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Magnetic nanoparticle hyperthermia enhancement of cisplatin chemotherapy cancer treatment

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Abstract

Purpose—The purpose of this study was to examine the therapeutic effect of magnetic nanoparticle hyperthermia (mNPH) combined with systemic cisplatin chemotherapy in a murine mammary adenocarcinoma model (MTGB).

Materials and methods—An alternating magnetic field (35.8 kA/m at 165 kHz) was used to activate 110 nm hydroxyethyl starch-coated magnetic nanoparticles (mNP) to a thermal dose of 60 min at 43 °C. Intratumoral mNP were delivered at 7.5 mg of Fe/cm³ of tumour (four equal tumour quadrants). Intraperitoneal cisplatin at 5 mg/kg body weight was administered 1 h prior to mNPH. Tumour regrowth delay time was used to assess the treatment efficacy.

Results—mNP hyperthermia, combined with cisplatin, was 1.7 times more effective than mNP hyperthermia alone and 1.4 times more effective than cisplatin alone ($p < 0.05$).

Conclusions—Our results demonstrate that mNP hyperthermia can result in a safe and significant therapeutic enhancement for cisplatin cancer therapy.

Keywords

Cisplatin; cumulative equivalent minutes; hyperthermia; iron oxide; nanoparticle

Introduction

Many chemotherapeutics such as cisplatin (CDDP) interact positively with therapeutic hyperthermia, and as a result are strong candidates for clinical hyperthermia adjuvant therapies [1–4]. Magnetic nanoparticle hyperthermia (mNPH) allows for greater tumour targeting than more conventional hyperthermia delivery techniques and the potential to achieve an improved therapeutic ratio. Despite the benefits of combining hyperthermia and chemotherapy, the difficulty of delivering a controlled and effective thermal dose has

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Declaration of interest

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hindered many adjuvant efforts. mNP hyperthermia is unique from other hyperthermia platforms. The heat sources may be located within, or in close proximity to, the targeted cells themselves and conform to the physical tumour boundary. We hypothesise that mNP hyperthermia will be able to deliver a less invasive and therefore more effective and controlled thermal dose than traditional hyperthermia platforms. The use of precise intratumoral delivery, static magnetic fields, and/or tumour antibodies will enable precise treatment of the tumour. If mNP are administered systemically, there is potential to treat metastatic masses if appropriate targeting techniques, such as antibodies and vascular alteration, are employed.

Magnetic nanoparticle hyperthermia therapy

The mNP hyperthermia technique we utilised for this study primarily relies on extracellular tumour heating. Experiments, included in an accompanying paper (this issue, pp. 819–27), suggest that when the mNP are extracellular, this tumour heating is primarily a ‘global’ tumour heating phenomena rather than intracellular, individual cell, heating. That said, the relative contributions of intracellular versus extracellular mNP therapeutic activity remain unclear. As mNP hyperthermia is generated by localised and internal heat sources, biological effects may exist which differ from those observed with conventional hyperthermia application. In this study it is our assumption that mNP heating interacted with the cisplatin in a manner similar to that of conventional hyperthermia and cisplatin.

Hyperthermia and chemotherapy

Hyperthermia is known to interact with chemotherapeutics through a variety of mechanisms, including, but not limited to, changes in cellular metabolism, membrane permeability and membrane transport, acceleration of primary modes of action, reversal of repair mechanisms, increasing oxygen radical production, and alterations of local tumour environment (pH and nutrient state) [5–7]. In addition, at a tissue-regional level, hyperthermia is known to modify blood flow in tumours and normal tissues, which is critical to the distribution of systemically delivered agents [8,9]. Hyperthermia has the capacity to both increase blood flow in response to elevated temperatures and induce vascular stasis. In comparison to normal tissue, tumours have a lower capacity to increase blood flow, with stasis occurring at lower thermal doses [10]. As blood flow may be either increased or decreased in response to heat, it is important to consider the temperatures and duration of thermal treatment when assessing systemically delivered chemotherapy.

Cisplatin mechanism of action

The primary mechanism of cisplatin tumour treatment effect is through the formation of DNA adducts, which consequently interferes with transcription and replication, resulting in cell death [11]. Although exposure to cisplatin often results in apoptotic cell death, cells may also die due to necrosis or other cell-cycle based mechanisms. Consequently, necrotic and apoptotic death may be observed within the same cell population [11,12].

Improving cisplatin efficacy and safety with mNP hyperthermia

Despite its efficacy, cisplatin treatment is limited by a number of clinically important side effects including nephrotoxicity, neurotoxicity, myelosuppression, and ototoxicity. Additional complications can be observed when cisplatin is used in conjunction with other therapies [13]. As with other chemotherapeutic agents, the vast majority of administered cisplatin does not reach the tumour. At 3 and 24 hours after intravenous administration, approximately 76% and 95% of the delivered cisplatin dose, respectively, is bound to plasma protein, potentially limiting the amount of drug reaching tumour cell DNA [11,14]. Intracellular mNP may be able to exploit this inherent situation for therapeutic gain.

It is thought that cisplatin enters into the cell through both passive transport and active transport systems. By increasing blood flow and membrane permeability, hyperthermia allows for greater cisplatin entry into the cell, leading to additional formation of adducts [7,15–18]. The use of hyperthermia with cisplatin has been shown experimentally to reduce cisplatin resistance in tumours. Cisplatin resistant cells exhibit fewer plasma membrane receptors and transporters, as well as reduced endocytosis [19]. The addition of hyperthermia ‘reverses’ resistance in cisplatin resistant cells, improving cytotoxicity by increasing cisplatin concentrations and adduct formation [16–18]. Because of the targeted and superior localisation of mNP hyperthermia, combining it with systemically delivered cisplatin is likely to improve the therapeutic ratio beyond that achieved with conventional hyperthermia. Furthermore, the unique nature of mNP hyperthermia may result in biological effects beyond those expected with tissue-level, global applications of hyperthermia, both with and without the addition of chemotherapeutics. It is also worth noting that cisplatin has been successfully loaded into magnetic nanoparticles. In these experimental systems mNPH is used to enhance drug release [20,21]. Cisplatin in combination with mNPH has also been demonstrated to be effective *in vitro* [22,23].

Although mNP hyperthermia has great potential, especially when used as part of an adjuvant treatment strategy, much work remains to be done in order to optimise this technology and to understand how the mechanisms of interaction may differ from traditional hyperthermia platforms. In particular, studies designed to address the effect of mNP incubation, intracellular/extracellular location, and biodistribution on mNP hyperthermia tumour treatment efficacy are critical for the optimisation of this technology.

Materials and methods

Model

Mouse mammary adenocarcinoma cells (MTGB) were used to grow syngeneic mammary tumours in the flanks of female C3H mice (Charles River Laboratories, Wilmington, MA) aged 6–8 weeks. These cells are a virally induced mouse breast tumour line that was originally derived in 1960 [24,25]. They were grown with modified alpha minimum essential medium (MEM) (Mediatech, Manassas, VA) with additives of 10% FBS (HyClone Laboratory, South Logan, UT), 1% penicillin-streptomycin (HyClone), 1% L-glutamine (Mediatech). The cells were treated with 0.25% trypsin in EDTA (HyClone). Cells were suspended in unaltered alpha MEM at a concentration of ten million cells/mL prior to

inoculation at 1×10^6 . Tumours were treated when they reached a volume of $150 \text{ mm}^3 \pm 40 \text{ mm}^3$, approximately 2 weeks following inoculation. Tumours were measured using digital calipers. Tumour volume was calculated using the measured perpendicular diameters (d_1 , d_2 , d_3) of the ellipsoidal tumour, found with digital calipers and the equation

$$\text{Volume} = \frac{\pi \times d_1 \times d_2 \times d_3}{6}$$

The mice were sacrificed and the tumours removed when the tumour reached three times the treatment volume (study end point).

mNP injection and dosimetry

The mNP used in these experiments had a 50-nm Fe_3O_4 core and 110-nm hydrodynamic diameter with a biocompatible hydroxyethyl starch coating (MicroMod, Rostock, Germany). Details regarding their manufacture are detailed by Grüttner et al. [26,27]. The mNP are ferromagnetic and heat via magnetic hysteresis when an alternating magnetic field (AMF) is applied. The mNP were suspended at a total mNP concentration of 42 mg/mL (28 mg of Fe/mL). Tumours were injected intratumorally in four equal quadrants at a dose of 7.5 mg/cm^3 . The total delivered volume of mNP was between 29 and 51 μL (depending on tumour size). mNP injections were performed 10 min prior to AMF activation.

Administration of AMF

The AMF was generated by a water cooled, circular coil (Fluxtrol, Auburn Hills, MI) capable of exposing the entire mouse to the AMF. This coil was comprised of 8 mm square tubing and a concentrator made of Ferrotron 559 [28]. It was 5 cm long, with a total of five turns resulting in a 3.6 cm internal diameter and 5.2 cm outer diameter. The coil was powered by a Huttinger TIG 10/300 generator (Freiburg, Germany) operating at 165 kHz and 35.8 kA/m and a constant temperature of 30 °C. A TKD250 chiller (Tek-Temp Instruments, Croydon, PA) was used to cool the generator and coil.

Temperature recording and thermal dose

Mouse core and tumour temperatures were measured continuously throughout the experimental period using a 560 μm diameter fibre-optic probe (FISO, Quebec, Canada). Tumour temperature measurements were taken using a single fibre-optic probe placed in the centre of the tumour. Tumour temperatures were measured in one central location. Mice were treated under anaesthesia using 1–3% isoflurane gas and 95% O_2 . Core temperatures were taken via the rectum.

The biological effects of heat on tissue are a function of time and temperature. As individual tumours heat at different rates, it is useful and more accurate to describe the treatment in terms of biological effect. The cumulative equivalent minute (CEM) relationship, proposed by Sapareto and Dewey, normalises hyperthermia treatments by describing the biological effect in terms of CEM at 43 °C [25]. The CEM relationship is $\text{CEM} = tR^{43^\circ\text{C} - T}$, where 't' is equal to the time interval at a specific temperature 'T', R equals 0.25 when temperatures

are below 43 °C and 0.45 when temperatures are above 43 °C [29]. The total thermal dose is equivalent to the summation of these values.

The majority of animals treated achieved a CEM of 60 within 20 min of AMF activation (average of 15.3 min, standard deviation (SD) of 3.8 min). Three animals, two from the mNP + AMF + cisplatin treatment group and one from the mNP + AMF treatment group, took significantly longer to reach a CEM of 60 (average of 51.6 min, SD 19.1 min). The average temperature throughout the treatment for the tumours which heated rapidly was $42.6\text{ }^{\circ}\text{C} \pm 2.4\text{ }^{\circ}\text{C}$, with a maximum temperature of $46.5\text{ }^{\circ}\text{C} \pm 0.7\text{ }^{\circ}\text{C}$. The average temperature throughout the treatment for the tumours which heated less rapidly was $42\text{ }^{\circ}\text{C} \pm 1.9\text{ }^{\circ}\text{C}$ with a maximum temperature of $45.6\text{ }^{\circ}\text{C} \pm 1.1\text{ }^{\circ}\text{C}$. As the treatment duration was shorter for animals which experienced rapid heating in the tumour, the average rectal temperature for this group was lower than for animals which took over 20 min to reach a CEM of 60 in the tumour. The average rectal temperature for animals in this group was $37.3\text{ }^{\circ}\text{C} \pm 0.6\text{ }^{\circ}\text{C}$, in comparison to $38.9\text{ }^{\circ}\text{C} \pm 1.2\text{ }^{\circ}\text{C}$ for animals which took over 20 min to reach a CEM of 60 in the tumour. A summary of tumour and core temperatures is included in Table I.

Cisplatin

Pharmaceutical grade cisplatin (Teva Parenteral Medicines, Haarlem, Netherlands) was administered intraperitoneally, in 1 mL PBS (phosphate buffered saline) (Corning Cellgro, Manassas, VA), 1 h prior to hyperthermia, at 5 mg/kg body weight.

Treatment groups

Seven treatment groups were studied: (1) mNP + AMF ($n = 6$), (2) mNP + AMF + cisplatin ($n = 7$), (3) cisplatin ($n = 4$), (4) cisplatin + AMF ($n = 4$), (5) cisplatin + mNP ($n = 6$), (6) AMF ($n = 5$), and (7) no treatment ($n = 6$). All tumours receiving hyperthermia were treated to CEM60 at the centre of the tumour. AMF treatment was scaled according to CEM values. AMF control animals received 30 min of AMF exposure at 165 kHz and 35.8 kA/m. Control/sham treated animals received PBS at the same volumes used for the mNP treatment. A summary of treatment groups is included in Table II.

Histology

Representative histological sections were taken from the following groups of mice: cisplatin, mNP + AMF, mNP + AMF + cisplatin 24 h after treatment. Tumour and peritumour tissue were fixed in 10% neutral buffered formalin, set in paraffin blocks and stained with haematoxylin and eosin (H&E).

Results

Tumours were treated when they reached a volume of $150 \pm 40\text{ mm}^3$. Control and AMF alone treatments reached the designated three-fold increase in size end point at 14 days (Figure 1). The administration of cisplatin alone or combined with mNP or AMF alone (no heat) increased tumour regrowth delay (25–27 days). The use of mNP with AMF activation (CEM60) resulted in a tumour regrowth delay of 21 days. The combination mNP and AMF

(CEM 60) and cisplatin achieved a tumour regrowth delay of 36 days. All of these groups are statistically different from each other at $p < 0.02$ (except for mNP and AMF alone versus cisplatin controls). The average regrowth delay for tumours which achieved a CEM of 60 (mNP + AMF) in less than 20 min was 21 days (SD 5 days). The regrowth delay for the one animal from this group which took longer than 20 min was 20 days. The average regrowth delay for tumours which achieved a CEM of 60 (mNP + AMF + cisplatin) in less than 20 min was 36 days (SD 2.9 days). The average regrowth delay for the two animals from this group which took longer than 20 min was 36 days (SD 0.7 days). From these results we conclude that the variation in treatment length did not affect the tumour regrowth delay.

Histopathological effects

As expected, cisplatin alone resulted in a combination of individual cell apoptotic and necrotic cytotoxicity (Figure 2). mNP hyperthermia alone demonstrated regional 'hyperthermia compatible' necrosis (Figure 3). The combination of cisplatin and mNPH resulted in extensive, near uniform tumour necrosis (Figure 4). The combined effect of the two modalities may have obscured/overwhelmed cells that may have also been undergoing cisplatin-based apoptosis.

Discussion

The addition of mNP hyperthermia to systemically administered cisplatin produced significant regrowth delay, in comparison to either modality alone, without any additional significant morbidity. mNP delivered directly into the tumour and activated by AMF appear to result in a thermal dose confined closely to the tumour boundary. Though the combination therapy resulted in significant therapeutic improvement, mNP hyperthermia is an emerging strategy that has yet to be optimised. We acknowledge that the use of mNPH in experimental tumours which are small and superficial lacks many of the complications which may be encountered in the clinic. However, additional ongoing work from our laboratory in spontaneous oral tumours (canine) has been promising. Improved mNP heat generation and localisation through particle design, treatment timing, particle uptake and antibody targeting have the potential to generate cytotoxicity beyond predicted by a measurable thermal dose. The potential for excellent localisation of the heat sources within the cells also produces the potential to improve hyperthermia–chemotherapeutic interactions. Improvements in mNP design and AMF delivery will likely improve treatment through greater energy release per mNP. Preliminary results suggest pretreatment strategies with chemotherapy, radiation and static magnetic fields will improve mNP uptake and distribution within the tumour.

Conclusion

These studies demonstrate mNP hyperthermia enhancement of systemically delivered cisplatin therapy in a murine breast tumour model. mNP hyperthermia, combined with cisplatin was 1.7 times more effective than mNP hyperthermia alone and 1.4 times more effective than cisplatin alone ($p < 0.05$). An increased understanding of treatment timing and mNP hyperthermia parameters (improved mNP distribution, mNP heating properties and AMF generation) will further improve treatment at even lower cisplatin doses.

References

1. Barlogie B, Corry PM, Drewinko B. In vitro thermochemotherapy of human colon cancer cells with cis-dichlorodiammineplatinum (II) and mitomycin C. *Cancer Res.* 1980; 40:1165–8. [PubMed: 7188883]
2. Herman TS, Teicher BA, Collins LS. Effect of hypoxia and acidosis on the cytotoxicity of four platinum complexes at normal and hyperthermic temperatures. *Cancer Res.* 1988; 48:2342–7. [PubMed: 3356002]
3. Hall, EJ. Radiobiology for the radiologist. 5. Philadelphia: Lippincott Williams & Wilkins; 2000.
4. van Bree C, Rietbroek R, Schopman E, Kipp J, Bakker P. Local hyperthermia enhances the effect of cis-diamminedichloroplatinum(II) on nonirradiated and preirradiated rat solid tumors. *Int J Radiation Oncology Biol Phys.* 1996; 36:135, 135–96.
5. Bull JM. An update on the anticancer effects of a combination of chemotherapy and hyperthermia. *Cancer Res.* 1984; 44:S4853–6.
6. Hahn GM. Metabolic aspects of the role of hyperthermia in mammalian cell inactivation and their possible relevance to cancer treatment. *Cancer Res.* 1974; 34:3117–23. [PubMed: 4138162]
7. Hildebrandt B, Wust P, Ahlers O, Dieing A, Sreenivasa G, Kerner T, et al. The cellular and molecular basis of hyperthermia. *Crit Rev Oncol.* 2002; 43:33–56.
8. Song CW, Park HJ, Lee CK, Griffin R. Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment. *Int J Hyperthermia.* 2005; 21:761–7. [PubMed: 16338859]
9. Ausmus PL, Wilke AV, Frazier DL. Effects of hyperthermia on blood flow and cis-diamminedichloroplatinum(II) pharmacokinetics in mammary adenocarcinomas. *Cancer Res.* 1992; 52:4965–8. [PubMed: 1516052]
10. Dudar TE, Jain RK. Differential response of normal and tumor microcirculation to hyperthermia. *Cancer Res.* 1984; 44:605–12. [PubMed: 6692365]
11. Fuertes M, Castilla J, Alonso C, Prez J. Cisplatin biochemical mechanism of action: From cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. *Curr Med Chem.* 2003; 10:257–66. [PubMed: 12570712]
12. Gonzalez VM, Fuertes MA, Alonso C, Perez JM. Is cisplatin-induced cell death always produced by apoptosis? *Mol Pharmacol.* 2001; 59:657–63. [PubMed: 11259608]
13. Hartmann JT, Kollmannsberger C, Kanz L, Bokemeyer C. Platinum organ toxicity and possible prevention in patients with testicular cancer. *Int J Cancer.* 1999; 83:866–9. [PubMed: 10597214]
14. Sooriyaarachchi M, Narendran A, Gailer J. Comparative hydrolysis and plasma protein binding of cisplatin and carboplatin in human plasma in vitro. *Metallomics.* 2011; 3:49–55. [PubMed: 21135941]
15. Meyn RE, Corry PM, Fletcher SE, Demetriades M. Thermal enhancement of DNA damage in mammalian cells treated with cis-diamminedichloroplatinum(II). *Cancer Res.* 1980; 40:1136–9. [PubMed: 7188882]
16. Hettinga J, Konings A, Kampinga H. Reduction of cellular cisplatin resistance by hyperthermia – A review. *Int J Hyperthermia.* 1997; 13:439–57. [PubMed: 9354931]
17. Van de Vaart P, Van der Vange N, Zoetmulder F, Van Goethem A, Van Tellingen O, ten Bokkel Huinink W, et al. Intraperitoneal cisplatin with regional hyperthermia in advanced ovarian cancer: Pharmacokinetics and cisplatin-DNA adduct formation in patients and ovarian cancer cell lines. *Eur J Cancer.* 1998; 34:148–54. [PubMed: 9624250]
18. Wallner KE, DeGregorio MW, Li GC. Hyperthermic potentiation of cis-diamminedichloroplatinum(II) cytotoxicity in Chinese hamster ovary cells resistant to the drug. *Cancer Res.* 1986; 46:6242–5. [PubMed: 3779644]
19. Gottesman MM. Mechanisms of cancer drug resistance. *Annu Rev Med.* 2002; 53:615–27. [PubMed: 11818492]
20. Taylor A, Krupskaya Y, Krämer K, Füssel S, Klingeler R, Büchner B, et al. Cisplatin-loaded carbon-encapsulated iron nanoparticles and their in vitro effects in magnetic fluid hyperthermia. *Carbon.* 2010; 48:2327–34.

21. Kettering M, Zorn H, Bremer-Streck S, Oehring H, Zeisberger M, Bergemann C, et al. Characterization of iron oxide nanoparticles adsorbed with cisplatin for biomedical applications. *Phys Med Biol.* 2009; 54:5109–21. [PubMed: 19661569]
22. Lee JS, Rodriguez-Luccioni HL, Méndez J, Sood AK, Lpez-Berestein G, Rinaldi C, et al. Hyperthermia induced by magnetic nanoparticles improves the effectiveness of the anticancer drug cis-diamminedichloroplatinum. *J Nanosci Nanotechnol.* 2011; 11:4153–7. [PubMed: 21780419]
23. Babincov M, Altanero V, Altaner C, Bergemann C, Babinec P. In vitro analysis of cisplatin functionalized magnetic nanoparticles in combined cancer chemotherapy and electromagnetic hyperthermia. *IEEE Trans Nanobioscience.* 2008; 7:15–19. [PubMed: 18334449]
24. Hoffman NA, Gollin FF, Clifton KH. Acquired radioresistance of transplanted mammary adenocarcinoma in inbred mice after serial irradiation. *Radiology.* 1967; 88:568–75. [PubMed: 6019522]
25. Cohn NK, Clifton KH. Aspects of the biology and radiation response of cloned C3H mouse mammary carcinoma cells in vitro and in vivo. *Eur J Cancer.* 1971; 7:505–14. [PubMed: 5143808]
26. Grüttner C, Müller K, Teller J, Westphal F, Foreman A, Ivkov R. Synthesis and antibody conjugation of magnetic nanoparticles with improved specific power absorption rates for alternating magnetic field cancer therapy. *J Magn Magn Mater.* 2007; 311:181–6.
27. micromod Partikeltechnologie, Rostock, Germany. [accessed 2013] Functionalized Micro- and Nanoparticles, product details. <http://www.micromod.de/en/functionalized-micro-and-nanoparticles-1.html>
28. Fluxtrol Center for Induction Technology, Auburn Hills, MI. [accessed 2013] Ferrottron 559H, product details, 2010. <http://fluxtrol.com/products/product-types/ferrotron-559h/>
29. Dewhirst MW, Viglianti BL, Lora-Michiels M, Hanson M, Hoopes PJ. Basic principles of thermal dosimetry and thermal thresholds for tissue damage from hyperthermia. *Int J Hyperthermia.* 2003; 19:267–94. [PubMed: 12745972]

Figure 1.

This figure represents the number of post-treatment days required for the tumours to reach three times treatment volume. mNP + AMF + cisplatin was 1.7 times more effective than mNP + AMF (36 versus 21 days), 1.4 times more than cisplatin + AMF (36 versus 25 days) and 2.6 times more than no treatment (36 versus 14 days). **p* values less than 0.003 when compared to mNP + cisplatin + AMF; ***p* values less than 0.02 when compared to no treatment.

Figure 2.

Low (10×, left) and high (100×, right) magnification photomicrographs of a MTGB female mouse mammary adenocarcinoma accessed 24 h following systemically administered cisplatin at 5 mg/kg body weight. Although some tumour cells remain morphologically normal, the majority of the cells have a reduced volume and both apoptotic and necrotic cells are present. H&E stain.

Figure 3.

Low magnification (2×, upper left) photomicrograph of a bi-lobed MTGB flank mammary adenocarcinoma treated with mNPH (CEM60) to the centre of the tumour 24 h following treatment. Regions 'A' and 'B', represented correspondingly by high magnification photomicrographs, demonstrate uniform tumour necrosis. The high magnification photograph of region 'C' demonstrates individual cell damage but also significant tumour viability. Regions 'A' and 'B' contained significant mNP/Fe, while region 'C' did not.

Figure 4.

The low magnification (2×) photomicrograph of a MTGB flank mammary adenocarcinoma (top) treated with mNPH (CEM60 to the centre of the tumour, 24 h after treatment) and cisplatin demonstrated uniform tumour necrosis. Higher magnification (100×) demonstrates a more detailed view of the necrosis (A) and the partially viable tumour present in isolated regions (B). The regions with viable tumour are located primarily at the deep tumour margin. This tumour was assessed 24 h following treatment, therefore additional treatment effect is likely ongoing. A normal hyperplastic lymph node is visible in the lower right quadrant of the low magnification image. H&E stain.

Table I

Summary of tumour and core temperatures of mice that received mNP + AMF or mNP + AMF + CDDP. The majority of tumours achieved CEM60 in the centre of the tumour. Total hyperthermia dose was delivered in less than 20 min. Three mice that received mNP only (1) and mNP + AMF + CDDP (2) had a reduced heating kinetic. Ten tumours achieved CEM60 in less than 20 min (average treatment was 15.3 min). Three tumours required more than 20 min (average treatment time = 51.6 min). In spite of this issue there was no difference for these groups with respect to treatment efficacy (tumour regrowth delay).

CEM60 in:	<20 min		>20 min	
	Average	SD	Average	SD
Tumour				
Average temperature (°C)	42.6	2.4	42.0	1.9
Maximum temperature (°C)	46.5	0.7	45.6	1.1
Starting temperature (°C)	32.9	1.3	33.4	2.4
Maximum temperature (°C)	13.7	1.7	12.2	2.5
Treatment time (min)	15.3	3.8	51.6	19.1
Rectal				
Average temperature (°C)	37.3	0.6	38.9	1.2
Maximum temperature (°C)	38.9	1.1	40.8	0.8
Starting temperature (°C)	35.8	0.5	36.3	0.7
Maximum temperature (°C)	3.1	1.4	4.6	0.4
Regrowth delay				
mNP + AMF	21.0	5.0	20.0	–
mNP + AMF + Cisplatin	35.8	2.9	35.5	0.7

AMF, alternating magnetic field; mNP, magnetic nanoparticles; SD, standard deviation.

Table II

Experimental groups, treatment summary and efficacy.

	Treatment description	Mice per group	Thermal dose?	Days to 3×	SD	Treatment ratio (versus no treatment)
mNP + CDDP + AMF	CDDP 1 h before AMF exposure. mNP injected immediately before activation. Treated to CEM60 at centre of tumour	7	Yes	36	2.4	2.6
mNP + AMF	mNP injected immediately before activation. Treated to CEM60 at centre of tumour	6	Yes	21	4.5	1.5
CDDP alone	CDDP given with no additional treatment/treatment controls	4	No	25	7.1	1.8
CDDP + AMF	CDDP 1 h before AMF exposure. PBS injected at the same volume as prescribed mNP for other groups. Thermal probes inserted and exposed to AMF for 30 min	4	No	25	6.3	1.8
CDDP + mNP	CDDP 1 h before mNP injection. Animal under anesthesia with body temperature maintained for 30 min after mNP injection	6	No	27	7.0	2.0
AMF	PBS injected at the same volume as prescribed mNP for other groups. Thermal probes inserted and exposed to AMF for 30 min	5	No	14	4.0	1.0
No treatment	No treatments/treatment controls	6	No	14	4.3	1.0