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Magnetic Microcapsules for Targeted Delivery of Anticancer Drugs

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ABSTRACT

To achieve targeted distribution of anticancer drugs with sustained activity, ferromagnetic ethylcellulose microcapsules containing an anticancer drug, mitomycin C (FM-MMC-mc), were prepared by a method based on phase separation principles. Two prototypes of FM-MMC-mc were made: one with the drug as the core and zinc ferrite on its capsular surface (outer type); the other with both the drug and zinc ferrite as the core (inner type). Both preparations provided a sustained-release property and a sensitive response to conventional magnetic force, although certain differences in the release rate of drug, magnetic responsiveness, and particle size were found between the two dosage forms. Animal studies showed that the magnetic microcapsules could be magnetically controlled in the artery and urinary bladder. VX2 tumors in the rabbit hind limb and urinary bladder were successfully treated with magnetic control of FM-MMC-mc. Pharmacokinetic study revealed that the targeting of the microcapsules markedly enhanced the drug absorption into the surrounding tissues for a prolonged period of time. The results indicate the feasibility and effectiveness of the magnetic microcapsules as a targeted drug delivery system.

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Index Entries: Magnetic microcapsules, for anticancer drug delivery; zinc ferrite, in encapsulated anticancer drugs; targeting, of encapsulated anticancer drugs; artery, use of encapsulated anticancer drugs in; urinary bladder, use of encapsulated anticancer drugs in; VX2 tumor, use of encapsulated anticancer drugs against; anticancer drugs, use of encapsulated; magnetic microcapsules, use of anticancer drugs in.

INTRODUCTION

The targeting of anticancer drugs with an appropriate release rate is a major challenge for cancer chemotherapists. To help solve this problem, we have introduced ethylcellulose microencapsulation of anticancer drugs for the purpose of selective infusion into tumor supplying arteries (*1*). The rationale for this approach is to target microcapsules containing drugs into the vascular beds of tumor lesions through arterial catheterization and embolization of arterioles in the target sites. It has been found that infarction and prolonged drug action produce an enhanced antitumor effect with a decreased systemic drug toxicity. This mode of treatment has been described as chemoembolization (*2,3*). Until March 1982, 285 patients with advanced carcinomas in various organs including the kidney, liver, and intrapelvic organs were subjected to microcapsule therapy with promising results (*4,5*).

Our early clinical experiences have indicated that transcatheter arterial chemoembolization with microcapsules is, thus far, the best means to provide practical effects with respect to targeting of drugs and enhancement of drug action. However, this approach has certain technical disadvantages as well. Selective arterial catheterization generally needs a skillful technique, and those tumors with complicated arterial blood supplies usually remain beyond the scope of this treatment. The frequent occurrence of arteriovenous fistula in the tumor, such as in hepatoma and renal cell carcinoma, causes another problem inasmuch as the microcapsules, which have a mean particle size of approximately 200 μm, will readily pass through the fistula.

Considering these problems, we initiated research aimed at developing a magnetic control system of microcapsules (*6*). The purpose of this approach is to guide the intravascular or intracavitary microcapsules into desired sites and/or to retain them at the target site by means of external magnetic force. Magnetic ethylcellulose microcapsules (FM-MMC-mc) containing an anticancer drug, mitomycin C (MMC), and ferromagnetic particles were prepared, and animal experiments were performed. The present paper will summarize our results (*7–11*).

MATERIALS AND METHODS

Preparation and Characterization of FM-MMC-mc

Two types of FM-MMC-mc were prepared based on the principles of coacervation with certain modifications: outer-type and inner-type (*6,9*).

The outer-type FM-MMC-mc were produced as follows: ethylcellulose (1 g), polyethylene (0.5 g), and cyclohexane (100 mL) were dissolved by heating to 80°C, and 2 g of MMC (Kyowa-Hakko Co. Ltd., Tokyo) was dispersed in this solution. By cooling to room temperature with gentle stirring, MMC particles were encapsulated with ethylcellulose. MMC microcapsules thus prepared were mixed with 100 mL of *n*-hexane containing 0.5 g of zinc ferrite ($Zn_{20}Fe_{80}{\cdot}Fe_2O_3$) with a mean particle size of 1.6 μm and heated again to 45°C. The mixture was cooled with gentle stirring, whereby the ferrite particles were attached to the capsular surface. The microcapsules were air-dried and collected through a 42 mesh screen.

The inner-type FM-MMC-mc were prepared by adding the ferrite particles to the initial solvent so that both the drug and ferromagnetic particles were encased with ethylcellulose membrane. This method could vary the ferrite-drug ratio from 1:0 to 1:4, thus modifying the magnetic responsiveness, drug content, and drug release rate.

The particle size represented by the longitudinal diameter was measured by micrometer, and the surface structure was examined with a scanning electron microscope following vacuum coating with carbon and gold–palladium (60:40).

FM-MMC-mc were characterized with a magnetic balance, and then ground down into powder of which MMC activity was determined by the agar plate diffusion method using E. coli B-ATCC 11303 as the target microorganism (*12*). Release rate of MMC from the microcapsules was assessed as follows: 20 mg of FM-MMC-mc was settled in 500 mL of 0.9% NaCl solution (saline) maintained at 37°C and rotated at 25 rpm, and MMC concentration in 5 mL saline sampled at determined intervals was assessed spectrophotometrically (*13*). The result was expressed as the percentage of MMC dissolved in NaCl solution.

Magnetic Control of FM-MMC-mc in the Aorta

Three heparinized mongrel dogs weighing 12–15 kg were laparotomized and all arterial branches, except for the bilateral femoral arteries, of the aorta below the renal arteries were ligated. The femoral artery and vein on each side were connected by an external shunt with an 80 mesh screen and a samarium magnet (mean magnetic gradient, 2000 kA/m^2; length, 2.5 cm) was placed adjacent to the left lateral wall of the aorta just above the bifurcation of the common iliac arteries. Twenty mg

of outer-type FM-MMC-mc suspended in saline was infused into the aorta at the level of the renal artery and the weight of FM-MMC-mc collected in the mesh screen on each side was compared. In each animal the infusion was repeated three times with and without the magnet, respectively.

Three mongrel dogs were laparotomized and a polyethylene catheter was inserted through the right femoral artery into the aorta up to 5 cm above the renal artery. The same magnet mentioned above was placed at the left lateral wall of the aorta just above the renal artery and 20 mg of outer-type FM-MMC-mc was infused into the aorta through the catheter. Bilateral nephrectomy was done 7 d later to observe the morphologic changes in the kidneys.

Treatment of VX2 Tumors in the Hind Limb and Urinary Bladder of the Rabbit

Nine Japanese white rabbits weighing 3 kg were transplanted with 10^7 VX2 tumor cells in the bilateral hind limbs. A tumor with an average diameter of approximately 2 cm appeared in each site 12 d later. Five animals were infused with inner-type FM-MMC-mc containing 4 mg of active MMC [composition: MMC 30% (w/w), ferrite 50%, ethylcellulose 20%] into the left femoral artery. During the period of the drug infusion and the subsequent 30 min, an electric magnet with a magnetic force of 100 kA/m was placed over the tumor. Another four animals were infused with 4 mg of nonencapsulated MMC into the left femoral artery. In both groups, the left femoral artery was ligated after drug infusions and the tumors in the right hind limb were used as the untreated control. The tumor size was periodically measured and expressed as the product of two dimensions.

Paired inocula of 10^7 VX2 tumor cells were made in the urinary bladder of Japanese white rabbits under laparotomy, and a round samarium magnet (280 kA/m, 10ϕ × 4mm) was fixed with surgical adhesive paste to the external bladder wall at one of the tumor inocula. Four rabbits received intravesical instillation of the inner-type FM-MMC-mc containing 10 mg MMC suspended in 20 mL saline, four received 10 mg of nonencapsulated MMC and placebo ferromagnetic microcapsules (composition; lactose, 30%, ferrite 50%, ethylcellulose 20%) suspended 20 mL saline, and four received 20 mL saline without drug. Cystectomy was done 2 wk after the treatment and tumor growth was assessed by microscopic observation of tissue specimens processed to hematoxylin-eosin staining. The tumor size was expressed by the product of maximum diameter and depth.

Drug Activity in the Urinary Bladder

A samarium magnet (280 kA/m, 10ϕ × 4mm) was fixed to the external bladder wall of the Japanese white rabbits and the intravesical urine

was thoroughly excluded. Four animals were instilled with the inner-type FM-MMC-mc containing 6 mg MMC, four with 6 mg nonencapsulated MMC, and 6 mg placebo ferromagnetic microcapsules, and four with 6 mg nonencapsulated MMC. Whole bladder tissues adjacent to and apart from the magnet were separately removed in individual animals 3 h after the instillations. The tissue specimens were thoroughly rinsed with saline to remove both the microcapsules and drug over the epithelial surface, and underwent homogenization. Drug activity in the tissue homogenate was measured by the bioassay method (*12*).

RESULTS

In Vitro Properties of FM-MMC-mc

The particle sizes of outer- and inner-type FM-MMC-mc were 308 ± 35 and 250 ± 43 μm (mean ± SD, n = 100), respectively.

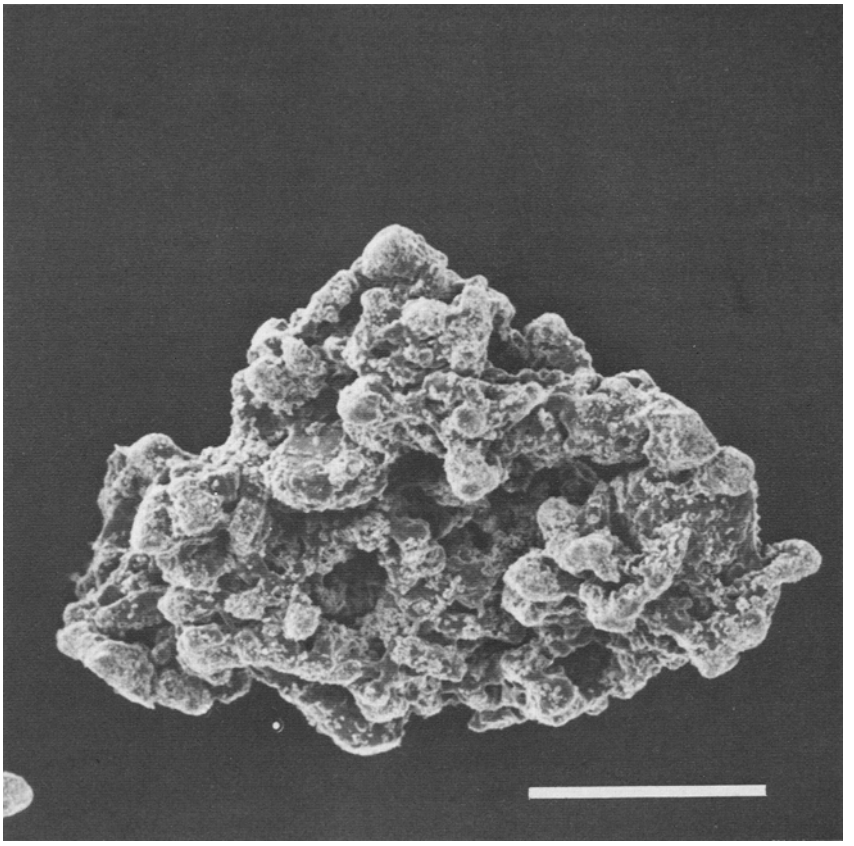

Fig. 1. Scanning electron microphotograph of outer-type FM-MMC-mc. Ferrite particles are attached to the surface. Bar indicates 100 μm.

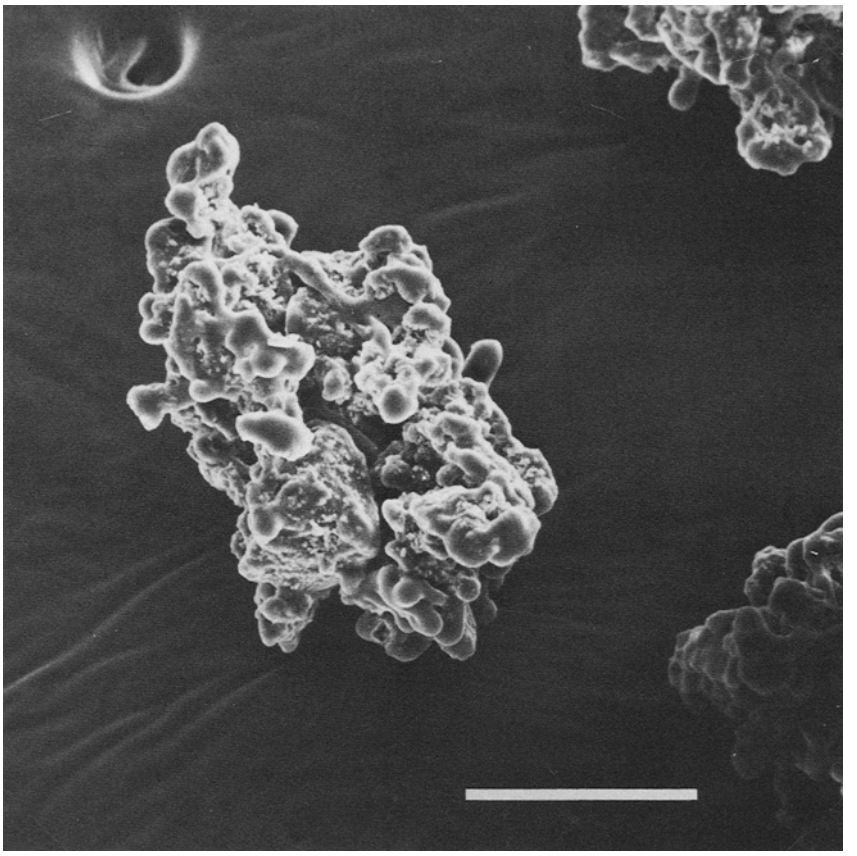

Fig. 2. Scanning electron microphotograph of inner-type FM-MMC-mc. Majority of ferrite particles are coated with ethylcellulose. Bar indicates 100 μm.

Scanning electron microscopy showed that the outer-type formed an irregular particle with ferrite particles solidly fixed to the capsular surface, and the inner-type had a rather smooth surface with occasional attachment of ferrite particles on the capsular surface (Figs. 1 and 2). These findings indicate that the majority of ferrite particles can be coated with ethylcellulose during the microencapsulation process of inner-type FM-MMC-mc, of which particle size is much smaller than that of outer-type. Though ferrite–MMC ratio in the inner-type FM-MMC-mc was varied from 1:0.5 to 1:4, the prescription of 1:0.5 was used in our studies with the inner-type FM-MMC-mc.

The composition of outer-type FM-MMC-mc analyzed with bioassay method and a magnetic balance was, on average, 50% (w/w) of active MMC, 34% of ethylcellulose, and 16% of ferrite particles, and that of the inner-type FM-MMC-mc was 30% of MMC, 50% of ferrite particles and 20% of ethylcellulose. Since the inner-type FM-MMC-mc contained a larger amount of ferrite particle within a smaller particle relative to the

outer-type FM-MMC-mc, the magnetic responsiveness of the former was markedly increased compared with the latter. For example, magnetization at a magnetic field of 40 kA/m was 0.9×10^{-5} Wbm/kg for the outer-type and 2.5×10^{-5} Wbm/kg for the inner-type, respectively (Fig. 3).

The sustained-release property was demonstrated in both the outer- and inner-type FM-MMC-mc, but the release rate of the former was much higher than that of the latter (Fig. 4). The amount of MMC released from the outer-type FM-MMC-mc was 70% after 4 h incubation in 37°C saline with stirring at 25 rpm, and that from the inner-type product was 19%. Since MMC content was 50% (w/w) for the outer-type and 30% for the inner-type, the release rate may be varied by altering both the structure and the composition of the microcapsules.

FM-MMC-mc in the Dog Aorta

The given magnetic field (2000 kA/m^2, length 2.5 cm) at the aortic bifurcation directed 89.0 ± 1.5% (n = 9) of the outer-type FM-MMC-mc from the abdominal aorta into the left femoral artery, while no difference between the amounts of FM-MMC-mc collected in the bilateral femoral arteries (50.9 ± 4.2% on the left side) was found without the magnet (*6*).

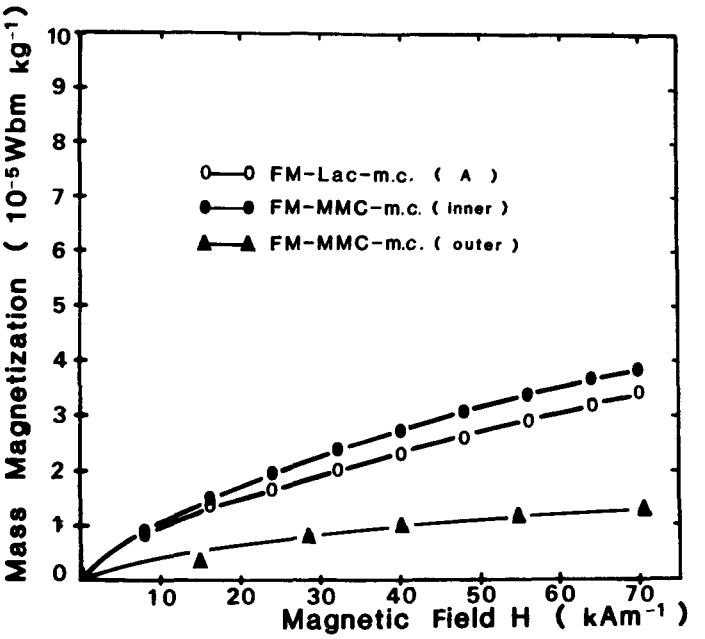

Fig. 3. Magnetic responsiveness of microcapsules: (○) capsules containing lactose; (●) inner-type FM-MMC-mc, and (▲) outer-type FM-MMC-mc [from Kato, T., et al. (1981), *J. Jpn. Soc. Cancer Ther.* **16**, 1351].

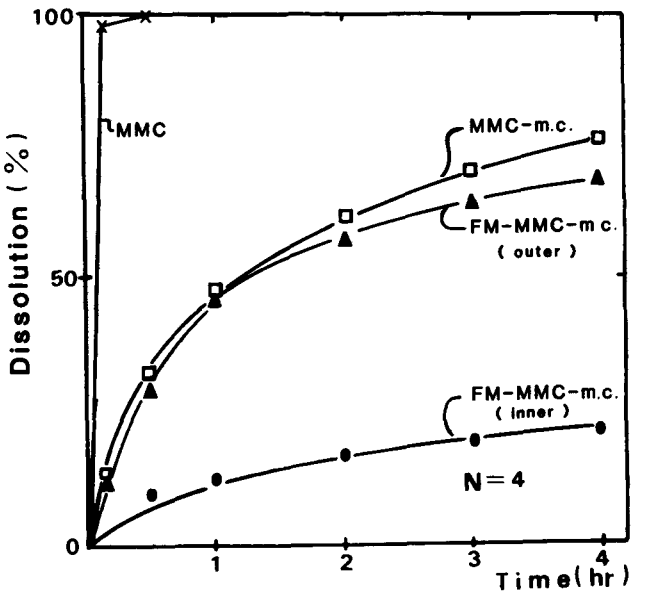

Fig. 4. Release rate of MMC from microcapsules in 37°C, 25 rpm saline: (●) inner-type FM-MMC-mc; (▲) outer-type FM-MMC-mc, and (□) nonferromagnetic MMC capsules [from Kato, T., et al. (1981), *J. Jpn. Soc. Cancer Ther.* **16,** 1351].

The left kidneys, to which the outer-type FM-MMC-mc were magnetically directed, showed extensive wedge-shaped necrotic areas 7 d after the intra-aortic infusion of the drug. In contrast, the right kidneys without magnetic control presented only a few minute necrotic areas (*6*). The necrotic change in the targeted kidney is considered to be produced by both the microembolization with the microcapsules and prolonged cytotoxic action of MMC released from the intravascular FM-MMC-mc.

These results indicate that, though complete guidance failed, the majority of the FM-MMC-mc can be controlled with a conventional magnetic force in the aorta, which generally has an average diameter of 1 cm and a blood flow of 50 cm/s at physiological situation.

Treatment of Tumors in the Rabbit Hind Limb

VX2 tumors transplanted in the rabbit hind limb grew up to 49 cm^2 35 d after the transplantation without treatment. One-shot infusion of nonencapsulated MMC into the femoral artery produced a temporary inhibition of tumor growth for 1 wk, but the tumors showed a regrowth reaching to 21 cm^2 35 d after the transplantation. In contrast, the inner-type FM-MMC-mc with the magnetic force exerted a marked antitumor effect; complete tumor remission was found in 2 of 5 animals and the mean tumor size in the remaining three animals was 3.8 cm^2 (Fig. 5).

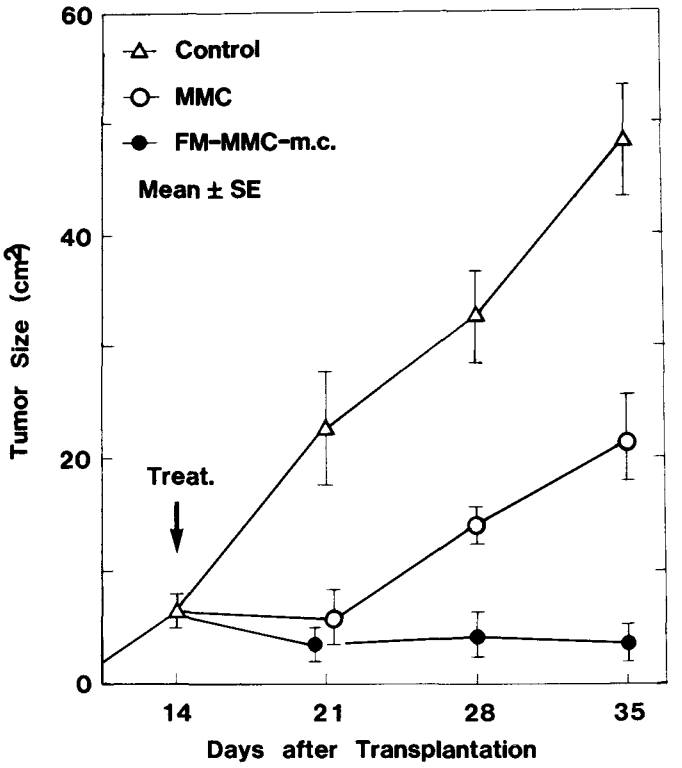

Fig. 5. Growth of VX2 tumors transplanted in rabbit hind limbs. Animals were treated with 4 mg FM-MMC-mc (●) or 4 mg nonencapsulated MMC (○) on day 12. Tumor size is expressed by product of two dimensions [from Kato, T., et al. (1981), *Gan to Kagakuryoho* **8**, 698].

Treatment of Bladder Tumors in the Rabbit

Complete remission of VX2 bladder tumors was found at the site of tumor inoculation where the inner-type FM-MMC-mc were magnetically guided in the bladder. Though there was a necrotic area (mean, 2.6 mm^2) at the concerned place in all of the four bladders, microscopic observation failed to find any intact tumor cells in these necrotic lesions. In the same experimental group, however, tumors were taken by the bladder tissue at the site apart from the magnet. The bladders of the other two experimental groups, treated with MMC combined with placebo ferromagnetic microcapsules or with saline, had paired tumors regardless of the magnetic field (Table 1). It should be noticed that, although intravesical instillation of encapsulated or nonencapsulated MMC exerted a slight degree of inhibitory effect on tumor growth, complete tumor remission was achieved exclusively by the targeting of FM-MMC-mc with an external magnetic force.

To confirm the therapeutic effect of intravesical FM-MMC-mc, drug activity in the bladder tissue was measured. MMC activity of

TABLE 1
Tumor Size[a] of VX2 Tumor in Rabbit Urinary Bladder 14 d After Transplantation and Intravesical Therapy

	Magnet (−)	Magnet (+)
Untreated control	53.6 ± 12.6	40.7 ± 12.4
MMC + placebo FM-mc	34.1 ± 5.1	21.5 ± 2.8
FM-MMC-mc	32.6 ± 7.5	0

[a]Expressed by product of maximum diameter and depth in tissue specimens (mm^2, mean ± SE, $n = 4$). See text.

0.102 ± 0.052 μg/g (mean ± SE, $n = 4$) was detected in the blader tissue under the magnetic field 3 h after intravesical instillation of inner-type FM-MMC-mc, while 3 of the 4 tissues apart from the magnetic field showed no drug activity (limitation of bioassay, 0.003 μg/g) and one had 0.01 μg/g of MMC. All samples, except for one, obtained from the bladders with intravesical nonencapsulated MMC either alone or in combination with placebo ferromagnetic microcapsules failed to show any drug activity regardless of the magnetic field (*9*).

VX2 bladder tumor grown in the bladder wall is a highly invasive tumor insensitive to intravesically instilled anticancer drugs (*14,15*). The results described here, however, demonstrated that the magnetically directed FM-MMC-mc slowly releases MMC that is restrictively absorbed into the bladder tissue through the epithelium, thus providing a marked therapeutic effect on such an invasive bladder tumor.

DISCUSSION

The feasibility of magnetic control of intravascular materials was demonstrated in early 1960s (*16*). This was followed by clinical trials with therapeutic vascular occlusion of intracerebral aneurysms using carbonyl iron suspended in albumin solution (*17*) and of a renal cell carcinoma using carbonyl iron mixed with liquid silicone (*18*). Intravascular carbonyl iron confined in the kidney (*19*) and intraperitoneal zinc ferrite (*9*) were shown to produce no systemic toxic reaction, and no significant toxicity was experienced in the patients exposed to transient magnetic fields (*18–20*). These studies might have stimulated an idea that drugs can be controlled by an extracorporeal magnetic force, but it should be realized that none of the drugs by themselves have magnetic responsiveness strong enough to control their physical behavior. The feasibility of magnetically controlled pharmacotherapy was proved by developing drug-carrier complexes sensitive to conventional magnetic fields; magnetic microcapsules (*21*), magnetic albumin microspheres (*22*), and FM--MMC-mc (*6*).

Independently of our research, Widder and associates prepared the magnetic albumin microspheres with a mean particle size of 1 μm (*22*).

This product contains approximately 9% (w/w) of adriamycin and 20–50% (w/w) of Fe_3O_4, being stored at 4°C in a lyophilized form. It was reported that, when the magnetic albumin microspheres were infused into the caudal artery of the rat tail, approximately 50% of the carriers was retained in the targeted tail segment exposed to a magnetic field of 8000 Oe (640 kA/m) for 30 min (*23*). This mode of treatment was then applied to Yoshida sarcoma grown in the rat tail; most of the treated animals had complete tumor remission in contrast to progressive tumor growth in the control group (*24*).

There are significant differences in the composition, stability, particle size, and magnetic responsiveness between the magnetic albumin microspheres and the FM-MMC-mc prepared by us. First, the drug content in the inner-type FM-MMC-mc can be varied from 30 to 67% and the product can be stored without loss of drug activity at room temperature under dry conditions for more than 1 yr (*9*), while the albumin microspheres can contain much smaller amount of drug and must be stored at 4°C in a lyophilized form. Second, the particle size of the two products is quite different. FM-MMC-mc are designed to be large enough to induce arteriolar embolization, thus enhancing the antitumor effects in combination with infarction and sustained drug action, and minimizing the frequency of treatment (*2,4*). On the other hand, the magnetic albumin microspheres are prepared to be small enough to avoid embolization in the capillary beds so that they can be administered frequently (*23*). Though both ideas are attractive, the merits and demerits of these two approaches could be discussed based on further detailed investigations. Third, magnetic responsiveness of the albumin microspheres seems considerably lower than that of FM-MMC-mc. For example, the magnetic control efficiency of the albumin microspheres at 640 kA/m magnetic field was 50% in the small artery of the rat tail with a flow rate of 0.6 mL/min (*23*), while that of FM-MMC-mc at 2000 kA/m^2 of mean magnetic gradient exerted from an approximately 240 kA/m magnetic force was 89% in the dog aorta with a flow rate of 240 mL/min (*6*). Since the magnetization of ferromagnetic materials depends on their mass size, the magnetic responsiveness of microspheres with a particle size of 1 μm may be limited to the above level. It seems most likely that the magnetic albumin microspheres can be magnetically retained in the capillary beds, but they cannot be directed from nontarget arteries into target arteries. Without the aid of superselective arterial catheterization, the majority of microspheres must escape from the target tumor lesion.

Our studies have shown that anticancer drugs such as mitomycin C can be prepared in the form of magnetic microcapsules, either an outer-type or an inner-type with respect to magnetic particles contained, which has sensitive magnetic responsiveness as well as a sustained-release property. Animal studies have proved that this product can be magnetically controlled in a large artery, producing an enhanced therapeutic effect on the target tumors through the mechanism of chemoembolization. Even with this system, intra-arterial infusion needs the aid of semisel-

ective arterial catheterization. The magnetic control system, however, appears to offer the possibility to perform targeted microcapsule therapy without the difficult arterial catheterization technique, and the possibility to extend the indication of this treatment to tumors that are outside the reach of arterial catheterization. In addition, the magnetic microcapsules can be effectively controlled in the urinary bladder, providing a complete remission of VX2 bladder tumor of the rabbit. This approach may be readily applicable to patients with locally advanced carcinoma in the urinary bladder or gastrointestinal tract.

In conclusion, the possibility and effectiveness of magnetically controlled chemotherapy in the vascular and luminal spaces have been experimentally demonstrated. Further integration of research between medicine and technology will expedite the approach to medical application of magnetism.

SUMMARY

1. Magnetic microcapsules, containing an anticancer drug and zinc ferrite particles, were prepared based on a coacervation method.
2. The product was sensitive to a conventional magnetic force and permits sustained-release of the encased drug.
3. The microcapsules were magnetically controllable in the dog aorta, and the intra-arterial microcapsules exerted an enhanced antitumor effect on VX2 tumors in the rabbit hind limb under an external magnetic field.
4. Intravesical magnetic control of the microcapsules resulted in complete remission of VX2 tumors in the rabbit urinary bladder.
5. Feasibility and effectiveness of the microcapsules with magnetic force were discussed.

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