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Radiation enhanced efficiency of combined electromagnetic hyperthermia and chemotherapy of lung carcinoma using cisplatin functionalized magnetic nanoparticles

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The effect of trimodality treatment consisting of hyperthermia, cisplatin and radiation was investigated in two non-small lung carcinoma cell lines with different sensitivities to cisplatin. Hyperthermia treatment was performed using heat released via Neél and Brown relaxation of magnetic nanoparticles in an alternating magnetic field. Radiation with dose 1.5 Gy was performed after 15 min electromagnetic hyperthermia and cisplatin treatment. Electromagnetic hyperthermia enhanced cisplatin-induced radiosensitization in both the cisplatin-sensitive H460 (viability 11.2 ± 1.8 %) and cisplatin-resistant A549 (viability 14.5 ± 2.3 %) lung carcinoma cell line. Proposed nanotechnology based trimodality cancer treatment may have therefore important clinical applications.

1. Introduction

Lung cancer remains the most common human cancer in the worldwide, with non-small cell lung cancer (NSCLC) accounting for ~80% of cases (Parkin et al. 2002; Jemal et al. 2008). Despite great achievements made over the past decades in surgery, radiotherapy and chemotherapy, the 5-year survival rate of lung cancer in many countries is < 15% (Erridge et al. 2007). Chemotherapy remains the mainstay of treatments of lung cancer and cisplatin is one of the most widely used first-line chemotherapeutic agents for NSCLC treatment (Schiller et al. 2002). Approval of cis-diamminedichloroplatinum(II) (cisplatin) for clinical use by the United States Food and Drug Administration (FDA) in 1978 revolutionized the treatment of a variety of malignancies including testicular, ovarian, bladder, and small cell lung, as well as head and neck cancer (Rosenberg 1985; Boulikas and Vougiuoka 2004). Detailed studies on its molecular mechanism of action, using a variety of spectroscopic methods including X-ray, NMR and other physico-chemical methods, revealed its ability to form irreversible crosslinks with bases in DNA. Most notable among the DNA changes are the 1,2-intrastrand cross-links with purine bases. Other adducts include inter-strand crosslinks and non-functional adducts that have been postulated to contribute to cisplatin's activity (Jordan and Carmo-Fonseca 2004). Platinum anticancer drugs are administered by intravenous injection, and within 1 day, 65–98 % of the platinum in the blood plasma is protein bound (Boffetta et al. 1998). The binding of cisplatin to proteins reduces the urinary excretion of platinum and causes the deposition of platinum in tissues. In addition, the binding of cisplatin to proteins and enzymes is believed to be the cause of many of the severe side effects exhibited by the drug, especially ototoxicity and nephrotoxicity (Ogilvie et al. 1992). So far, attempts to prevent neuro- and nephrotoxicity have failed. Acquired multi-drug resistance is known to develop in patients receiving cisplatin chemotherapy. Although extensive efforts were devoted to overcoming these major issues by development of new generations of platinum derivatives which are less toxic and more active than cisplatin and/or do not display cross-resistance, the improvements is still rather small. An alternative approach is the encapsulation of cisplatin in sterically stabilized liposomes (Pinzani et al. 1994), having excellent stability in plasma, a much longer circulation time, better efficacy and lower toxicity than free cisplatin.

Recently, especially magnetic nanoparticles have attracted attention because of their potential as contrast agents for magnetic resonance imaging (MRI), magnetic drug targeting, and heating mediators for cancer therapy (hyperthermia) (Pankhurst et al. 2003; Babincová 1995; Babincová and Babinec 1995; Babincová et al. 2001, 2002, 2008; Ito et al. 2007; Garćia-Jimeno et al. 2012; Safarik and Safarikova 2009; Safarik et al. 2012). Many studies exist on the interaction of hyperthermia with cisplatin or radiation and of cisplatin with radiation (Kampinga and Dikomey 2001; Raaphorst et al. 1997), therefore our aim in this study is to use cisplatin-functionalized magnetic targetMAG nanoparticles for an *in vitro* evaluation of combined electromagnetic hyperthermia, chemotherapy, and radiotherapy in the treatment of non-small lung carcinoma A549 and H460 cell culture.

2. Investigations, results and discussion

2.1. Cisplatin sensitivity

To determine the sensitivity of A549 and H460 cells for cisplatin, MTT assay was prformed after one-hour incubation (Fig. 1 and 2). The results show that H460 cells are more sensitive

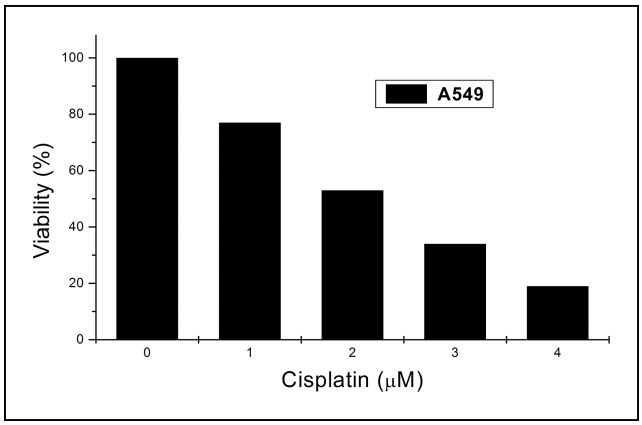

Fig. 1: Viability analysis for A549 non-small lung carcinoma for different concentrations of cisplatin.

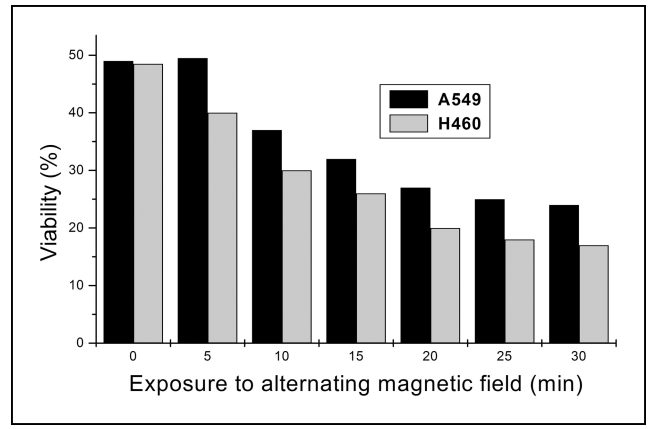

Fig. 3: Viability of A549 and H460 cells for different time of exposure to alternating magnetic field.

for cisplatin than A549 cells. This difference was statistically significant ($p < 0.05$).

2.2. Combined effect of ciplatin functionalized targetMAG and electromagnetic hyperthermia

Viability 50 % was obtained using cisplatin concentrations 2.1 μM and 59 μM for H460 and A549 cells, respectively. To investigate the effect of electromagnetic hyperthermia we prepared targetMAG nanoparticles with these concentrations of cisplatin and iron concentration of 0.3 mg/ml. Cell culture was exposed to an alternating magnetic field, and again viability was determined in a MTT assay. In Fig. 3 it is shown that addition of hyperthermia to cisplatin treatment leads to an enhanced cisplatin effect in both cell cultures.

2.3. Gamma-irradiation of cells

The radiation dose survival plots for both cell lines are shown in Fig. 4.

2.4. Trimodal therapy – combined effect of ciplatin functionalized targetMAG, electromagnetic hyperthermia and gamma-irradiation

We prepared targetMAG nanoparticles with cisplatin concentrations 2.1 μM and 59 μM for H460 and A549 cells, respectively, and iron concentration of 0.3 mg/ml, applied alternating magnetic field for 15 min, and gamma-irradiated cells with dose 1.5 Gy. The viability of cells determined by MTT assay was 11.2 ± 1.8 %, and 14.5 ± 2.3 % for H460 and A549 cells, respectively. Proposed trimodality treatment consisting of radiation, cisplatin and electromagnetic hyperthermia therefore improve treatment outcome by increasing the cellular radiosensitivity compared with cisplatin alone. Observed synergism between these there modalities can be explained in addition to the cytotoxic properties of cisplatin, that it also acts as a radiosensitizer. The implicated interaction mechanisms are enhanced formation of toxic platinum intermediates in the presence of radiation-induced free radicals, and the capacity of cisplatin to scavenge free electrons formed by the interaction between radiation and DNA that may fixate otherwise reparable damage to DNA. Moreover, iron forming magnetic nanoparticles with the strong photoelectric absorption and secondary electron caused by gamma or X-ray irradiation can further accelerate DNA strand break.

Concerning the further utilization of these data, the formulation of targetMAG cisplatin complexes for use in a drug delivery system *in vivo*, it is important to note that normal human extracellular fluids contain a high chloride concentration (100 mM). Under these conditions, one would expect that cisplatin bound to targetMAG would due to physical-chemical interactions provide a relatively long local release of cisplatin. Once complexes enter the cell in which the chloride concentration is low a necessity arises to generate a positively charged, aquated species, that can react with nucleophilic sites on intracellular macromolecules, forming adducts with proteins, RNA and DNA. TargetMAG-cisplatin complex can dissociate spontaneously in the intracellular matrix generating reactive Pt species, but heating of targetMAG nanoparticles in an electromagnetic field can lead to a controlled generation of active cisplatin.

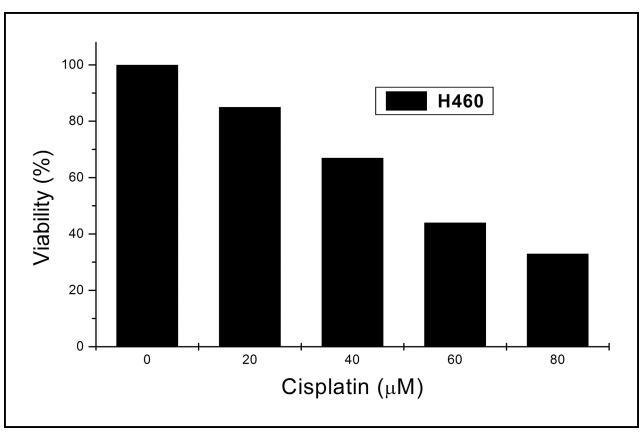

Fig. 2: Viability analysis for H460 non-small lung carcinoma for different concentrations of cisplatin.

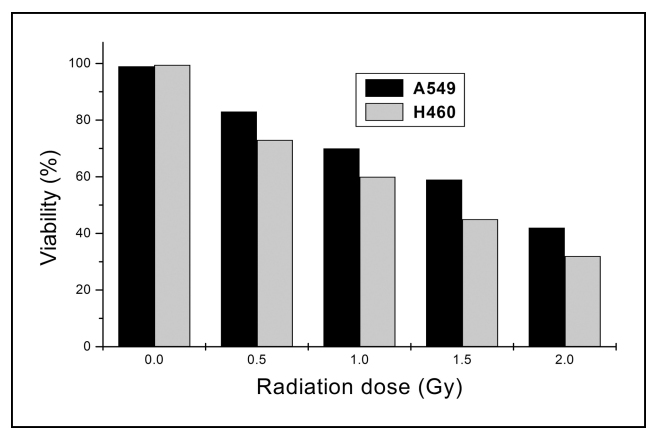

Fig. 4: Dependence of viability of A549 and H460 cells on radiation dose.

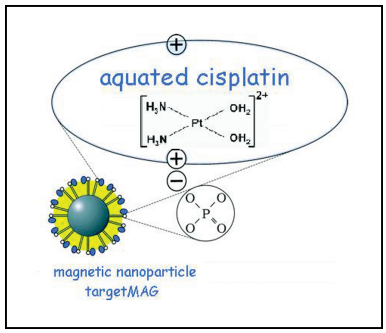

Fig. 5: Structure of cisplatin functionalized magnetic nanoparticle targetMAG.

2.5. Conclusions

In conclusion, combined cisplatin and electromagnetic hyperthermia treatment increased the radiosensitivity of non-small lung carcinoma cells. Besides the heating and drug-release properties, targetMAG nanoparticles have another important feature — the possibility of drug targeting using a static high-gradient magnetic field. This would be helpful in treating a diseased organ by first targeting targetMAG with drug and subsequently exposing to the field for drug release. This trimodal cancer treatment using targetMAG nanoparticles, electromagnetic hyperthermia, and gamma-radiation may have promising clinical applications.

3. Experimental

3.1. Material

Cis-diamminedichloroplatinum(II) (cisplatin), 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other reagents of highest purity were procured locally.

3.2. Magnetic nanoparticles

The biocompatible colloidal suspension of magnetic nanoparticles (targetMAG) used in experiments was produced by Chemicell GmbH (Berlin, Germany; German patent application no. 19624426.9) by wet chemical methods from iron oxides and hydroxides to produce special multidomain particles. These nanoparticles are covered with hydrophilic starch polymers coupled with endstanding phosphate functional group allowing ionic binding of positively charged species (Fig. 5), in our case aquated cisplatin (Sigma, St. Louis, MO, USA). Pyrogenicity and sterility tests were performed by the Pharmacy Department of the Virchow Medical School (Berlin, Germany) according to good practice guidelines.

3.3. Binding of cisplatin to targetMAG nanoparticles

The possibility of neutral cisplatin binding to negatively charged phosphate groups at the surface of targetMAG may be explained by the hydrolysis of cisplatin in aqueous solutions in the absence of Cl ions. It has been reported that when cisplatin [$(NH_3)_2PtCl_2$] is dissolved in aqueous solvents, the Cl ions are replaced by water molecules and, resulting in positively charged species, the monohydrated-monochloro derivative $[(NH_3)_2PtCl(H_2O)]^{+1}$, and dihydrated species $[(NH_3)_2PtCl(H_2O)_2]^{+2}$ (Barrough and Glimcher 2002) which are able to interact with phosphate groups. Unfortunately the concentration of charged aquated cisplatin is about 15 %. To prepare a 100 % solution of $[(NH_3)_2PtCl(H_2O)_2]^{+2}$ cisplatin solution was added to $AgPF_6$ at a molar ratio 1:2 (ciplatin: $AgPF_6$). After mixing, the solution was left to settle at 4 °C overnight. To this solution suspension of targetMAG nanoparticles was added for the binding of desired concentration of cisplatin.

3.4. Cell culture

Human non-small cell lung carcinoma H460 and A549 cells were maintained in RPMI-1640 supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The effect of cisplatin, cisplatin-targetMAG, gamma-radiation and their combination with electromagnetic hyperthermia on the proliferation of H460 and A549 cells was measured by MTT assay. Cells were plated at a density of $1x10^4$ cells per well in 96 well plates overnight and then treated with cisplatin, cisplatin bound to magnetic nanoparticles, and irradiated with an electromagnetic field and gamma radiation. 20 µL of MTT solution (2 mg/ml in PBS) were added to each well and the cells were cultured for another 4 h at 37 °C. The medium was completely removed and 150 µl DMSO were added to solubilize MTT formazan crystals. The plates were then agitated and the optical density was determined at 570 nm (OD570) using an ELISA plate reader (Model 550; Bio Rad, Hercules, CA, USA). At least five independent experiments were performed. Cell viability was determined as:

$$\text{Cell viability (\%)} = (\text{number of viable treated cells}/\text{number of control}) \times 100.$$

3.5. Setup for application of electromagnetic hyperthermia

Electromagnetic heating was performed using a 412 MHz radiofrequency generator (GV6A, ZEZ Rychnov, Czech Republic) with a power dissipation of 6 kW. The coil-shaped and water-cooled antenna was made of 3 copper windings with a diameter of 15 cm, connected to a water-cooled resonance circuit which produced the electromagnetic field. The temperature of the suspension was measured by contactless infrared thermometer. The system created a magnetic field with induction of 1.5 mT in the centre of the coil, where the cell culture was inserted (Fig. 6).

3.6. Gamma irradiation

Gamma-irradiation was performed with 250 kV x-rays through a Thoreaus-1 filter at 5 Gy min^{-1} using a Siemens Stabilipan x-ray generator (Siemens AG. Munich, Gemany).

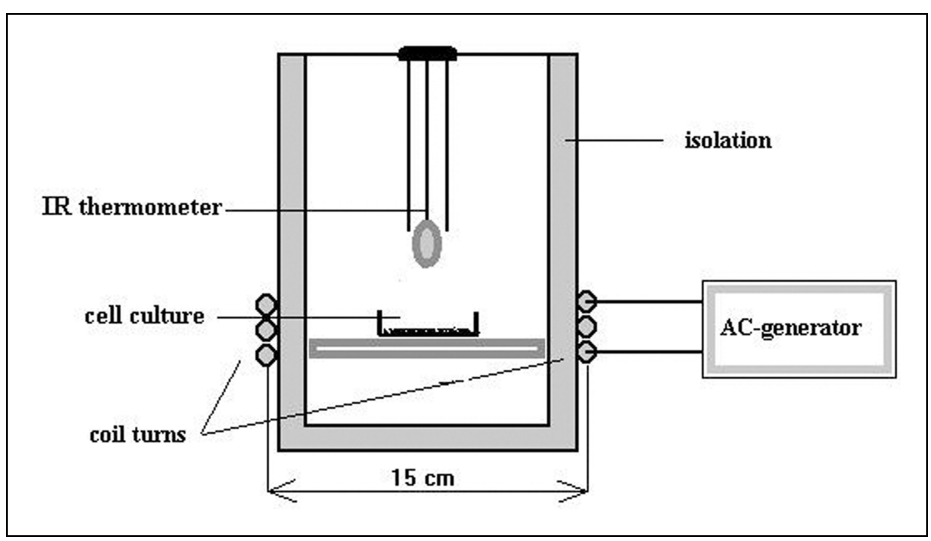

Fig. 6: Experimental setup for the application of electromagnetic hyperthermia.

3.7. *Statistical analysis*

The experiments were performed at least for three times. Data were expressed as the mean ± standard deviation (SD). Statistical correlation of data was checked for significance by ANOVA and Student's t test. $P < 0.05$ was considered to indicate a statistically significant difference.

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