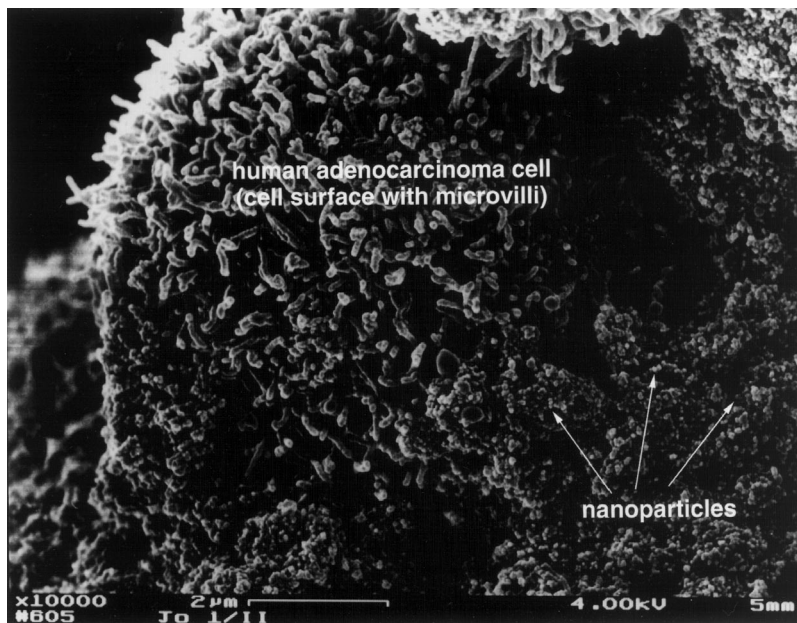


Fig. 3. Scanning electron micrograph of a colon adenocarcinoma cell, which has been grown in a ferrofluid containing growth medium (modified aminosilan shell magnetite, core diameter around 10 nm, 0.6 mg/ml) for 72 h. Note, that the particles are highly adhesive to the surface of the cell, which was not or far less observed with normal cells.

different cellular adhesion and internalization processes, which may become especially important in view of diagnostic and therapeutic purposes. However, further pharmacological studies must show, how blood circulation time and scavenging activity of the reticuloendothelial system may limit this promising approach *in vivo*. Further carcinoma cell types are now under investigation such as colorectal and mammary carcinomas. A sample scanning electron micrograph (SEM) of a colorectal carcinoma cell is shown in Fig. 3. Note, that the magnetite particles are adhesive on the natural surface of the cells without any changes of normal cell structures, like the microvilli as has been observed by comparable micrographs of normal cells in regular culture (not shown).

5. Perspectives

It is still a fascinating concept, that tumor cells could be loaded with thousands of particles, which would become activated – comparable to genes

– only by a specific signal, yielding the death of all particle containing cells as soon as an AC magnetic field is applied. Our cellular observations so far indicate that a tumor, which has taken up these particles, will not be able to get rid of them. Daughter cells from a particle containing parent cells should therefore contain up to 50% of the particle amount of the parent cell. Hence, particle loaded tumor cells may not only contain the ‘markers’ for their own death, the descendants would still have a higher risk of dying from future AC magnetic field applications. It is therefore an exciting challenge for future research to increase the biological efficacy and particle SAR in order to achieve advanced magnetic fluids of which only a few particles are required for selective tumor cell inactivation.

Future animal studies and further investigations on cells and tissues with a broad spectrum of functionalized nanoparticles will elucidate the overall potential of MFH. Besides these preclinical studies, which may yield benefits not before many years of research, today large efforts are needed to start a clinical study with existing biocompatible ferro-fluids

and a clinical prototype of the AC magnetic field applicator. Suitable tumor entities should then be carefully chosen embedded into actual oncological concepts, i.e. tumors with bad prognosis and tumors which are difficult to heat, like brain tumors and tumors of high perfused organs (kidney, liver, lung).

In conclusion, the generation of functionalized surfaces of those particles for cancer cell targeting is the next challenge for the future. Nanotechnology will offer new strategies towards these functionalized particle systems, which might be able to circumvent the known scavenging effects of the reticuloendothelial system. The ensemble of biological strategies, clinical hyperthermia experience, the discovery of the ‘thermal bystander effect’, combined with methods of interventional radiology, microsurgery and the use of precisely operating navigation systems, will give us a new weapon against cancer, which is called MFH.

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