Site-Specific *in vivo* Targeting of Magnetoliposomes Using Externally Applied Magnetic Field

Melánia Babincová[a,*], Veronika Altanerová[b], Miloš Lampert[c], Čestmír Altaner[b], Eva Machová[d], Miron Šrámka[c] and Peter Babinec[a]

[a] Department of Biophysics, Comenius University, Mlynská dolina F1, 842 15 Bratislava, Slovakia. Fax: +(421)-7–654–25–882. E-mail: babincova@fmph.uniba.sk
[b] Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovakia
[c] Department of Neurosurgery, Derer's Faculty Hospital, Limbová 5, Bratislava, Slovakia
[d] Institute of Chemistry, Slovak Academy of Sciences, Dúbravská 9, Bratislava, Slovakia

* Author for correspondence and reprint request

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Human serum albumin labeled with technetium-99m was encapsulated together with magnetite particles into phosphatidylcholine/cholesterol liposomes. In order to investigate the stability of this complex and its ability to be used for magnetic drug targeting, the *in-vivo* distribution after intravenous administration in rats was estimated. For *in-vivo* targeting an SmCo permanent magnet with intensity ~0.35 T was attached near the right kidney. Difference between the relative radioactivity in the magnetically targeted right kidney (25.92±5.84%) and non-targeted left kidney (0.93±0.05%) is sufficiently high for relevant clinical applications.

Introduction

One of the major challenges in cancer treatment is the delivery of anti-neoplastic agents to remote, difficult-to-reach anatomic sites. The aim of the specific cell targeting is to enhance efficiency of drug delivery and at the same time to reduce the toxicity and side effects to normal tissues. Although many attempts have been made to find a clinically applicable way the problem is still not fully clarified. Conjugation of drugs to antibodies specific to target cell antigens is one example that is clinically being explored (Siegall, 1994). Another example is magnetic targeting based on magnetic pharmaceuticals (e.g. magnetic albumin and polymer microspheres) targeted after injection to the tumor region using external magnetic field. Increase of the therapeutic index of associated drugs using magnetic carriers is up to 100-fold (Schütt *et al.,* 1997) and has been shown by no other drug-targeting device.

Blood vessels in tumors are often leaky and have a higher permeability to circulate large molecules up to sizes of 400 nm in diameter (Brown and Giaccia, 1998). For a long time this property was used for delivery of drugs in liposomes (Lasic, 1998). To increase the therapeutic index of liposomes, these were prepared with encapsulated magnetite particles (Babincová, 1993; Shinkai *et al.,* 1994; Viroonchatapan *et al.,* 1995, 1996; Babincová and Babinec, 1997; Babincová and Machová, 1998; Babincová *et al.,* 1999; Bulte *et al.,* 1999). These magnetically-responsive liposomes (magnetoliposomes) have several advantages: An external magnetic field may be used for their targeting to the desired site and alternating high-frequency magnetic field or laser pulses for the release of the encapsulated drug.

Higher therapeutic doses of anti-cancer drugs are often toxic also for the normal cells, therefore even a slightly modified drug distribution (more of the drug delivered to the tumor) may results in a beneficial effects. One of the most important characteristics of an ideal targetable drug delivery system is its ability to traverse the endothelium of the targeted tissue and to release the encapsulated drug at the cellular level.

The objective of this study was to investigate the distribution, stability and specificity of magnetoliposomes (MLs) in rats. The targeting of intrave-

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nously injected ^{99m}Tc labeled MLs was provided by an external magnetic field applied near the right kidney.

The kidney was chosen as a target organ to open an avenue to treat renal tumors, which represent seventh leading cause of cancer and ome renal tumors, e.g. rhabdoid kidney tumor are extremely aggressive and their therapy remains inadequate (Figlin 1999. Therefore delivering and confining chemotherapeutic agent to kidney could be effective for treatment of these malignancies.

Materials and Methods

Preparation of ^{99m}Tc-labeled magnetoliposomes

50 mg of soybean phosphatidylcholine (Sigma, St. Louis, USA) and 10 mg of cholesterol (Sigma, St. Louis, USA) was dissolved in chloroform/methanol (2:1 v/v) in a round bottom flask and the solvent was evaporated in a rotary evaporator, so that a thin lipid film was formed. To study biodistribution of Mls we have used their radioactive labeling with ^{99m}Tc. Its gamma radiation (140 Kev) is suitable for the detectors used and its period (6 hours) is sufficient for conveniently carrying out biodistribution examinations while avoiding any residual activity in vivo and avoiding any risk of contamination. The preparation of ^{99m}Tc is particularly easy, since it can be extracted from ^{99}Mo, using commonly available generators and can be obtained by simple elution in the form of pertechnetate in sterile, neutral, isotonic solution. To prepare ^{99m}Tc labeled human serum albumin (HSA) provided by Mallinckrodt Medical (Petten, The Netherlands), 0.5 mg of HSA was dissolved in 100 ml of physiological buffered saline containing 64 MBq (1.7 mCi) of fresh radioactive sodium pertechnetate of ^{99m}Tc (Phillips *et al.*, 1992). After 15 minutes the resulting ^{99m}Tc-HSA was added to the lipid film and diluted with 10 ml of physiological buffered saline. Finally 30 mg of magnetite (Fe_3O_4) particles of dimensions 3–30 nm (prepared by co-precipitation of $FeCl_2$ and $FeCl_3$ in the presence of an excess of ammonia) was added. The solution was sonicated in a water bath for 10 min at the highest output of MSE disintegrator.

In vivo magnetoliposome targeting

Adult female Sprague Dawley rats 200 g of weight were used throughout the experiments. For each experimental group two animals were used. 1 ml of ^{99m}Tc-HSA-MLs were injected intravenously into the rat tail. To study magnetoliposome distribution the two rats each were sacrified by decapitation at 20, 40, 60, 80, 100 and 120 minutes after injection. The blood was taken from heart, the lung, liver, spleen, heart, left and right kidney were immediately removed, blotted, weighted, and carefully minced. The ^{99m}Tc radioactivity of aliquots from obtained tissues was measured using a Clini Gamma Counter (LKB). The amounts of radioactivity retained in all tissue samples were measured and divided by the total activity administered (approximately 2.5 MBq in each rat) to obtain the percentage distribution of the ^{99m}Tc in every tissue. These percentages were then normalised with individual tissue sample weight to compensate for animal-to-animal variations. As a result the amount of radioactivity detected in the tissues represents the amounts of intact MLs taken from the blood-stream. Effects of magnetic field on MLs biodistribution were followed after attachment of 1.5x1.5x2 cm SmCo magnet (surface magnetic intensity ~0.35 T) close to the right kidney. During magnetic targeting the rats were anaesthetized by i.p. administration of 0.1 mg/kg atropine, 10 mg/kg Rometar (Xylzainum) and 90 mg/kg Narkamon (Ketaminum hydrochloridum) (all obtained from Spofa a.s., Prague, Czech Republic), this step is not necessary but allows to achieve fixed experimental conditions during the study.

Results and Discussion

Intravenous administration of a magnetically responsive liposomes results in retention of this carriers in the blood vessels of the target tissue when an extracorporeal magnetic field is applied. The greater the carrier uptake, the lesser would be its availability at non-targeted sites.

As is known (Scherphof *et al.*, 1978), liposomes become permeable or destabilised in the presence of blood (allowing entrapped solutes to leak out) because plasma high density lipoproteins remove phospholipid molecules from the vesicle bilayer. In order to stabilise liposomes we have prepared them from a mixture of phosphatidylcholine/cho-

lesterol. Using a 1 hr centrifugation at 12,000×*g* obtained MLs were separated from non-encapsulated magnetite particles and HSA. Comparing the ^{99m}Tc radioactivity in supernatant and pellet a 49% efficiency of ^{99m}Tc-HSA encapsulation has been found. In the physiological saline buffer these MLs remain stable and retain the encapsulated ^{99m}Tc for at least 12 hrs.

The kinetics of *in vivo* MLs distribution are summarised in Table I for all tissues examined. The large values for the liver are due to the fact that MLs which are not endocytosed or lodged between endothelial cells probably travel to the liver for reticulo-endothelial clearance. On the other hand the negligible gamma-radioactivity in the brain is probably due to the existence of the blood-brain barrier, impermeable for liposomes of this composition (Kobayashi *et al.*, 1996). Small values measured are due to leakage of free ^{99m}Tc to this tissue. Because the life-time of liposomes of this composition in the blood-stream is about one hour (Liu *et al.*, 1995), we have applied a magnetic field generated by a small permanent magnet fixed for 45 min near the right kidney, to study the influence of magnetic field on the *in vivo* MLs distribution in rats. The obtained results are shown in Figure 1. The value of 25.92±5.84% for the magnetically-targeted right kidney is significantly higher than 0.93±0.05% for the non-targeted left kidney. The values for other studied organs are similar to that obtained in the MLs distribution study without a magnetic field.

The results of this study validate the usage of MLs for their targeting to desired sites in the body by application of an external magnetic field.

The approximate expression for the force F_{mag} acting on the MLs (Häfeli *et al.*, 1997) is

$$F_{mag} = V_{magnetite}\, \chi\, H\, (\partial H/\partial x)$$

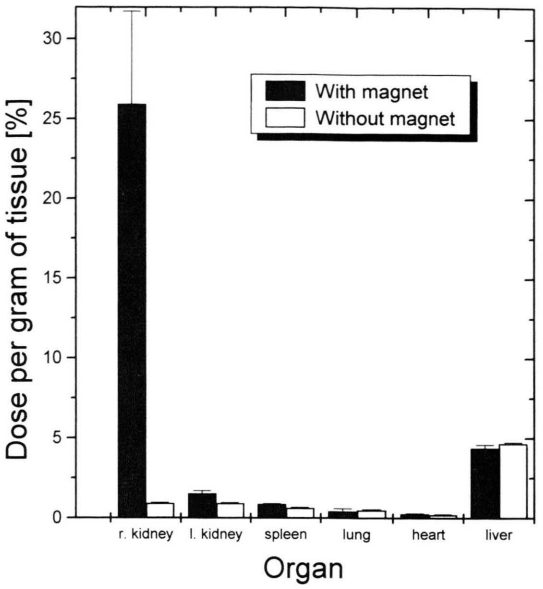

Fig. 1. Retention of ^{99m}Tc-labeled MLs in the rat tissues 45 min after single dose intravenous administration, with and without magnetic field externally applied near the right kidney. Each column represents the mean value from 2 independent experiments ± calculated standard deviations.

where $V_{magnetite}$ is the total volume of magnetite encapsulated in the MLs, χ is the magnetic susceptibility, H the strength of magnetic field and $(\partial H/\partial x)$ the magnetic field gradient. It is clear that the magnetic responsiveness of MLs is determined by many factors including strength of the applied magnetic field and physical properties of encapsulated magnetic nanoparticles.

In related studies (Babincová and Babinec, 1997; Viroonchatapan *et al.*, 1995) it was shown that ML-encapsulated drugs may be released in response to electromagnetically induced heating of magnetic particles. Therefore our future studies

Table I. Kinetics of *in vivo* distribution of ^{99m}Tc radioactivity in rat tissues after the single dose MLs intravenous administration (each data represents the mean value from 2 independent experiments ± standard deviations).

	Dose per gram of tissue [%]							
Time [min]	Right kidney	Left kidney	Liver	Spleen	Heart	Lung	Brain	Blood
20	0.78±0.007	0.77±0.045	4.83±0.478	0.81±0.036	0.13±0.007	2.14±0.194	0.042±0.023	0.43±0.039
40	0.57±0.03	0.46±0.032	3.70±0.498	0.73±0.028	0.08±0.023	1.11±0.065	0.009±0.002	0.32±0.011
60	0.98±0.04	0.83±0.035	4.23±0.162	1.09±0.049	0.13±0.014	1.18±0.088	0.003±0.001	0.33±0.017
80	1.0±0.017	1.25±0.26	5.76±0.01	1.5±0.053	0.13±0.003	1.94±0.22	0.008±0.023	0.31+0.000
100	0.87±0.12	0.9±0.007	4.98±0.021	0.71±0.01	0.1±0.004	1.02±0.003	0.007±0.000	0.30±0.01
120	1.26±0.049	1.29±0.237	3.84±0.049	1.03±0.123	0.07±0.003	1.50±0.065	0.005±0.001	0.17±0.014

should be directed to the investigation of cancer treatment using both magnetic guidance and electromagnetic drug release.

In conclusion, the obtained results demonstrate the usefulness of MLs for *in-vivo* targeting. The therapeutic benefit of such system will be further investigated by the application of doxorubicin-containing MLs in the treatment of rat sarcomas.

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