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CONTACTLESS RADIO-CONTROL OF COLCHICINE AND CISPLATIN RELEASE FROM MAGNETOLIPOSOMES: NEW TECHNOLOGIES CAN IMPROVE PERFORMANCE OF OLD DRUGS

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Large unilamellar magnetoliposomes with encapsulated colchicine or cisplatin (anticancer drugs) were prepared by reverse-phase evaporation. They were exposed to an alternating magnetic field with frequency 3.5 MHz and induction 1.5 mT produced in three turn pancake coil. The results showed that magneto-liposomes could be specifically heated to 42 ^{o}C (phase transition temperature of a used lipid) in a few minutes and during this time encapsulated anticancer drugs are massively released and strong anticancer effects are observed.

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1. Introduction

It is now well recognized that when systemic chemotherapy is used for the treatment of solid tumors, it is almost impossible to achieve therapeutic levels of drug at the tumor site without damaging healthy tissues [1, 2]. One solution is to encapsulate anticancer drug into liposomes which after injection into the blood stream preferentially accumulate in the tumor. Drug is slowly released from accumulated liposomes providing therapeutic benefit. However, the ability to control and produce efficient release would be extremely advantageous. In [3] we have proposed a method for microwave mediated drug release from liposomes with enwrapped ferromagnetic microparticles. Although microwave radiation is preferentially absorbed by these particles and produced heat release encapsulated drug, the surrounding tissue is also substantially heated and moreover particles from μm range are only partially biocompatible. To overcome these shortcomings we have developed a new method using magneto-liposomes (MLs) with encapsulated stabilized superparamagnetic magnetic fluid with average particle diameter 8 nm and saturation magnetization 800 G, and instead of microwaves (2.45 GHz) we have used AC-magnetic field with the frequency ~ 1 MHz. We have achieved almost three order higher specific absorption power than using large ferromagnetic particles [4-8], and moreover the surrounding tissue is not heated because at these frequencies the heat is produced almost exclusively due to the Néel relaxation [9].

Our aim in this study is to show that the produced heat may be used for extremely efficient colchicine and cisplatin release from magnetically responsive liposomes.

Colchicine (Fig. 1, left), a heterocyclic alkaloid isolated from Colchicum automnale [10], is a well known antimitotic poison and is also commonly used for its anti-inflammatory properties. Its antitumor activity derives from its tubulin binding activity [11,12]. Colchicine binds specically to tubulin KL heterodimers with a high afinity.

Colchicine is a mitotic poison which has been much used in research as a chemotherapeutic agent for cancer. This alkaloid, which is extracted from the autumn crocus, is

probably the most powerful "radiomimetic " drug known-that is, it reproduces the cellular changes induced in cells by X rays. Even at dilutions of 1 in 100,000,000 it can bring about the typical mitotic arrest at the metaphase stage. However, the therapeutic value of colchicine against cancer is (as is typical with chemotherapy agents) limited by its toxicity against normal cells.

As a chemical entity cis-diamminedichloroplatinum(II) (cisplatin) was first described by M. Peyrone in 1845 (known as Peyrone's salt). The structure (Fig.1, right) was elucidated by Alfred Werner in 1893. In the 1965 Rosenberg *et al*. [13] discovered that electrolysis products from a platinum electrode inhibited binary fission in *Escherichia coli* bacteria. The bacteria grow to 300 times their normal length but cell division fails. In the late 1960s, a series of experiments were to test the effects the cispatin, along with other platinum coordination complexes, on sarcomas artificially implanted in rats. These studies found that cispatin was the most effective out of this group, which started the medicinal career of cisplatin. Approved for clinical use by the United States Food and Drug Administration (FDA) in 1978, it revolutionized the treatment of a variety of malignancies including testicular, ovarian, bladder, and small cell lung, as well as head and neck cancer [14, 15]. Detailed studies on its molecular mechanism of action, using a variety of spectrocopic 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 nonfunctional adducts that have been postulated to contribute to cisplatin's activity [16]. Platinum anticancer drugs are administered by intravenous injection, and within 1 day, 65–98% of the platinum in the blood plasma is protein bound. 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. So far, attempts to prevent neuro- and nephrotoxicity have failed.

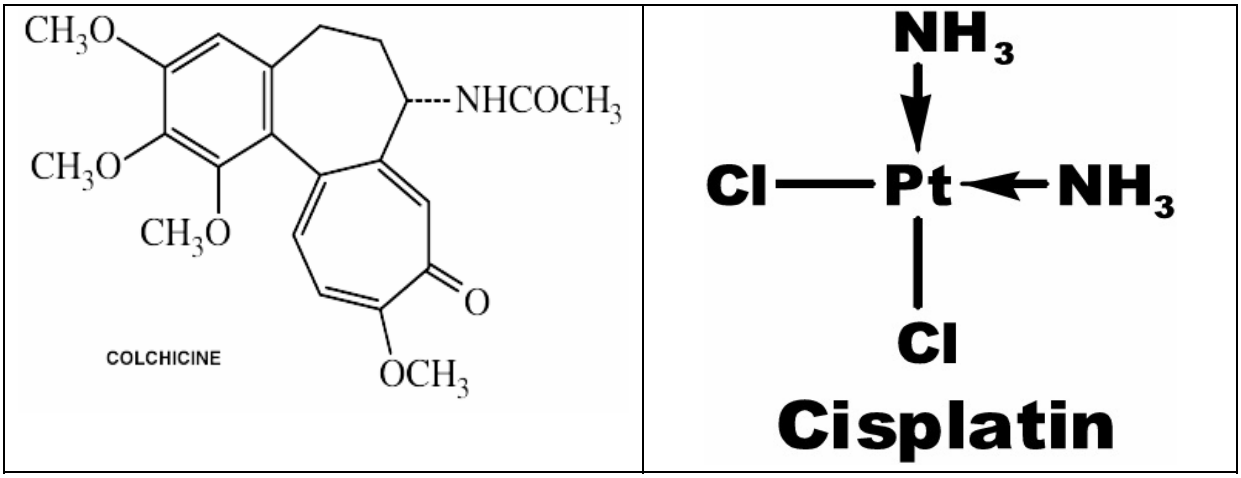

Fig.1. Chemical structures of colchicine and cis-diamminedichloroplatinum(II) (cisplatin).

2. Experimental

2.1 Magnetoliposome preparation

70 mg of dipalmitoyl-phosphatidylcholine (Sigma, USA) was dissolved in 20 ml of a diethyl ether and chloroform mixture (1:1, v/v) in rounded bottom flask. Then 3 ml of dextran-magnetite with varying total Fe_3O_4 concentrations and desired amounts of colchicine and cisplatin (Sigma, USA) in Tris-HCl buffer of pH 7.4 (Radelkis, Budapest) was added to the flask and emulsified in a Labsonic 2000 sonicator bath (Branson Ultrasonics, Danbury) at 100 W for 5 min at 45^{o}C. The emulsion was then evaporated at 45 ^{o}C under a reduced pressure (30 mm Hg) in a

rotary evaporator until an opaque suspension of magneto-sensitive liposomes (magnetoliposomes) with entrapped anticancer drugs had formed. Magnetoliposomes were separated from non-encapsulated magnetite (and drug by centrifugation (10,000g) and resuspended in Tris-HCl buffer. The final concentration of dipalmitoyl-phosphatidylcholine was 20 mM.

2.2. Radio-frequency magnetic field exposure experiments and release measurement

Alternating magnetic field was generated using experimental setup shown in Fig. 1.The equipment based on the RF generator GV6 (ZEZ Rychnov, Czech Republic) was adjusted to the following parameters:

- oscillation frequency f = 3.5 MHz
- maximal high frequency output power P_{mvf} = 6 kW, (P_{mvf} a U_{mvf} possible to regulate)
- maximal input power P_{max} = 10 kVA
- maximal high frequency potential in resonance coil U_{mvf} = 450 V
- magnetic induction B = 1.13 mT.

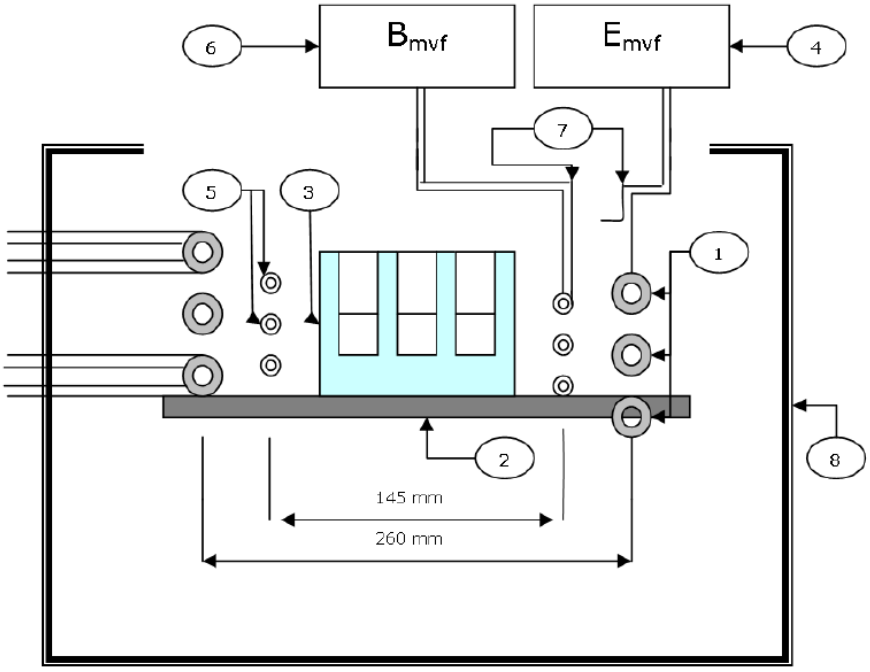

Fig. 2. Experimental setup for the application of RF-magnetic hyperthermia: (1. Three-turn cooper induction coil (Φ24 mm/ Φ24 mm) cooled with water; 2. Carrier board from teflon, with sample holder; 3. Foamy polystyrene; 4.Amplitude of high frequency potential E_{mvf} meter, it measures Umvf of 1/3 thread of induction resonance coil circuit; 5. Induction thread of cooper cable (d = 140 mm, Φ = 0.7 mm); 6. Amplitude of magnetic induction of high magnetic field B_{mvf} meter; 7. High-potential conductor (15 kV) connected to E_{mvf} and B_{mvf} meter; 8. Ground insulator or board capacitor).

2.3. Cell line and growth condition

C rat glioma cell culture (ATCC) maintained in Dulbecco minimal essential medium (Gibco, Gaithesburg, MD, USA) supplemented with 10 % fetal calf serum in 5 % CO_2 humidified atmosphere at 37 ^{o}C. The viability of cells was assessed using Trypan blue dye exclusion assay, despite some drawbacks of this method. It was not possible to used MTT test, due to the fact that magnetic nanoparticles internalized in the cells impaired tests based on production of chromogen. The 2.5 x10^6 cells were plated on 6 cm Petri dish with experimental media containing colchicine or cisplatin with magnetoliposomes at various concentrations. The antiproliferative effects of various treatments (including electromagnetic hyperthermia) were determined after a 2-day incubation period. Relative number of dead cells was determined as a:

Relative cell number (%)=(number of dead treated cells/number of control cells)x100.

3. Results and discussion

The first task of this work was investigation of the effect of RF field application on heating fluid with a frequency of 3.5 MHz for different concentrations of ferrofluid. We determined temperature of ferrofluid for magnetosomes concentrations: C_1=3 mg/ml, C_2=15 mg/ml and C_3=30 mg/ml. Heating up of ferrofluid was made for four independent experiments and its dependence of RF action time is showed in Fig. 3. From the figure is possible to distinguish the rise of RF field action with the increasing of temperature for each concentration. Thus, as long as the concentration increases, the fluid more rapidly gets to higher temperatures. This is also true if we use ferrofluids with diverse nanoparticles concentrations. Ferrofluid temperature goes ups due RF action field. This is possible to explain thanks to the Brown and Néel relaxation effect. For each concentration was detected a temperature rising on dependence of time exposure. To more contained particles at ferrofluid which reacted with magnetic field, the macroscopic increment of temperature of the samples is higher until it reaches its maximum values: and 39.3 °C, 57.7 °C and 73.5 °C for C_1, C_2 and C_3 ferrofluid concentrations, respectively.

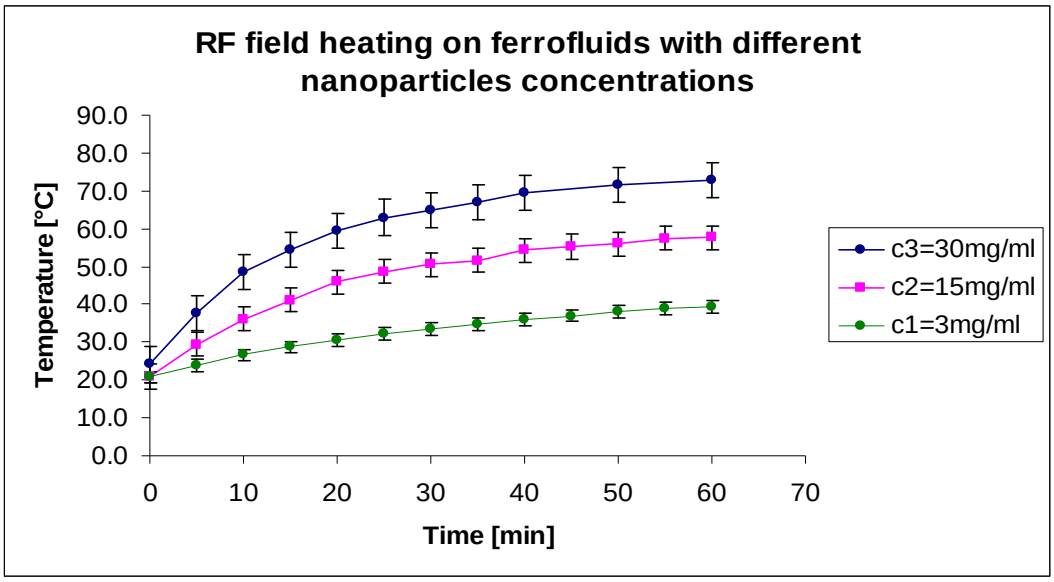

Fig. 3. Ferrofluid temperature dependence on RF field time exposure. Nanoparticles concentrations in ferrofluids were: C_1=3 mg/ml, C_2=15 mg/ml, and C_3=30 mg/ml, respectively.

Fig. 4. shows extent of colchicine release from MLs containing varying amounts of ferrocolloid (quantified according to iron content) under the influence of AC-magnetic field. As can be seen the release is very fast, due to the fact that superparamagnetic particles are embedded within the lipid bilayer, therefore the heat produced via Néel relaxation is directly used for the heating of lipid to its phase transition temperature (42 ^{o}C) which leads to massive release of encapsulated colchicine, and very similar results were obtained also for cisplatin release. It should be stressed that we have not observed substantial macroscopic heating of the MLs suspension.

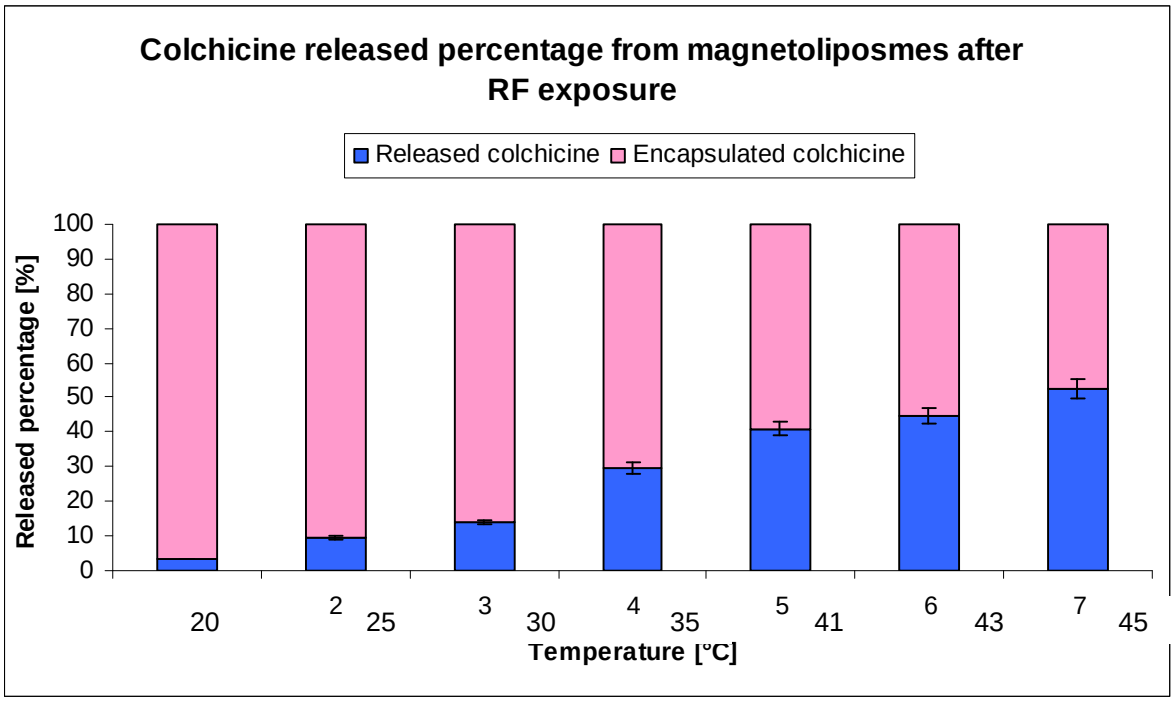

Fig. 4. Contactless release of colchicine from magnetoliposomes after application of radiofrequency field.

In Fig. 5 and Fig. 6 we have compared the efficiencies of single therapies for colchicine and cisplatin, respectively. For colchicine (Fig.5) with hyperthermia we have observed 40.6 % and with chemotherapy 61.8 % death cells. The highest percentage of tumour cells decease was observed at combined therapy 98.6 %. For cisplatin (Fig. 6) with hyperthermia we have observed 40 % and with chemotherapy 60 % death cells. The highest 98 % of tumour cells decease was again observed using combined therapy.

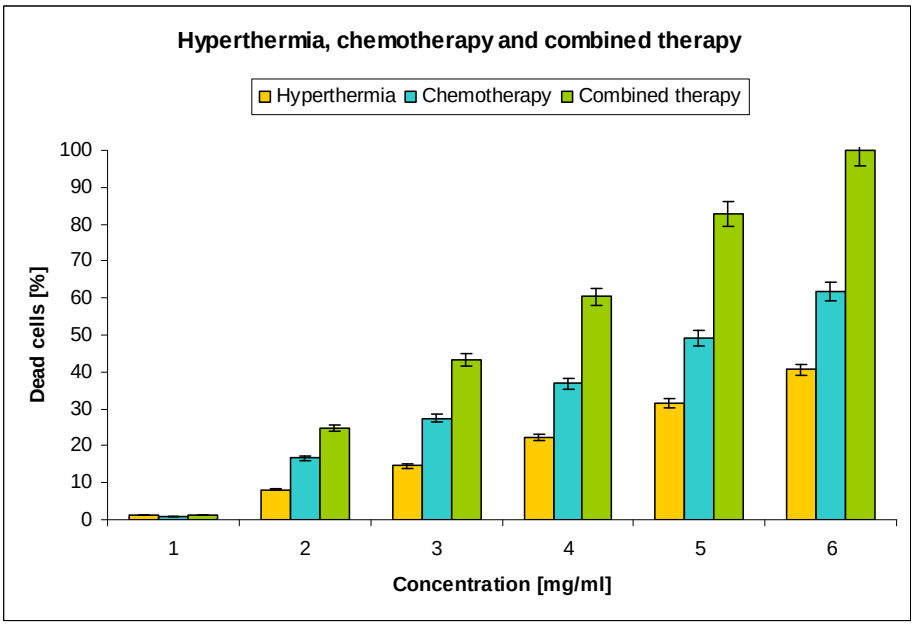

Fig. 5. *Comparison of mortality among C6 glioma cells (in percentage calculated from the 12 independent values) in combined therapy (hyperthermia plus chemotherapy) on colchicine magnetoliposomes concentrations: 1-control sample, C_2 = 0.02 mg/ml, C_3 = 0.04 mg/ml, C_4 = 0.06 mg/ml, C_5 = 0.08 mg/ml, and C_6 = 0.10 mg/ml.*

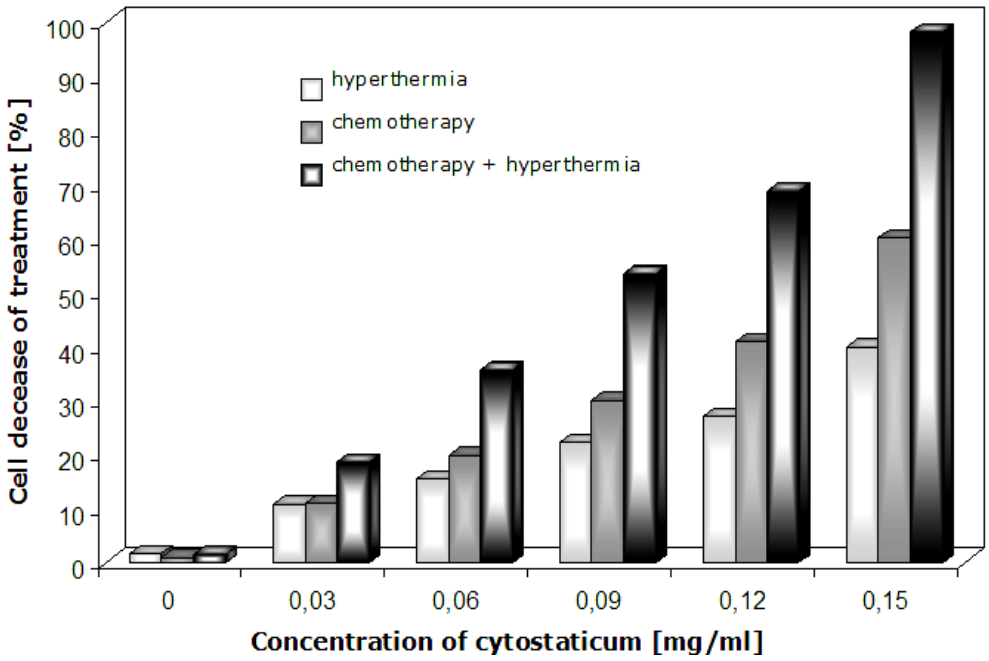

Fig. 6. Comparison of mortality among C6 glioma cells (in percentage calculated from the 12 independent values) in combined therapy (hyperthermia plus chemotherapy) on cisplatin magneto-liposomes concentrations.

We have already developed a portable coil system through which the radiofrequency field may be focused to the desired site (tumor) at a suitable time intervals to release drug from circulating MLs. Besides the heating and drug release properties, MLs have another important feature - possibility of drug targeting using a static magnetic field [16]. This would be helpful in treating a diseased organ by first targeting MLs and subsequently exposing to the field. The possibility of targeting MLs to kidney was already provided [17] and also Lübbe at al. [18] achieved complete tumor remission in animals using a new kind of ferrofluid associated with epirubicin and external magnetic field at 0.5-0.8 T.

Colchicine and cisplatin are most potent anticancer agents known; however, their full therapeutic exploitation is limited by their toxicity in healthy tissues. As we have demonstrated our approach represents a novel versatile tool for the cancer treatment.

Acknowledgments

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