ELSEVIER

Bioelectrochemistry 55 (2002) 17–19

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

AC-magnetic field controlled drug release from magnetoliposomes: design of a method for site-specific chemotherapy

M. Babincová [a,*], P. Čičmanec [b], V. Altanerová [c], Č. Altaner [c], P. Babinec [a]

[a] *Department of Biophysics and Chemical Physics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia*
[b] *Department of Solid State Physics, Comenius University, Mlynská dolina F1, 842 48 Bratislava, Slovakia*
[c] *Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovakia*

Received 1 June 2001; received in revised form 11 July 2001; accepted 16 July 2001

Abstract

Large unilamellar magnetoliposomes (MLs) with encapsulated doxorubicin (DOX) (anticancer drug) were prepared by reverse-phase evaporation. They were exposed to an alternating magnetic field with a frequency of 3.5 MHz and an induction of 1.5 mT produced in three-turn pancake coil. The results showed that magnetoliposomes could be specifically heated to 42 °C (phase transition temperature of a used lipid) in a few minutes and during this, the encapsulated doxorubicin is massively released.

Keywords: Magnetoliposomes; Alternating magnetic field; Drug release; Doxorubicin; Hyperthermia; Cancer therapy

1. Introduction

It is now well recognized that when systemic chemotherapy is used in the treatment of solid tumors, it is almost impossible to achieve therapeutic levels of drug at the tumor site without damaging healthy tissues [1]. 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.

Yatvin et al. [2] used heat to induce rapid release of pharmaceuticals from thermosensitive liposomes composed of phospholipids having transition temperatures slightly above normal physiological temperature. Local hyperthermia, heating of the target area to a temperature of 42–44 °C, would cause the liposome lipids to ''melt'', and the liposomes flowing through the vascular bed of a hyperthermized area would rapidly release the entrapped drug into the surrounding medium. This approach substantially depends on the ability to apply hyperthermia to the tumor area in a targeted manner; unfortunately, none of the existing techniques of hyperthermia offers a general and satisfactory way to do so [3].

In Ref. [4], 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 particles with μm range are only partially biocompatible. To overcome these shortcomings, we have developed a new method using magnetoliposomes (MLs) with encapsulated stabilized superparamagnetic fluid with average particle diameter of 8 nm and saturation magnetization of 400 G, and instead of microwaves (2.45 GHz), we have used AC-magnetic field with a frequency of ~1 MHz. We have achieved almost three-order higher specific absorption power compared to using large ferromagnetic particles [5]. Moreover, the surrounding tissue is not heated because at these frequencies, the heat is produced almost exclusively due to the Néel relaxation [6].

Our aim in this study is to show that the produced heat may be used for extremely efficient doxorubicin (DOX)-release from MLs.

* Corresponding author. Tel.: +421-2-60295685; fax: +421-2-65412305.
E-mail address: babincova@fmph.uniba.sk (M. Babincová).

1567-5394/02/$ - see front matter
PII: S1567-5394(01)00171-2

2. Material and methods

Large unilamellar MLs with encapsulated DOX were prepared by reverse-phase evaporation.

First, colloidal gamma-ferric oxide was synthesized by alkaline coprecipitation from the solution of ferric and ferrous salts as described in Ref. [6]. The ferrocolloid precipitate was extensively washed with distilled water, methanol, and stored in 100% methanol. Necessary amount of ferrocolloid precipitate was washed several times with 100% chloroform; the lipid (dipalmitoyl-phosphatidylcholine) was dissolved in chloroform and added to the chloroform-washed ferrocolloid precipitate. Phospholipids have strong affinity to the surface of iron oxide particles and transfer them into colloidal state again, now in chloroform. Finally, the chloroform ferrocolloid–lipid solution was evaporated under the stream of nitrogen and held under vacuum for 2 h to remove the traces of solvent to obtain iron oxide colloidally dispersed in the lipid film. This film was dissolved in isopropyl ether/chloroform, mixed with 300 mM citric acid (pH 4.0), and extensively sonicated. Organic solvent was evaporated in a rotary evaporator to obtain large unilamellar MLs. The encapsulation of DOX was made using pH gradient method [7]. Briefly, pH of the MLs suspension, initially at 4.0, was raised to 7.8 with 1 M NaOH and heated to 60 °C. MLs were then mixed with preheated DOX at a drug/lipid ratio of 0.2. Free and encapsulated DOX were determined spectrophotometrically employing Specol 210 (Carl Zeiss Jena, Germany). This procedure utilizes pronounced changes in absorbance (absorbance maxima 480 nm, pH 7.8; 550 and 592 nm, pH 10.5) observed on increasing the pH of DOX solution from 7.8 to 10.5. One hundred percent release was achieved upon the addition of Triton X-100.

Iron concentration is determined by *o*-phenantroline method after digestion of an aliquot with concentrated H_2SO_4 and reduction with ascorbate [8]. All used chemicals were obtained from Sigma (USA).

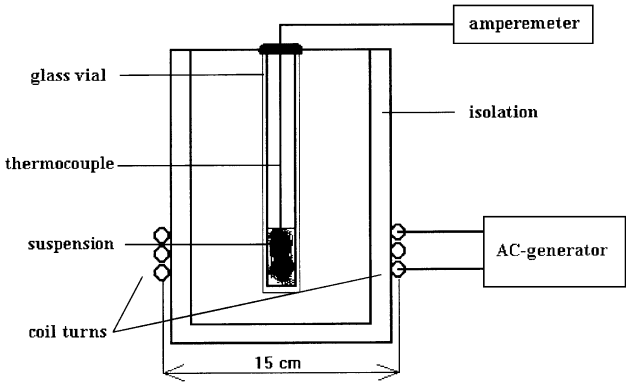

Fig. 1. Experimental setup for the generation of an alternating magnetic field with frequency of 3.5 MHz and induction of 1.5 mT produced in three-turn pancake coil cooled with water and isolated from magnetoliposome suspension by styrofoam-covered tube. Temperature inside suspension was measured using nonabsorbing thermistor.

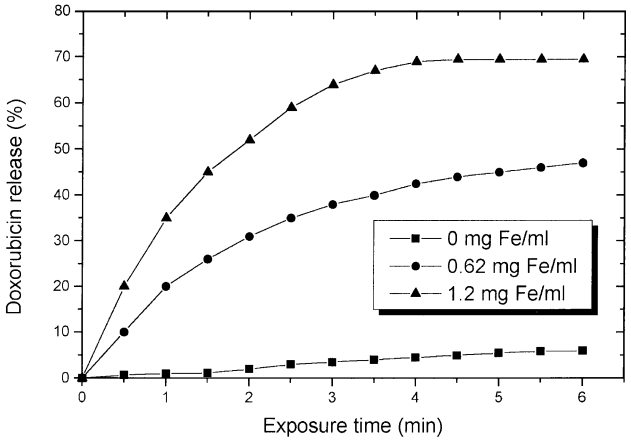

Fig. 2. Dependence of doxorubicin release from magnetoliposomes with various concentrations of ferrocolloid. The results are mean values from four independent measurements.

Alternating magnetic field with a frequency of 3.5 MHz and an induction of 1.5 mT was generated using experimental setup shown in Fig. 1, as described in details in Ref. [6].

3. Results and discussion

Fig. 2 shows extent of DOX 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 °C) which leads to massive release of encapsulated DOX.

It should be stressed that we have not observed substantial macroscopic heating of the MLs suspension. The increase of temperature using the highest ferrocolloid concentration (1.2 mg Fe/ml) was only 2 °C after 6 min (the initial temperature in all experiments was 25 °C, we have also performed experiments with 37 °C as an initial temperature, and the release was about two times faster). Substantial heating is measurable using iron concentrations > 10 mg Fe/ml, which may be useful for combined hyperthemia and drug release [6].

We have already developed a portable coil system through which the AC-field may be focused to the desired site (tumor) at suitable time intervals to release the drug from circulating MLs. Besides the heating and drug-release properties, MLs have another important feature—the possibility of drug targeting using a static magnetic field. 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 the kidney was already provided [9]. Moreover in 1996, Lübbe et al. [10] already 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. In a second step [11] conducted by the same authors, the first Phase I clinical trial using this approach was performed on patients with advanced, unsuccessfully treated cancers or sarcomas with very promising results. Magnetic targeting may have wide applications because it is not organ-specific. MLs, therefore, represents a novel versatile tool for cancer treatment.

Acknowledgements

The authors would like to thank VEGA grant 1/8310/01 and Všeobecná úverová banka, a.s., for the financial support.

References

[1] R.K. Jain, Barriers to drug delivery in solid tumors, Sci. Am. 271 (1994) 58–65.
[2] M.B. Yatvin, J.N. Weinstein, W.H. Dennis, R. Blumenthal, Design of liposomes for enhanced local release of drugs by hyperthermia, Science 202 (1978) 1290–1293.
[3] G. Kong, M.W. Dewhirst, Hyperthermia and liposomes, Int. J. Hyperthermia 15 (1999) 345–370.
[4] M. Babincová, Microwave induced leakage of magnetoliposomes, Possible clinical implications, Bioelectrochem. Bioenerg. 32 (1993) 187–189.
[5] M. Babincová, E. Šusteková, P. Babinec, P. Čičmanec, V. Craciun, D. Leszczynska, Heating of superparamagnetic colloid in high-frequency magnetic field: implications for electromagnetic hyperthermia, Czech. J. Phys. B 50 (2000) 979–981.
[6] M. Babincová, P. Čičmanec, P. Babinec, V. Altanerová, Č. Altaner, Magnetoliposomes mediated local electromagnetic hyperthermia, Radio Eng. 9 (2000) 12–13.
[7] L.D. Mayer, L.C.L. Tai, M.B. Bally, G.N. Mitilines, R.S. Ginsberg, P.R. Cullis, Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients, Biochim. Biophys. Acta 1025 (1990) 143–151.
[8] H.H. Willard, L.L. Merritt, J.A. Dean, Instrumental Methods of Analysis, Van Nostrand, New York, 1974.
[9] M. Babincová, V. Altanerová, M. Lambert, Č. Altaner, M. Šramka, E. Machová, P. Babinec, Site specific in vivo targeting of magnetoliposomes in external magnetic field, Z. Naturforsch. 55c (2000) 278–281.
[10] A.S. Lübbe, C. Bergemann, W. Huhnt, T. Fricke, H. Riess, J.W. Huhn, D. Huhn, Preclinical experiences with magnetic drug targeting: tolerance and efficacy, Cancer Res. 56 (1996) 4694–4701.
[11] A.S. Lübbe, C. Bergemann, H. Riess, F. Schriver, P. Reichardt, K. Matthias, M. Matthias, B. Dorken, F. Herrmann, R. Gurtler, P. Haas, N. Haas, R. Sohr, B. Sander, A.J. Lemke, D. Ohlendorf, W. Huhnt, D. Huhn, Clinical experiences with magnetic drug targeting: a Phase I study with 4′-epidoxorubicin in 14 patients with advanced solid tumors, Cancer Res. 56 (1996) 4686–4693.