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Towards breast cancer treatment by magnetic heating

Ingrid Hilger[a,*], Rudolf Hergt[b], Werner A. Kaiser[a]

[a]*Institut für Diagnostische und Interventionelle Radiologie des Klinikums der Friederich-Schiller-Universität Jena, Bachstraße 18, D-07740 Jena, Germany*
[b]*Institut für Physikalische Hochtechnologie e.V., Winzerlaer Straße 10, D-07745 Jena, Germany*

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Abstract

Recent studies encourage the application of minimal-invasive techniques for the treatment of breast cancer. Therefore, two different approaches related to the use of magnetic heating (hyperthermia and thermoablation) are proposed. Hereby, the tumour is loaded with a magnetic material (iron oxide) and exposed to an alternating magnetic field in order to generate heating. Different therapeutic conditions will be discussed.
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Keywords: Magnetic heating; Hyperthermia; Thermoablation; Nanoparticles; Breast cancer; Minimal-invasive therapy; Specific heating power; Temperature

1. Introduction

Every woman in the Western world worries about breast cancer. It is still one of the most important issues medicine is dealing with. Nevertheless, the latest developments are promising. As a result of the implementation of newer techniques, cancers are being diagnosed at earlier stages, and, consequently, fewer women are dying. Mostly, the detected tumours are associated with a good prognosis [1,2], which implicates that the possibility of a minimal-invasive treatment without deformation of the morphological structure of the organ is of special interest for the patient's emotional and physical welfare. Several minimal-invasive techniques have been developed using different therapeutic agents. For example, the intratumoural injection of ethanol, chemoembolisation, interstitial chemotherapy as well as temperature-based techniques as cytotoxic agents, such as cryotherapy (low temperatures between -20 and $-30\,^{\circ}C$), locoregional treatments applying ultrasound radiofrequency waves or laser as energy sources have been reported (e.g. [3]). Concerning the deposition of energy at the target, the use of a magnetic material (iron oxide, e.g. magnetite) was proposed. The magnetic material absorbs energy from an alternating magnetic field

*Corresponding author. Tel.: +49 3641 9325920/21; fax: +49 3641 9325922.
E-mail address: ingrid.hilger@med.uni-jena.de (I. Hilger).

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to convert it into heat that is used to eliminate the tumour. The magnetic approach was first investigated by Gilchrist et al. in the year 1957 [4]. They injected iron oxide particles into lymph nodes of dogs and observed temperature increases of approximately 5 °C after the exposure of the animals to an alternating magnetic field.

For the implementation of this method for the breast, basically two different situations are likely to take place: the treatment of in situ tumours on one hand, multi-focal ones on the other. For each situation an accurate clarification of influencing parameters and treatment conditions is required, which will be discussed in the text.

2. Treatment of non-invasive, in situ breast tumours

Non-invasive, in situ tumours, which remain in the site of origin, are frequently detected at a stage, when they are still small and confined. This is the case where cancer cells have not grown into the surrounding tissues and remain within the borders of a duct or lobule. Such tumours are the ideal targets for the first implementation of the magnetically induced minimal-invasive treatment. Using radiological stereotactic methods for tumour puncture, the magnetic material can be directly applied to the tumour. In this context, extensive examinations have been performed for the determination of the temperature regime, the monitoring of the localisation of the magnetic material, as well as experimental testing in mice and rats (e.g. [5]). As useful magnetic material, our investigations were focused on nanoparticles of magnetite and maghemite with core diameter range of 10–20 nm. These particles have a coating of dextran, which is a good base for further coupling of, e.g., antibodies. In order to hold the particles dose in the body as low as possible, a high value of the specific heating power (SHP) is necessary [6]. Although SHP increases both with AC-magnetic field amplitude as well as frequency, one cannot increase these parameters above a critical value in order to avoid inductive heating of the healthy tissue. As useful values an amplitude of 10 kA/m and a frequency of 410 kHz have been proved in animal experiments [5,7].

Since the intratumoral loading of in situ tumours with magnetic nanoparticles could be easily controlled, the generation of thermoablasive temperatures is feasible (see below). In order to be effective, the implementation of adequate time–temperature regimes is crucial. Intensive studies considering time-dependent temperature dosages have been widely performed for hyperthermic treatments (using temperatures up to 42 °C for approximately 60 min). Nevertheless, since systematic data regarding thermoablasive tumour treatments are rare, we performed corresponding heating experiments on cells in culture in order to clarify the corresponding relationships. It could be shown that after heating at temperatures, starting from 37 to 62 °C for 4 min, a decreasing cell survival with increasing culture times after heating takes place. The critical temperature leading to cell death was found to be between 51 and 55 °C for treatment times of several minutes [8]. It was concluded that cell viability is strongly related to the thermostability of defined vital proteins (e.g. [9]). Considering the clinical situation, thermoablasive temperatures between 51 and 55 °C should be generated at the tumour region. In analogy to the currently established surgical procedures, the range of the critical temperatures should be extended to a security rim of 1 cm around the tumour border. Ideally, and looking at current discussions related to the treatment of early breast tumour stages with a good prognosis [10], the implementation of the proposed method as a single therapy modality is conceivable and under progress now.

The intrinsic potential of the magnetic approach is high. Distinct effects of magnetic induced heating (2 min, magnetic field amplitude: 6.5 kA/m, frequency: 400 kHz) were observed when defined iron oxide masses (ferromagnetic material, main grain size 1 μm) were injected into the heat sensitive muscle tissue showing the formation of distinct fringes of necrotic tissue around the particle agglomerations [11]. From experiments with magnetic nanoparticles (particle core diameter: 10 nm; 7–112 mg/ approx. 4 cm^3 tissue specimens) applied to breast tissue, temperature increases up to the boiling point of water were observed [7]. Moreover, it could be shown that the

maximum temperature rise achieved in the target region depends on the square of the target radius as well as on the heat conductivity of the environment [12]. Therefore, adequate magnetic materials with high heating potential are necessary, particularly for the treatment of small tumours.

The visualisation of the distribution of magnetic nanoparticles at the target is an important precondition for an appropriate therapeutic quality control related to the particle distribution. In comparison to other methods, e.g. magnetic drug targeting, larger amounts of magnetic material will be needed for magnetic thermoablation, depending on the tumour size and the heating power of the magnetic material used. By injecting defined amounts of magnetic nanoparticles in lymph nodes from swine—an in vitro tumour model—it could be shown that radiography is the method of choice for the depiction of magnetite agglomerates. Magnetite amounts (superparamagnetic material, core diameter of 10 nm) as low as 1 mg were clearly detected [13].

The data from animal experiments revealed that the generation of localised heat spots is feasible even in the in vivo situation. On an average, after intratumoral application of magnetic nanoparticles with a core diameter of 10 nm (21 mg ± 9 magnetite per 299 mm^3 target tissue), temperatures up to around 71 °C [7] were measured at the tumour centre during magnetic heating (field amplitude: 6.5 kA/m, frequency: 400 kHz, treatment time: 242 s, Fig. 1). Considering the in vivo situation for the breast, a favourable situation emerges from the fact that the breast is mainly composed of fat with high insulation properties, so that the influences due to thermal conduction and convection are less pronounced as those known from thermal treatments of liver tumours. However, an adequate particle distribution within the tumour according to its shape is necessary in order to avoid the occurrence of areas with temperature underdosage [5].

Typically, using the aforementioned experimental conditions, a collapse of the subcutaneously implanted tumours as a result of destruction of tumour cells was observed after magnetic heating of subcutaneously implanted tumours in mice

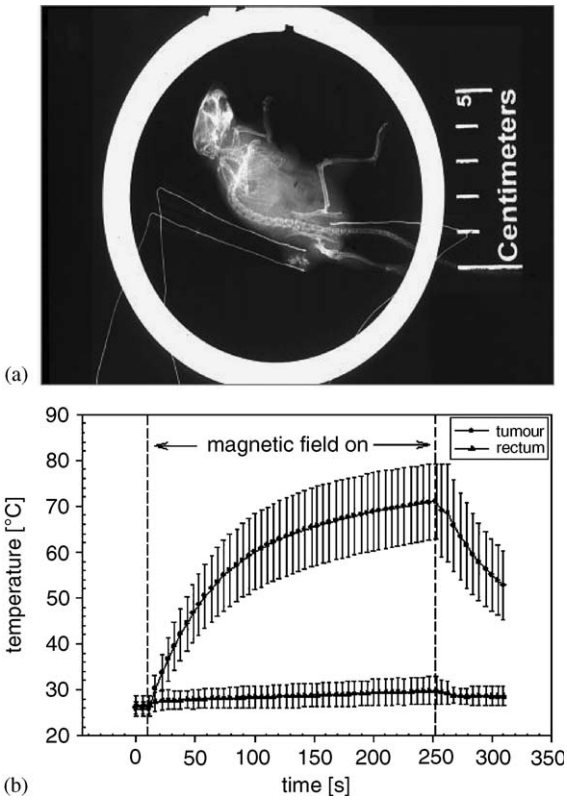

Fig. 1. Magnetic heating of tumours in mice. (a) Radiography showing the position of thermosensors at the distal and proximal tumour periphery. (b) Intratumoural and rectal temperature courses during the exposure of 10 tumour bearing mice to an alternating magnetic field (frequency: 410 kHz, amplitude: 8.8 kA/m) for 242 s. Intratumoural application of 21 ± 9 mg dextran coated magnetite (with permission).

(Fig. 2). Microscopically, those tumours showed typical signs for the induction of coagulation necrosis, such as chromatin migration along the nuclear envelope and nuclear pyknosis [7] and DNA damages [14]. These are further evidences for the potential of the proposed method.

The long-term effects resulting from heating could be deduced from previous observations in relation to the morphological features. According to this, the occurrence of hyperthrophic granulation tissue followed by healing through the formation of keloid is conceivable. In unfavourable cases side effects like fever are likely to occur [15].

All in all, investigations indicate that the minimal-invasive treatment of early stages of in

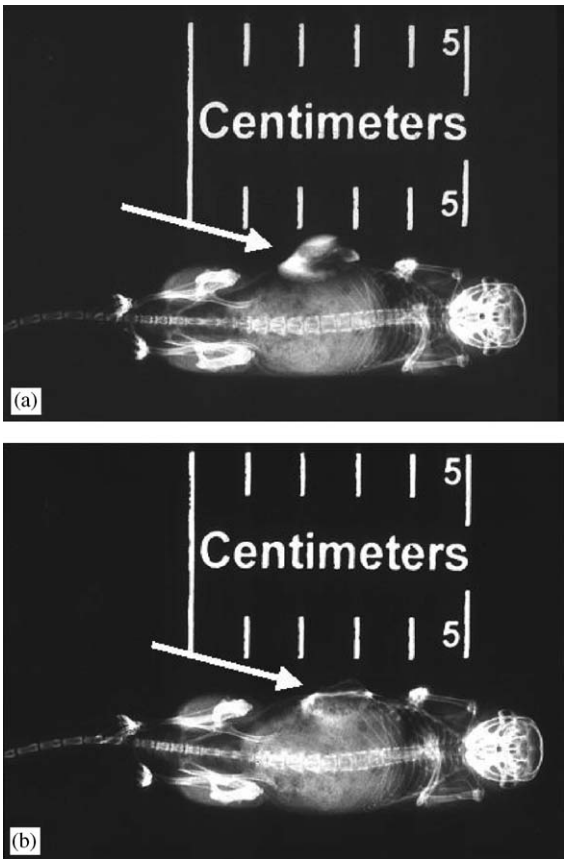

Fig. 2. Radiograph showing the typical macroscopic observations of a tumour bearing mice before (a) and after (b) magnetic tumour heating. Arrow: tumour (with permission) form (7).

situ breast tumours is a promising method in the near future. Corresponding activities for first clinical applications are currently being performed. Recently, the treatment of human tumours in the brain by magnetic fluid hyperthermia carried out by a group in Berlin further reinforce the feasibility of our approach.

3. Treatment of multi-focal tumours

The minimal-invasive treatment of multi-focal tumours in the breast is still an unsolved problem, since their access using current radiological stereotactic tumour puncture is difficult. One solution to this problem is the intravasal accumulation of the magnetic material. Micrometastatic tumours with passive diffusion for oxygen and nutrient supply have a limited growth up to $2\,mm^3$. In order to be able to grow larger, tumours generate a new vasculature system. This process was called "tumorangiogenesis" [16]. According to this, tumours larger than $2\,mm^3$ are connected to the vessel system and, consequently, accessible to magnetic nanoparticles, if applied into the vessel system. When reaching the tumour region, those magnetic nanoparticles should have access to specific structures on cell membranes at the target region, particularly when binding is mediated by highly specific biomolecules. Ideally, the therapeutic agents could find the target structure by itself regardless of their localisation within the breast.

With this background, initial in vitro experiments were performed for demonstrating the basic capabilities of the aforementioned approach. Modelling the nanoparticle binding (core diameter: 3–15 nm) on the cell surface, cell labelling using the periodate method was performed. By this method, a periodate oxidation of the coating polymer is performed which reacts with amino groups on the cell surface to form a Schiff's base. The Schiff's base is subsequently stabilised by reduction. Distinct cell labelling could be detected by magnetorelaxometry (MRX)—which measures the relaxation of particle magnetisation by means of a SQUID sensor after switching off an external magnetic field (e.g. [17])—as well as by electron microscopy and Prussian blue staining [18]. It could be shown that the magnetic nanoparticles are predominantly localised at the cell surface while some of them were already endocytosed (Fig. 3). In the in vivo situation, cells in the tumour region exposed to the blood vessel system are the first target structures magnetic nanoparticles could bind to. It was further shown that cells labelled with magnetic nanoparticles could also be detected by a clinical magnetic resonance tomography system. T1-weighted images showed distinct signals for cell amounts as low as 10 labelled cells per voxel ($0.039\,cm^3$) or 0.01 μmol iron per ml [19]. This preliminary data demonstrate that, in the long term, there are also good possibilities for an adequate imaging using established radiological imaging systems.

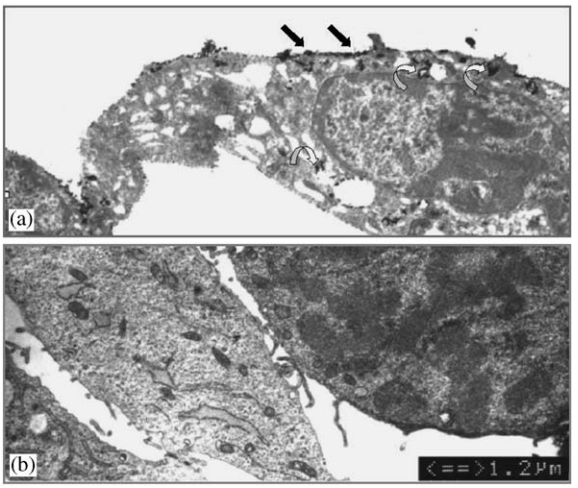

Fig. 3. Electron microscopy micrographs showing the features of mouse endothelial cells labelled (a) with magnetic nanoparticles (periodate method, core diameter: approx. 10 nm, dextran coated) and not labelled (b, control cells). Arrow: nanoparticle bound at the plasmalemma, curved arrow: endocytosed nanoparticles (modified after [18], with permission).

The in vitro magnetic heating treatments of the aforementioned magnetically labelled cells (containing iron amounts of up to 107 pg/cell) revealed temperature increases—depending on the magnetic nanoparticle sample—between 2 and 13 °C after the exposure to the magnetic field (amplitude: 11 kA/m, frequency: 410 kHz) for 3.2 min [18,20]. It should be considered, that the suspension of cells in buffer lead to a temperature increase of almost 1 °C as a result of the occurrence of eddy currents. In the light of the in vivo situation, temperatures at least over 40 °C are necessary to increase the in vivo sensitivity of tumour cells to radiation and chemotherapy [21]. Moreover, taking into account that the accumulated iron oxide mass at the target will be limited—particularly due to the number of antigens expressed on the cell surface—the use of nanoparticles with high heating potential is imperative. In addition, thermal convection and conduction take place in the in vivo situation, particularly when hyperthermic temperatures (approximately 45–47 °C for treatment times over 30 min) are used, which comprises a further challenge for the design of potent magnetic nanoparticles. In so far, the in vitro observed temperature increases are, of course, still too low for the intended therapeutic application.

Our data has shown that cell labelling, magnetic heating (so called "targeted magnetic hyperthermia") and imaging could be generated in a cell model system for the binding of magnetic nanoparticles as it is likely to occur after administration of the magnetic material to the vessel system.

Taking together, it is conceivable that the minimal-invasive treatment of in situ breast cancers could be implemented in the clinical practice in the near future. Compared to the therapy of non-invasive in situ breast tumours, the treatment of multi-focal ones is determined by a higher complexity of interacting parameters so that the way to the clinical implementation using the accumulation of the magnetic material through the vessel system is still a longer one to cover.

Acknowledgements

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References

[1] C. Harmer, M. Staples, A.M. Kavanagh, Cancer Cause. Control 10 (1999) 333.
[2] E.S. Hwang, H.S. Cody, Southern Med. J. 91 (1998) 522.
[3] E.A. Dick, S.D. Taylor-Robinson, H.C. Thomas, et al., Gut 50 (2002) 733.
[4] R.K. Gilchrist, R. Medal, W.D. Shorey, et al., Ann. Surg. 146 (1957) 596.
[5] I. Hilger, R. Hiergeist, R. Hergt, et al., Invest. Radiol. 37 (2002) 580.
[6] R. Hergt, R. Hiergeist, M. Zeisberger, et al., J. Magn. Magn. Mater. (this issue).
[7] I. Hilger, W. Andrä, R. Hergt, et al., Radiology 218 (2001) 570.
[8] I. Hilger, S. Frühauf, W. Andrä, et al., Thermology Int. 11 (2001) 130.
[9] M.A. Joly, A Physico-chemical Approach to Denaturation of Proteins, Academic Press, New York, 1965.
[10] L. Tabar, G. Fagerberg, N.E. Day, et al., Lancet 339 (1992) 412.
[11] I. Hilger, R. Hergt, W.A. Kaiser, Invest. Radiol. 35 (2000) 170.
[12] R. Hergt, W. Andrä, C.G. d'Ambly, et al., IEEE Trans. on Magn. 34 (1998) 3745.

[13] I. Hilger, F. Hofmann, J.R. Reichenbach, et al., Fortschr. Röntgenstr. 173 (2002) 101.
[14] I. Hilger, A. Rapp, K.O. Greulich, W.A. Kaiser. Assessment of DNA damages on target tumor cells after thermoablasive heating. Radiology (2005), in press.
[15] U.-N. Riede, H.-E. Schaefer, in: U.-N. Riede, H.-E. Schaefer (Eds.), Allgemeine und Spezielle Pathologie, Georg Thieme Verlag, Stuttgart, 1995, p. 154.
[16] J. Folkmann, Nature Medicine 2 (1996) 167.
[17] E. Romanus, M. Hückel, C. Groß, et al., J. Magn. Magn. Mater. 252 (2002) 387.
[18] I. Hilger, A. Kießling, E. Romanus, et al., Nanotechnology 15 (2004) 1027.
[19] I. Hilger, J.R. Reichenbach, B. Danz, et al., Eur. Radiol. 13 (Suppl. 1) (2003) 131.
[20] I. Hilger, S. Polloczek, C. Fritsche, et al., Eur. Radiol. 14 (Suppl. 2) (2004) 331.
[21] P. Wust, M. Molls, Hyperthermie in Kombination mit Radiotherapie und/oder Chemotherapie, third ed., Springer, Berlin, 1999.