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Magnetic Guidance of Ferro-colloid-entrapped Emulsion for Site-specific Drug Delivery¹⁾

YASUNORI MORIMOTO,* KENJI SUGIBAYASHI and MASAYUKI AKIMOTO

Faculty of Pharmaceutical Sciences, Josai University,
1-1 Keyakidai, Sakado, Saitama 350-02, Japan

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Magnetic guidance of magnetic emulsions with site specificity was investigated *in vitro* and *in vivo*. The emulsions consisted of ethyl oleate-based magnetic fluid as the oily dispersed phase and 1% casein solution as the aqueous continuous phase. The magnetic emulsion was characterized *in vitro* for magnetic responsiveness by using a constant flow apparatus, and its high retention by a magnetic field was confirmed. Ethyl oleate in the magnetic emulsion was labelled with ¹⁴C-palmitic acid and a selective localization of the magnetic emulsion at a predetermined site (lungs) by application of an electromagnet to the lungs was demonstrated. Such preferential localization by magnetic means suggested that this new carrier system can provide a highly specific delivery system for chemotherapeutic agents in cancer chemotherapy.

Keywords——magnetic emulsion; electromagnet; target site; carrier system; cancer chemotherapy

The ideal dosage form in chemotherapy is one that provides specific drug delivery to the desired target sites in a sufficient amount, for a long period of time, with minimum side effects. Recently, several types of drug carriers intended to modify the systemic distribution of chemotherapeutic agents have been proposed as possible drug delivery system. Encapsulation carriers such as liposomes,²⁾ albumin microspheres^{3,4)} and microspheres in oil emulsions⁵⁾ offer promise for attaining site specificity. However, controlled localization of the drug carriers has been difficult to achieve, because intravenous injection of such carriers results in their rapid clearance by the reticuloendothelial system.

One way to achieve the targeting of a drug is to use magnetizable drug carriers in combination with an external magnetic field. Widder *et al.*⁶⁾ suggested that since magnetic microspheres injected into the ventral caudal artery could be localized to some extent to a predetermined target site by an externally applied magnetic field, magnetically guided albumin microspheres containing drug might be useful as a drug delivery system with site specificity. We also investigated the drug carrier properties of magnetic albumin microsphere by measuring microsphere levels in the lung and kidney after intravenous or intra-renal-arterial administration, respectively,^{7,8)} and showed that adriamycin entrapped in magnetic albumin microspheres had pronounced antitumor activity on AH 7974 lung cancer in rats.⁹⁾ More recently, we reported the development of a magnetic responsive oil-in-water type emulsion with the capacity to localize lipid-soluble drugs at a target site with the aid of a magnetic field, and suggested that magnetic emulsions might be utilized as a drug carrier for site-specific drug delivery.¹⁰⁾

Magnetic control of intravascular particles has proven feasible in diagnostic and therapeutic applications with no discernible toxicity.¹¹⁾ If site-specific drug delivery of anticancer agents could be achieved by magnetic means, this delivery system would eliminate side effects that are often the sequelae of systemic drug distribution. The present report describes the magnetic responsiveness of magnetic emulsions *in vitro* and *in vivo*.

Experimental

Materials— ^{14}C -Palmitic acid was purchased from the Japan Radioisotope Association. Casein prepared by the method of Hammersten was obtained from Wako Pure Chemicals Industries, Ltd. Ethyl oleate-based magnetic fluid was prepared by the modified method of Shimoiizaka *et al.*¹²⁾ Magnetic powder and ethyl oleate-based magnetic fluids were prepared by the method described previously.^{1,13)} Ethyl oleate-based magnetic fluid contained 20.2% (w/w) magnetites in ethyl oleate. All other chemicals were reagent-grade products.

Preparation of Magnetic Emulsions—Two ml of ethyl oleate-based magnetic fluid containing ^{14}C -palmitic acid (20 μCi) as a radiolabelled tracer (oily phase) and 8.0 ml of 1% casein solution (aqueous phase) were mixed and emulsified with an Ultra Turrax (Janke & Kunkel, Co., Ltd.) at 10000 rpm for 5 min. In all cases, emulsification was carried out in a water bath at 37°C. The size of oil droplets varied from about 1 to 16 μm and the average diameter was 5.0 μm . All of the magnetic emulsions prepared by this method show similar particle-size distribution patterns, and there was no great difference of size distribution among batches used.

In Vitro Model for Targeting—The magnetic emulsion was characterized *in vitro* for magnetic responsiveness by using a constant flow apparatus. The retention by a magnetic field is represented as % ^{14}C -radioactivity or magnetites relative to the initial amount. The radioactivities and magnetites levels retained were determined by the method described previously.¹⁾

In Vivo Model for Targeting—Female Donryu rats, weighing about 140–150 g, were used in all experiments. The site utilized for *in vivo* targeting of the emulsions was rat lung. Each rat was anesthetized with pentobarbital sodium at a dose of 60 mg/kg *i.p.*, and both poles of an electromagnet (Nihon Denji Sokki Co., Ltd.) were directly applied to the sides of each rat, that is, the breast and back of the rat, for 10 or 60 min after injection of the emulsions so as to concentrate the emulsions into the lungs. The value of magnetic induction developed in the thorax of the rat was adjusted to 2000, 4000 or 6000 gauss (G). For determining the tissue distribution of emulsions, magnetic emulsions containing ^{14}C -palmitic acid in the oily phase were used. The emulsions (0.2 ml) were injected into the rat through the tail vein. Following injection, the electromagnet was retained for 10 or 60 min, and the rats were sacrificed by decapitation. The organs were immediately removed. For measurement of ^{14}C -levels, each aliquot of tissue was solubilized with Protosol (New England Nuclear) and diluted directly with a scintillator (Aquasol 2, New England Nuclear), and the radioactivity was determined with a liquid scintillation counter (Model LSC-703, Aloka, Japan). The distribution of radioactivity to various organs was represented as % of dose per gram of tissue.

Histological Examination—At 10 min after intravenous injection of magnetic emulsion, the lungs and liver were excised, fixed in 10% formalin, stained with Berlin Blue and microscopically examined.

Results

Magnetic Responsiveness of Magnetic Emulsions

The magnetic responsiveness of the emulsions diluted in 1.0% casein solution is shown by

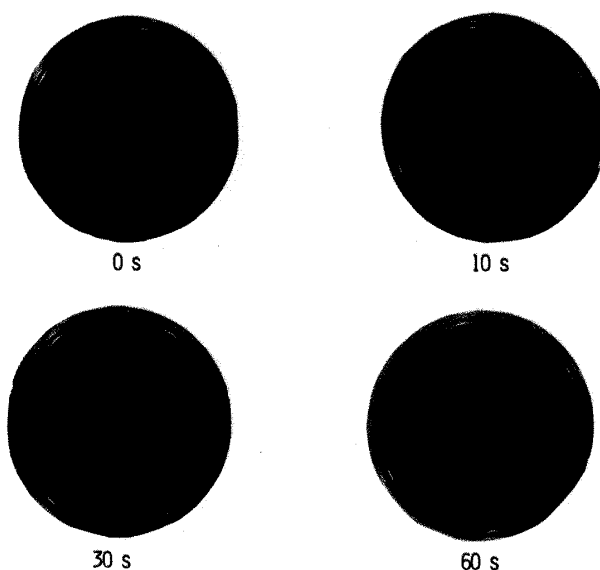


Fig. 1. Time Sequence Photographs of Magnetic Emulsions after Application of a Disc Magnet

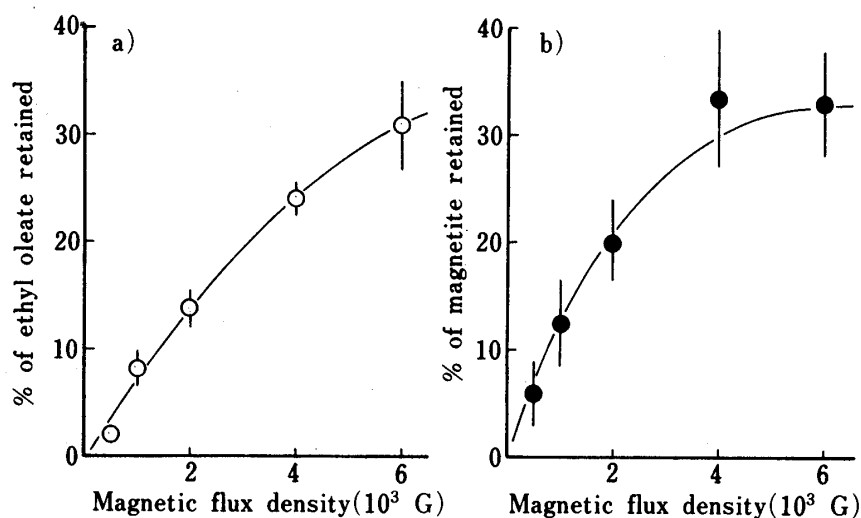


Fig. 2. Retention of Magnetic Emulsion flowing in pH 7.4 Isotonic Buffer as a Function of Magnetic Flux Density

a) Ethyl oleate. b) Magnetites.

Results are expressed as the means $\pm$ S.E. of five experiments.

the time sequence photographs in Fig. 1. It is clear that the emulsions were sequentially localized on and around a disc-magnet (inner radius 5 mm, outer radius 9.5 mm, thickness 6 mm). Details of their magnetic responsiveness under flow conditions are described below.

***In Vitro* Model for Targeting**

The results of *in vitro* retention of emulsions are shown in Fig. 2. The data demonstrate that magnetic retention varies reproducibly with magnetic induction. The amounts of magnetites and ethyl oleate retained increased steadily with magnetic flux density. However, magnetites and ethyl oleate were not completely retained, and about 30.8% of ethyl oleate and 33.0% of magnetites were retained at the target site at 6000 G.

Next, we investigated whether the emulsions with high magnetic responsiveness could be retained in the lungs by magnetic means after intravenous injection or not.

***In Vivo* Model for Targeting**

The tissue distribution of radioactivity at 10 min after intravenous injection of magnetic emulsions is shown in Fig. 3. After injection of the emulsion into rats without the electromagnet (control group), about 16.2% of magnetic emulsions injected was found in the lungs. When the electromagnet was applied to the lungs (magnet group), the emulsion level in the lungs was 28.9, 32.5 or 32.7% at 2000, 4000 or 6000 G, respectively, *i.e.*, about twice the level in the control group. The emulsion levels in the liver and spleen in the magnet groups were slightly lower than that in the control group. However, there was not a clear correlation between the emulsion level in the lungs and the magnetic flux density.

Fig. 4 shows the tissue distribution of the emulsions at 60 min following intravenous injection with application of the electromagnet to the lungs for 60 min. Lung emulsion levels in the magnet group were found to be about 10–11% at 60 min after injection regardless of the intensity of magnetic induction. However, about 8.0% was found in the lungs at 60 min after injection of the emulsions into rats without the electromagnet. The difference between the magnet and control groups was less than we had expected.

Fig. 5 shows photomicrographs of the lungs and liver at 10 min after intravenous injection of magnetic emulsions. This study showed that magnetites contained in emulsions reached the lungs, and lodged in pulmonary capillary beds or were retained in the immediate vicinity of alveoli. However, magnetites that passed the pulmonary filter were mainly trapped in the

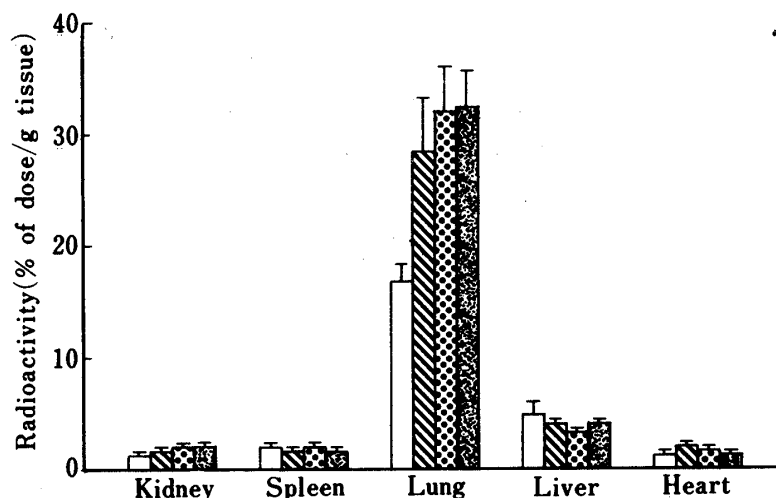


Fig. 3. Tissue Distribution of Radioactivity 10 min after the Injection of a magnetically Responsive Emulsion containing ^{14}C -Palmitic Acid through the Tail Vein

□ : control; ▨ : magnet (2000 G);
 ▩ : magnet (4000 G); ▤ : magnet (6000 G).
 Results are expressed as the means $\pm$ S.E. of 6–7 animals.

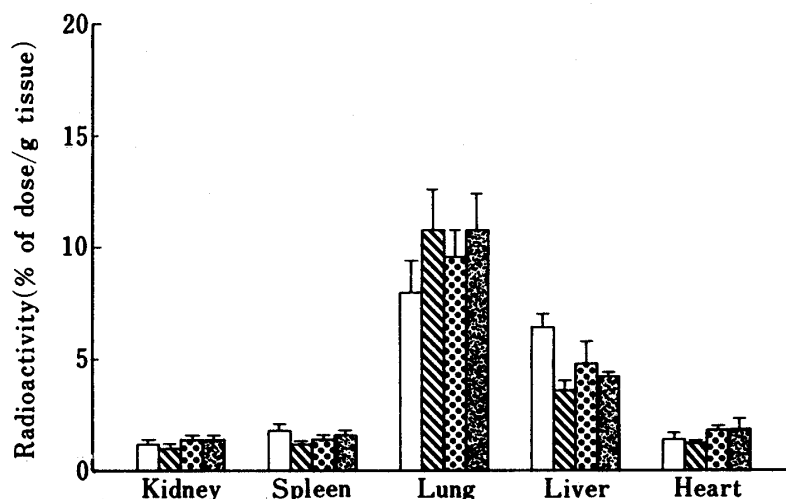


Fig. 4. Tissue Distribution of Radioactivity 60 min after the Injection of a magnetically Responsive Emulsion containing ^{14}C -Palmitic Acid through the Tail Vein

Column: as in Fig. 3.
 Results are expressed as the means $\pm$ S.E. of 6–7 animals.

liver. The retention of magnetic emulsions in the lungs was indicated by these results, but the difference between control and magnet groups was not substantial.

Discussion

In recent years, encapsulation of drugs into carriers has found increased use in diagnosis and therapy.¹⁴⁾ For successful application, however, controlled localization of carriers for treatment of a variety of focal lesions should be achieved.¹⁵⁾

In pharmaceutical practice, various types of emulsion have been used as dosage forms for administering drugs. Elson *et al.*¹⁶⁾ reported that the use of methotrexate in the form of a multiple emulsion (w/o/w type) gave a prolongation of pharmaceutical action as there was a

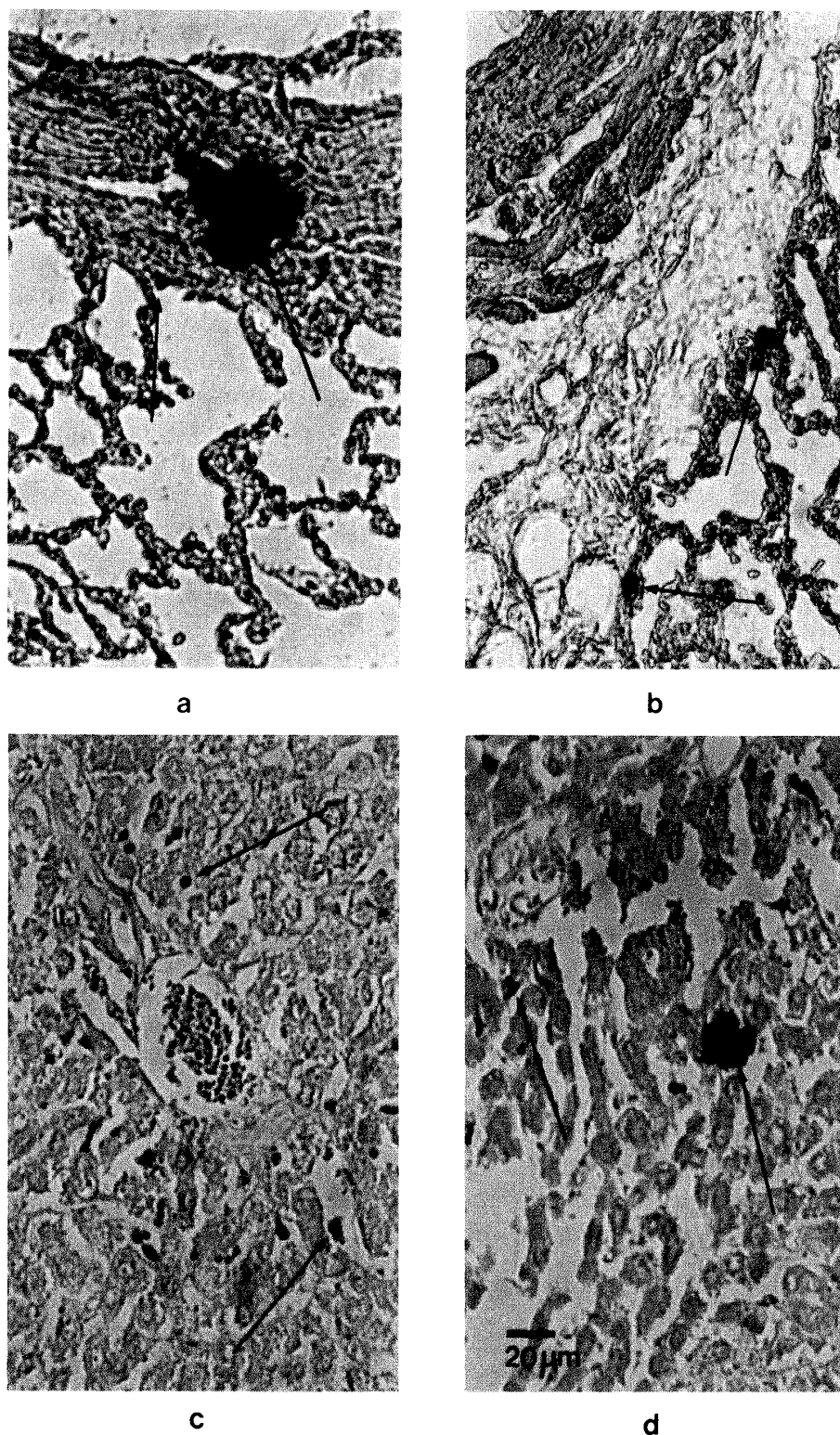


Fig. 5. Photomicrographs of the Lungs and Liver (Fe Stain) at 10 min after Intravenous Injection of Magnetic Emulsions

a, Lung (magnet); b, Lung (non-magnet); c, Liver (magnet); d, Liver (non-magnet).
Arrows indicate magnetites retained.

slow release of the drug in its water-soluble active form. Moreover, Hashida *et al.*⁵⁾ suggested that the use of emulsion formulations was very promising in the targeting of anticancer agents to the lymphatics and in preventing metastasis. Recently, we developed magnetically responsive emulsions prepared with ethyl oleate-based magnetic fluids.¹³⁾ Targeting with the magnetic emulsions by magnetic means does not depend on biological function, but depends on the magnetic properties of the emulsion itself and the applied magnetic flux density (Figs. 2 and 3). Therefore, a magnetic emulsion with high magnetic responsiveness and good stability as a drug carrier for lipid-soluble drugs might be useful as a delivery system for cancer chemotherapy.

At 10 min after intravenous injection, 16.2% of magnetic emulsions had accumulated in the lungs in the control group (Fig. 3). The distribution of magnetic emulsions can be explained by the trapping phenomenon at the pulmonary capillary bed before distribution to the whole body and subsequent phagocytosis of entrapped material at the liver (Fig. 5). The distribution pattern might depend on the particle size of emulsion droplets, and this might explain the greater accumulation of magnetic emulsions in the lungs as compared with other fat emulsions, which had a small average diameter and narrow size distribution.^{17,18)} The amount of emulsions retained at the targeting site (lung) in the magnet group was about twice that in the control group, regardless of the intensity of magnetic induction. Modification of the relative tissue distribution by magnetic means was thus demonstrated (Fig. 3). Since a lipid-soluble anticancer agent could be delivered with the emulsions, it can be concluded that magnetic emulsions might be useful as a drug carrier.

At 60 min post-injection, however, there was only a small difference between the emulsion levels in the lungs of the control group and the magnet groups (Fig. 4). The decrease in the lungs observed from 10 to 60 min following administration of the emulsions may be due to a washout effect of blood flow on the emulsions within the lungs, so that material initially trapped was subsequently recirculated in the blood-stream.⁷⁾ When magnetic fluids contact water, they degrade within a few days, resulting in the decomposition of the colloidal suspension in water.¹⁹⁾ Moreover, application of potential magnetic forces to the lungs might accelerate this phenomenon and result in washout within the lungs due to the separation of magnetites and base oil from magnetic fluids. The results obtained from Fig. 4 indicate that the application time of the electromagnet to target sites is important for retention of emulsions in those sites. However, the effect of magnet application time on the retention of emulsions seems to be very complicated, and further experiments are necessary.

The application of magnetic emulsions as a drug delivery system for lipid-soluble drugs could be advantageous in the treatment of tumors due to the concentration of the drug at the site of carrier localization. Moreover, since reduction of drug concentration in the plasma and non-target organs is most desirable to reduce side effects, a specific carrier system which delivers the anticancer agent to a predetermined target-site would be very valuable. An extended investigation of the therapeutic index of anticancer agents when given in the magnetic emulsion form is being carried out.

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