[Chem. Pharm. Bull. 34(10)4253—4258(1986)]

Feasibility of Magnetic Liposomes as a Targeting Device for Drugs

HIROSHI KIWADA,*[1] JUNKO SATO, SUNJU YAMADA and YURIKO KATO

Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya Funagawara-machi, Shinjuku-ku, Tokyo 162, Japan

(Received March 5, 1986)

Liposomes containing ultrafine magnetite were prepared and their transport characteristics in a magnetic field were examined *in vitro* and *in vivo*. About 15 μg of magnetite (Fe_3O_4) could be entrapped in the liposomes per μmol of lipid without impairing the liposomal encapsulation capacity for the aqueous marker (^{3}H-inulin). In *in vitro* experiments, when 0.1 ml of liposome suspension (0.86 μmol of lipids and 13.3 μg of magnetite) was applied to a glass capillary at a flow rate of the medium of 0.17 ml/min, about 35% of the liposomes was found in the region of the applied magnetic field (4000 G).

It became apparent that the ability of the magnetic field to hold the liposomes was influenced by the flow rate of the medium and the magnitude of the magnetic field. The viscosity of the medium and diameter of the capillary where the magnet is applied for fixation of the liposomes may also affect the holding capacity.

In vivo experiments were carried out with rats having Yoshida sarcoma implanted in a foot pad. The liposomes were injected from the branch of the femoral artery without impairing normal blood flow in the tumor tissue. A very small amount of the liposomes, but significantly more than the control, was trapped at the tumor tissue.

The trapped amount of liposomes in the present experiments appeared to be insufficient for practical therapy, but a more powerful magnet may give better results.

Keywords——liposome; magnetic liposome; magnetite; targeting; Yoshida sarcoma; magnetic field

Introduction

It is desirable in chemotherapy to deliver drugs to a specific organ or tissue. For this purpose, many types of carrier having an affinity for the target site have been proposed.[2] Many of these drug carriers utilize a specific affinity for antigens or carbohydrate moieties as a probe for targeting. However, Widder *et al.* proposed the use of magnetic force for the site-specific drug delivery by using albumin microspheres containing magnetite (Fe_3O_4).[3,4] Magnetic fields are believed to be harmless to biological systems and adaptable to any part of the body.

Senyei *et al.*[5] reported that labeled marker contained in magnetic microspheres was significantly fixed at a part of the rat tail where a magnetic field was applied after injection into the tail artery. Ovadia *et al.*[6] also reported the localization of magnetic microspheres in the head and hind leg besides the tail after arterial infusion, by the use of a powerful samarium-cobalt permanent magnet, even though injected foreign particles are known to be mainly taken up by the reticuloendothelial system (RES)[7] in the liver and spleen in normal circumstances. In these reports the uptake in these organs was suppressed by applying a magnetic field at other parts of the body.

Selective targeting of magnetic albumin microspheres to tumors implanted in the tail of rats was reported by Widder *et al.*[8] and they succeeded in suppressing tumor growth.[9]

On the other hand, liposomes have recently received much attention as potential drug

NII-Electronic Library Service

carriers *in vivo*.[10,11] It may be worthwhile to explore the possibility of controlling liposomal disposition in the body with magnetic force. In this experiment, liposomes containing ultrafine magnetite were prepared and fixation of the liposomes by magnetic force was observed in an *in vitro* experiment. The effects of the magnetic force, flow rate of the medium and amount of liposomes applied in the experimental system were examined. The targeting efficiency to Yoshida sarcoma implanted in the rat hind foot pad was also examined.

Experimental

Materials——Reagents and lipids were obtained as described in the previous paper.[12,13] Ultrafine magnetite (Fe_3O_4, mean diameter 250 Å) was a gift from Kanto Denka Kogyo Co., Ltd. (Tokyo). Triton X-100 and *o*-phenanthroline were purchased from Nakarai Chem. Ltd. (Kyoto) and hydroxylamine hydrochloride and sodium citrate were from Kanto Chem. Co. (Tokyo).

Preparation of the Magnetic Liposomes——Egg L-α-phosphatidylcholine (20 μmol) and D-α-tocopherol (0.5 μmol) were dissolved in 10 ml of chloroform, and the magnetite suspended in methanol and ^{14}C-cholesterol as a lipid marker (*in vitro* experiments) were added. The mixture was sonicated in a bath-type sonicator (Tocho, IUC-2811, Tokyo) to disperse the magnetite, then it was evaporated to dryness to form a lipid thin film containing the magnetite on the inner wall of a round-bottomed flask. One milliliter of phosphate-buffered saline (PBS) containing ^{3}H-inulin as an aqueous marker was transferred into the flask and agitated with a vortex mixer until the lipid film was completely hydrated. A 1 ml aliquot of the liposome suspension was applied to a gel column (Sephacryl S-1000, Pharmacia, 1.0 cm × 10 cm) for separation of liposomes from free inulin and aggregated magnetite. The aggregated magnetite was retained on top of the gel column. Fraction of 2 ml of liposomal eluate were collected. In the standard preparation (1.9 mg of magnetite in total lipid used), the final concentrations of total lipid and magnetite were 8.6 μmol/ml and 133 μg/ml, respectively. The mean diameter of the liposomes was 1.54 μm (determined with a photon correlation spectrometer; model N_4, Coulter, Hialeah, FL).

Assay of Magnetite——The amount of encapsulated magnetite was determined based on ferrous ion by using *o*-phenanthroline[14] as follows. A liposomal sample (0.1 ml) was mixed with 0.1 ml of Triton X-100 solution (5%), then the magnetite was ionized by adding 0.5 ml of conc. HCl, and 1.0 ml of hydroxylamine hydrochloride solution (10%) was added to reduce ferric ion. After 15 min, 1.0 ml of *o*-phenanthroline solution (0.5%) was added, the mixture was neutralized with 0.5 ml of 12 N NaOH, and the pH was adjusted to about 4.0 with sodium citrate solution (30%). The volume of the sample was adjusted to 10 ml, and the absorbance was determined at 509 nm.

Apparatus——The apparatus used in this experiment is illustrated in Fig. 1. The capillary was made with glass tubing (i.d. 1 mm) and it was positioned perpendicularly. The liposome suspension was injected as a bolus at the top during the infusion of PBS with an infusion pump (Natsume Seisakusho, type KN-202, Tokyo). If the capillary was set horizontally, the liposomes accumulated on the bottom of the capillary because of their high density, and the effect of the magnet did not appear clearly. The electric magnet was located at the middle of the capillary, which was widened (i.d. = 4 mm, length = 2.0 cm) at this point so that fixed liposomes would not be washed away by the water stream. A preliminary experiment showed that a straight capillary was unsatisfactory.

The electric magnet was made by the authors with 1.7 kg of cuprum wire and is illustrated in Fig. 1. This magnet was able to generate a magnetic field of about 4000 G (determined with a gaussmeter; Maezumi Electric Co. Ltd., Tokyo) at 10 V.

Animal Experiment——Yoshida sarcoma (gift from Dr. Umemura at Toho University, Chiba, Japan) was

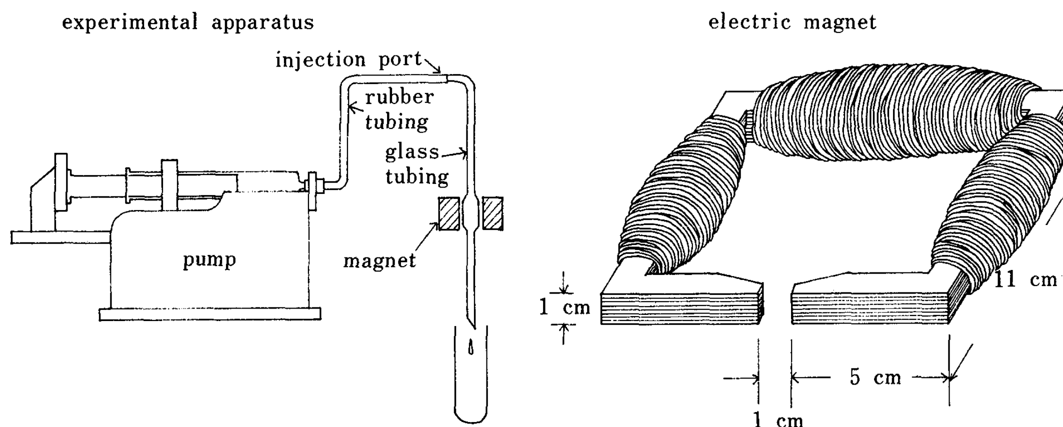

Fig. 1. Illustration of the Apparatus and the Electric Magnet Used

NII-Electronic Library Service

implanted in a hind foot pad of Donryu male rats (body weight = 250—280 g). After 7—10 d, when the tumor had reached 10 × 10—15 × 20 mm in size, the animals were anesthetized with pentbarbiturate (Abbot Lab., IL) and the branch of the femoral artery was cannulated with polyethylene tubing (o.d. = about 0.3 mm, thinned by heating). Then 0.2 ml of the liposome suspension labelled with ^{3}H-inulin (prepared by the standard method; 1.72 μmol of lipid and 26.6 μg of magnetite) was infused through the tubing with a constant-flow syringe pump (Natsume Seisakusho, type KN-201, Tokyo) at the flow rate of 0.024 ml/min while the tumor was positioned in the gap of the magnet. After the infusion, animals were sacrificed immediately by puncturing the carotid artery. Blood was removed rapidly with a heparinized pipet. Liver, lung, spleen and tumor were subsequently removed and rinsed with PBS. Then 0.2 ml of whole blood or 1 ml of liver homogenate (total volume, 50 ml) was transferred into liquid scintillation vials. Each sample was digested with 2 N KOH in isopropanol, bleached with 30% H_2O_2 overnight at room temperature, and neutralized with acetic acid. Liquid scintillation cocktail (Scintisol EX-H, Wako Pure Chem. Co., Osaka) was added and the radioactivity was counted with a liquid scintillation counter (Aloka, LSC-637, Tokyo) after a delay of at least 3 h to allow chemiluminescence to dissipate. Other tissues and organs were digested and bleached overnight at 37 °C. After neutralization the volume of the digested sample was adjusted to 10 ml water. One milliliter of the sample was used for counting the radioactivity as described for blood.

Results and Discussion

Effect of Magnetite on Liposome Preparation

It seems likely that the use of a greater amount of magnetite in the liposomes would allow more effective fixing of the liposomes by the magnetic force.[15] Therefore, it is necessary to know how much magnetite can be incorporated into liposomes. The amount of magnetite in liposomes was examined as a function of the initial amount of magnetite while the amount of lipid was fixed (45.5 μmol).

The ratio of magnetite entrapped to the initial amount used did not vary much for various initial amounts. However, the amount of magnetite per unit lipid increased with increasing initial amount of magnetite. On the other hand, the encapsulation capacity of the liposomes for inulin was not affected. The entrapped amount of the magnetite per unit lipid was highest in the case of the preparation of liposomes with 4.2 mg of magnetite in 45.5 μmol of lipid, as shown in Table I. However in this preparation, complete hydration of the liposomes was not achieved, and many aggregates were observed. Therefore in the following experiments, the liposome suspension prepared with 1.9 mg of magnetite in 45.5 μmol of lipids was used as the standard preparation.

Holding of Liposomes by Magnetic Force

A 0.1 ml aliquot of the magnetic liposome suspension prepared by the standard method (13.3 μg of magnetite and 0.86 μmol of lipids) was injected at the top of the capillary shown in Fig. 1. The flow rate of the medium was at 0.08 ml/min. The effluent from the bottom of the capillary was collected in 1 ml fractions, and the radioactivity of the encapsulated ^{3}H-inulin

TABLE I. Effect of the Amount of Magnetite on the Encapsulation Ratio

Amount of magnetite[a] (mg)	Availability of magnetite[b] (%)	Encapsulation ratio in unit lipid[c] (μg/μmol)	Encapsulation capacity[d] (%)
0	—	—	7.4 ± 0.6
0.31 ± 0.14	11.7 ± 4.7	2.2 ± 0.9	8.5 ± 0.7
0.64 ± 0.18	16.3 ± 0.5	5.8 ± 1.2	7.1 ± 1.0
1.90 ± 0.36	14.1 ± 0.4	15.4 ± 3.5	10.7 ± 1.2
4.20 ± 0.42	22.8 ± 6.7	41.1 ± 13.4	9.3 ± 2.7

a) Amount of magnetite used (total lipid used was 45.5 μmol in each preparation). *b*) Percentage of magnetitie found in the liposomal fraction per total magnetite. *c*) Amount of magnetite per unit lipid. Recovery of liposomes was measured in terms of radioactivity of ^{14}C-cholesterol. *d*) Percentage of aqueous marker (^{3}H-inulin) encapsulated. All values are expressed as the mean ± S.E. of three experiments.

NII-Electronic Library Service

was counted in each fraction. The effluent pattern with or without the magnet was shown in Fig. 2. After collection of 10 fractions, the medium retained in the capillary was washed out and the radioactivity was counted.

As shown in Fig. 2(b), most of the liposomes flowed out of the capillary in fractions 1 and 2 without the magnet, though appreciable radioactivity was observed in the following fractions. This tailing of the effluent liposomes is considered to be due to stagnation at the widened part of the capillary (Fig. 1). On the other hand, little radioactivity was found in the first fraction with the magnet on, as shown in Fig. 2(a). This result shows that many liposomes were trapped by the magnetic force at the widened part of the capillary (indeed, gathered liposomes were visually apparent at this region). Appreciable radioactivity found in the following fractions seems to be due to washing out of liposomes by the medium. About 50% of the injected liposomes was found in the retained medium.

Effects of Magnetic Force, Flow Rate and Injected Amount

Senyei *et al.* reported that the ability of an external magnet to hold magnetic microspheres was affected by the flow rate and magnitude of the magnetic force.[16] A limited

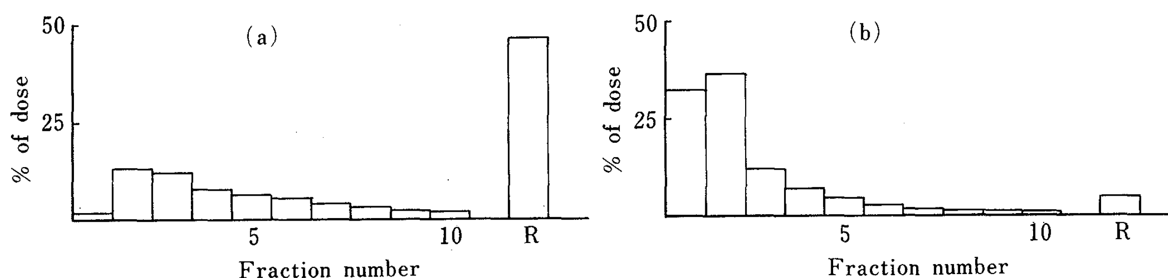

Fig. 2. Elution Patterns of the Magnetic Liposomes from the Apparatus Shown in Fig. 1 with (a) and without (b) the Magnet (4000 G)

A 0.1 ml aliquot of the magnetic liposome suspension was injected at the top of the capillary and fractions (1 ml each) were collected from the bottom of the capillary. The flow rate of medium was 0.08 ml/min. R is the retained radioactivity (^{3}H-inulin) in the capillary after infusion.

TABLE II. Effect of Magnetic Field Strength on the Holding of the Magnetic Liposomes

Magnetic field (G)	Flow rate (ml/min)	Injected volume (ml)	Holding ratio[a] (%)
4000	0.17	0.1	34.5±2.4
2000	0.17	0.1	19.6±3.4
0	0.17	0.1	4.3±2.2

a) Percentage of radioactivity retained in the capillary per dose after the infusion of 10 ml of medium. Values are expressed as mean ± S.E. of three experiments.

TABLE III. Effect of Flow Rate of Medium on the Holding of the Magnetic Liposomes

Flow rate (ml/min)	Magnetic field (G)	Injected volume (ml)	Holding ratio[a] (%)
0.51	4000	0.1	15.2±3.3
0.17	4000	0.1	34.5±2.4
0.08	4000	0.1	47.6±5.7

a) See footnote to Table II.

NII-Electronic Library Service

Table IV. Effect of Applied Amount of Liposomes on the Holding of Magnetic Liposomes

Injected volume (ml)	Magnetic field (G)	Flor rate (ml/min)	Holding ratio[a] (%)
0.1	4000	0.17	34.5 ± 2.4
0.05	4000	0.17	37.8 ± 0.7

a) See footnote to Table II.

Table V. Effect of Magnetic Force on *in Vivo* Distribution of the Magnetic Liposomes

Organ or tissue	Distribution (% of dose)[a]	
	With magnet	Without magnet
Blood	1.73 ± 0.11[b]	0.59 ± 0.10
Liver	54.44 ± 5.12	69.53 ± 4.59
Spleen	6.07 ± 0.89	4.91 ± 0.44
Lung	3.38 ± 0.24	2.45 ± 0.53
Tumor	0.28 ± 0.06[c]	0.15 ± 0.04

a) Percentage of radioactivity (^{3}H-inulin) per dose in whole organ or tissue except for blood. In blood the values are expressed as percentage in 1 ml of blood. Values are expressed as means ± S.E. of four experiments with the magnet (4000 G) and three experiments without the magnet. c) Significant difference ($p < 0.01$). c) Significant difference ($p < 0.05$).

holding capacity of this system was suggested by the result described above. Therefore, the effects of these factors on the holding of the magnetic liposomes were examined in more detail. The results were evaluated in terms of percent of retained radioactivity in the capillary per injected activity after the infusion of 10 ml of medium.

The effect of the magnetic field is summarized in Table II. The fixation of the liposomes increased almost proportionally to the magnetic field. Flow rate also affected the holding capacity, and lower flow rate resulted in higher fixation as shown in Table III. The viscosity of the medium should also affect the holding ability of the magnet as mentioned by Driscoll *et al.*[15]

The effect of injected amount was also examined, but the injected amount did not appear to affect the percentage trapped amount per injected dose (Table IV). This result suggests that the holding capacity may be primarily dependent on the magnetic field applied.

In Vivo Study

The application of magnetic liposomes in an *in vivo* system was attempted. Widder *et al.*[8,9] used Yoshida sarcoma implanted in rat tail as a model system for magnetic albumin microspheres. They inserted a polyethylene catheter into the ventral caudal artery and the microspheres were infused through the catheter. However, the blood flow into the tumor tissue might have been limited by the insertion of the catheter in their system. The holding ability of the magnet may be affected by the flow rate and viscosity of the blood.[15] Therefore, their system may not reflect the normal physiological condition. We implanted the tumor in the hind foot pad, and the catheter was inserted in the branch of the femoral artery; magnetic liposomes were infused at the dose of 0.2 ml of the liposome suspension (1.72 μmol of lipid and 26.6 μg of magnetite). In this system, the blood flow in the tumor tissue should be unaffected. Therefore, the results presented in Table V should indicate the effect of the magnet under conditions of normal blood flow.

NII-Electronic Library Service

Very little radioactivity was found in the tumor tissue and most was found in the liver, as would be expected for particulate substances, either with or without the magnet. However, significantly more radioactivity ($p<0.05$) was trapped at the tumor tissue with the magnet compared to that without the magnet. The reasons for the very small effect of the magnet in this experiment are considered to be as follows. 1) The size of the liposomes used in this experiment (1.54 μm) is smaller than the microspheres of Widder *et al.* (about 10 μm).[8] The liposomes may pass through the capillaries of the tumor tissue. 2) The linear flow rate and viscosity of the blood in the tissue may be higher than those of the medium used in the *in vitro* experiment, and the capillary bed in the tissue is smaller in volume than the glass capillary used in the *in vitro* experiment. Therefore, most of the magnetic liposomes seemed to be washed out by the blood flow. 3) Capillaries in the tumor tissue may not be well connected with capillaries originating from the femoral artery. 4) The magnitude of the magnetic field may not be high enough. In this experiment, about 30% of injected radioactivity was not recovered in the tissue or organs listed in Table V. This seems to be due to the degradation of liposomes in the circulation and excretion of released inulin in the urine, rather than accumulation in other tissues, as in the case of conventional liposomes.[17]

A more powerful magnet may allow magnetic liposomes to be targeted at the desired site more effectively. Use of oscillating magnetic fields to regulate the release of drugs was reported by Edelman *et al.*[18] The application of this regulation of release to magnetic liposomes is very interesting. The amount of the magnetic liposomes trapped at the tumor tissue by the magnet was very small compared with the *in vitro* results, for the reasons discussed above. The correlation of the Reynolds' number and holding efficiency by magnetic force is currently under study.

Significantly higher radioactivity was observed in the blood with the magnet on, but the reason for this is not clear.

Acknowledgement The authors thank Dr. Umemura (Toho University, Chiba) for the gift of Yosida sarcoma and advice on the treatment of the tumor.

References and Notes

1) Present address: *Faculty of Pharmaceutical Sciences, University of Tokushima, 1–78–1 Sho-machi, Tokushima 770, Japan.*
2) "Targeting of Drugs," ed. by G. Gregoriadis, J. Senior and A. Trouet, Plenum Press, New York, 1982.
3) K. J. Widder, A. E. Senyei and D. G. Scarpelli, *Proc. Soc. Exp. Biol. Med.*, **58**, 141 (1978).
4) K. Widder, G. Flouret and A. Senyei, *J. Pharm. Sci.*, **68**, 79 (1979).
5) A. E. Senyei, S. D. Reich, C. Gonczy and K. J. Widder, *J. Pharm. Sci.*, **70**, 389 (1981).
6) H. Ovadia, P. Y. Paterson and J. R. Hale, *Israel J. Med. Sci.*, **19**, 631 (1983).
7) I. Carr, "Kupffer Cells and Other Liver Sinusoidal Cells," ed. by E. Wisse and D. L. Knook, Elsevier/North-Holland Biomed. Press, Amsterdam, 1877, pp. 355—364.
8) K. J. Widder, P. A. Marino, R. M. Morris, D. P. Howard, G. A. Poore and A. E. Senyei, *Eur. J. Cancer Clin. Oncol.*, **19**, 141 (1983).
9) K. J. Widder, R. M. Morris, G. A. Poore, D. P. Howard and A. E. Senyei, *Eur. J. Cancer Clin. Oncol.*, **19**, 135 (1983).
10) "Liposomes: from Physical Structure to Therapeutic Applications," ed. by C. G. Knight, Elsevier/North-Holland Biomed. Press, Amsterdam, 1981.
11) G. Gregoriadis, "Drug Carriers in Biology and Medicine," ed. by G. Gregoriadis, Academic Press, New York, 1979, pp. 287—341.
12) H. Kiwada, H. Niimura, Y. Fujisaki, S. Yamada and Y. Kato, *Chem. Pharm. Bull.*, **32**, 753 (1984).
13) H. Kiwada, H. Niimura and Y. Kato, *Chem. Pharm. Bull.*, **33**, 2475 (1985).
14) I. M. Kolthoff, D. L. Leussing and T. S. Lee, *J. Am. Chem. Soc.*, **72**, 2173 (1950).
15) C. F. Driscoll, R. M. Morris, A. E. Senyei, K. J. Widder and G. S. Heller, *Microvascular Res.*, **27**, 353 (1984).
16) A. E. Senyei, K. J. Widder and G. Czerlinski, *J. Appl. Phys.*, **49**, 3587 (1978).
17) Y. Sato, H. Kiwada and Y. Kato, *Chem. Pharm. Bull.*, **34**, 4244 (1986).
18) E. Edelman, H. Bobeck and R. Langer, *Polym. Prep.*, **24**, 49 (1983).

NII-Electronic Library Service