PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 58, 141–146 (1978)

# Magnetic Microspheres: A Model System for Site Specific Drug Delivery *in Vivo*[1] (40158)

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Delivery of chemotherapeutic agents to desired target sites for the treatment of localized disease with a minimum of systemic side effects constitutes one of the major challenges of chemotherapy. Chemical approaches to targeting of chemotherapeutic agents are based on biochemical differences that exist between normal and diseased cells (1–5). In most cases, such differences are more quantitative than qualitative, such that drugs administered frequently exert undesirable toxic effects. Localization of chemotherapeutic agents to specific sites would reduce the systemic dose of a given agent while still achieving effective local concentrations of drug.

An alternative to chemical targeting of drugs is inclusion of chemotherapeutic agents within carriers. Recent examples include inclusion of mercaptopurine and daunomycin into albumin microspheres (6), and other anticancer agents into liposomes (7, 8). These are internalized by tumor cells *in vitro* and *in vivo* following ip injection. However, controlled localization of drug carriers has been difficult to achieve. Despite efforts to impart specificity by modifying surface charge (9, 10) and varying vesicle size (10, 11), intravascular administration of such carriers results in their uptake predominantly by the reticuloendothelial system (11–14).

Magnetic control of intravascular particles has proven feasible in both diagnostic and therapeutic applications with no discernible toxicity (15). Carbonyl iron particles, 1–3 $\mu$m in diameter, administered intravenously as a contrast medium were held *in situ* with an externally placed electromagnet in the dog (15). Alksne, using carbonyl iron, was able to produce magnetically localized thrombi in intracranial aneurysms in both animal and human subjects (16). Externally guided ferromagnetic silicone has been used to accomplish selective vascular occlusion and necrosis of hypernephromas in man (17).

This communication describes the development of a magnetically responsive biodegradable drug carrier with the capacity to localize both carrier and chemotherapeutic agent by magnetic means to a specified *in vivo* target site. The carrier consists of albumin microspheres 0.2–2 micrometers in diameter containing both $Fe_3O_4$ particles (10–20 nm in diameter) and a chemotherapeutic agent entrapped in the albumin matrix. Albumin microspheres have been shown to be biodegradable, a property which can be altered by preparing them at different temperatures (18, 19). Altering the nature of the matrix by heat denaturation also allows control of the rate of diffusion of drug from the carrier.

*Materials and methods. Microsphere preparation.* One hundred and twenty five milligrams of human serum albumin labeled with 5 $\mu$l of $^{125}$I-bovine serum albumin (1.51 mCi/mg New England Nuclear), 10 mg of bulk purified adriamycin HCl (Adria Laboratories) and 36 mg $Fe_3O_4$ particles were added to 0.5 ml distilled water. To this, 30 ml of cottonseed oil (Sargent Welch) was added and the mixture stirred to disperse the aqueous phase in oil. The resulting emulsion was homogenized by sonication for one minute at 100 W at 4° using a Branson Model 185 Sonifier. The homogenate was added dropwise over a 10-minute period into 100 ml of continuously stirred oil at 25°. The suspension was washed four times in anhydrous diethyl ether by centrifugation (Sorvall, model RC2-B) for 15 min at 2000*g*. Heat hardening of microspheres was accomplished by exposure to temperatures of 120° and 135–140° for 10 min. The stabilized microspheres were again washed four times with

[1] This work was supported by funds from The Department of Pathology and The Cancer Center, Northwestern University Medical School.



0037-9727/78/1582-0141$01.00/0

anhydrous ether and the resulting microspheres lyophilized and stored at 4°. The amount of free label associated with the carrier was assessed by sonicating an aliquot of the microspheres suspended in normal saline containing 0.1% Tween 80 in an ultrasonic water bath (Mettler Electronics Corp.) for 2 min. The resultant suspension was centrifuged for 10 min at 2000*g*. The supernatant was decanted and a volume of 24% trichloroacetic acid equal to twice the volume of the supernatant was added. After two hours at 4°, the TCA-treated supernatant was centrifuged for 10 min at 2000*g*. The resultant supernatant was assessed for $^{125}I$ activity in a gamma counter (Packard, Model 568). The percent free label was determined by the counts obtained from this fraction compared to the total counts from the starting carrier.

*Toxicity of carrier.* Toxicity of the microspheres containing $Fe_3O_4$, was measured by animal survival and histopathological studies in female $BDF_1$ mice (15–20 g). The carrier and appropriate controls were injected iv using the tail vein. Both an acute (7 days) and a chronic (90 days) evaluation of toxic effects were performed.

*Magnetic responsiveness of microspheres.* The carrier was characterized *in vitro* for its magnetic responsiveness using a constant flow apparatus (20). Percent retention was determined by counting the $^{125}I$ labeled carrier retained by the magnetic field and dividing by the total starting counts. In all preparations no more than 1% of the counts obtained represented free label as determined by the method described previously.

*Quantitation of adriamycin in microspheres.* The concentration of adriamycin in microspheres was determined by measuring the amount of drug incorporated during preparation of microspheres as described previously. Adriamycin was extracted from microspheres by incubation in a 5% solution of HCl in ethanol for 24 hr at 4° followed by centrifugation for 10 min at 2000*g*. The amount of adriamycin in the supernatant was determined by specific fluorescence using an Aminco SPF-125S spectrophotofluorometer (excitation = 470 nm, emission = 585 nm).

*In Vivo model for carrier targeting.* The model utilized for *in vivo* testing of the microspheres was the rat tail. The tail was demarcated into four equal segments each measuring approximately 3.5 cm in length and the third segment from the base was the site at which a permanent bipolar magnet (Edmund Scientific) was placed. The experimental animals were ~400 g female retired breeder Sprague–Dawley rats. The ventral caudal artery was partially exposed at the base of the tail and a polyethylene catheter (Clay-Adams, PE 10) presoaked in a solution of 0.6% heparin 1000 in saline was inserted caudally for a distance of 5 cm. The magnet with a field strength of 8000 Oe was then placed 6.5 cm distal to the point of insertion of the catheter. Microspheres (0.5 mg) suspended in 0.1% Tween 80 in normal saline (1 mg/ml), were infused by a constant flow syringe pump (Sage, Model 341) at a rate of 0.6 ml/min which corresponds to the rate of blood flow in this artery. Following infusion, the catheter was removed and the magnet was retained in position for 30 min. A transcutaneous Doppler apparatus (Parks Electronic Lab., Model 881-A) was used to document resumption of blood flow following removal of the catheter. After 30 min, the rat was sacrificed by intracardiac injection of saturated KCl, the organs immediately removed and counted for $^{125}I$ in a $\gamma$ counter. The tail was cut into the designated segments and each was counted individually. Distribution of $^{125}I$ labeled carrier in the various tail segments was examined by stripping the skin from the vertebrae and counting each separately. In separate experiments, animals were sacrificed 24 hr after the tail was removed from the magnetic flux.

*Drug localization.* To determine the relationship between drug localization and carrier distribution, the concentration of adriamycin was determined in the various tail segments. Tail segment 3 was the target site selected for carrier localization. Tissue extraction of adriamycin was accomplished by a modification of the method of Bachur (21) in which skin collagen was solubilized by soaking the skin overnight in 0.5 *N* acetic acid at 4°. The concentration of adriamycin was determined as stated previously. Control experiments showed that treatment of adriamycin with 0.5 *N* acetic acid did not change its fluorescence characteristics at the wave lengths employed. The level of adriamycin detectable by this method is 0.25 $\mu$g/ml.

Adriamycin encapsulated in microspheres was infused at a dosage of 0.05 mg/kg body weight with and without the application of a magnetic field of 8000 Oe to tail segment 3 for 30 min. In separate experiments free adriamycin was administered via catheter into the ventral caudal artery (0.05 mg/kg) and femoral vein (5 mg/kg).

*Results and discussion.* Microspheres (Fig. 1A) produced by our method have a relatively homogeneous size distribution (1 $\mu$m average diameter) which allows the carrier to penetrate to the capillary level. The distribution of the entrapped magnetically responsive $Fe_3O_4$ (10–20 nm in diameter) is presented in Fig. 1B.

Toxicity studies performed in female $BDF_1$ mice revealed negligible adverse effects both in acute studies, 0/40 mice dead, and chronic studied 0/20 mice dead, even at the highest dose tested (400 mg microspheres per kg body weight). No significant changes were noted on histopathological study of representative organs.

Area specific drug release depends on the ability to arrest carrier flow at the level of arterioles, capillaries, and venules in order to allow maximum localized drug saturation. Based on the known flow rates in the arteriovenous branches of the circulatory system, an appropriate magnetic field strength was selected which afforded maximum retention of carrier in capillaries while not affecting its flow at the arterial level. *In vitro* studies in our laboratory have demonstrated that a field strength of 8000 Oe produced from a bipolar permanent magnet was sufficient for 99% retention of the carrier at a linear flow rate normally found in capillaries (0.5 cm/sec) with less than one percent retention at the higher flow rates (10 cm/sec) present in small arteries (20).

Quantitative analysis of acid alcohol extracts from adriamycin-containing microspheres showed that essentially all (more than 99%) of the drug was incorporated. Adriamycin was present in the microspheres at a concentration of 58 $\mu$g/mg albumin–$Fe_3O_4$ complex. Upon suspension in normal saline 23.2 $\mu$g was released rapidly presumably because it was loosely bound to the sphere surface while 34.8 $\mu$g was released slowly because it was entrapped in the microspheres. The amount of adriamycin released after heat hardening was quantitated following incubation of microspheres in saline at 37° for four hours. The results shown in Table I indicate that heat denaturation decreased the release of adriamycin.

Results of *in vivo* targeting of the carrier are shown in Table II. These included experiments in which the magnetic field strength was varied; approximately 50% of the carrier injected was retained at the target site (tail section 3) at a field strength of 8000 Oe. Failure to achieve greater retention of carrier is most probably due to physiological shunting of blood via perforating arterial branches communicating the superficial and deep branches of the caudal artery (22), and as predicted the retention of microspheres decreased significantly at lower field strengths. Approximately 60% of the counts were routinely localized in the skin of tail segment 3, whereas 40% were present in bone, muscle and tendons of segment 3. All other segments had low background counts.

When animals were sacrificed 24 hr after the tail was removed from the magnetic flux, approximately 50% of the injected microspheres were localized in tail segment 3. Protracted retention of carrier in the absence of a magnetic field as well as carrier distribution in the skin suggested to us that the carrier might be lodged in the vascular endothelium or had possibly traversed the vascular basement membrane into interstitial tissue. This would be desirable since the microspheres would then serve as extravascular depots releasing the drug at the desired site. Alternatively, partial thrombosis due to conglutination of the microspheres could also be responsible for the continued retention of carrier. Electron microscopic study of tail skin

TABLE I. RELEASE OF ADRIAMYCIN FROM MICROSPHERES HEAT HARDENED AT VARIOUS TEMPERATURES.

| Hardening temperature°C | Adriamycin released[a] ($\mu$g/mg carrier) |
|---|---|
| 25 | 33 |
| 120 | 18 |
| 135 | 11 |
| 140 | 10 |

[a] Adriamycin released following incubation in normal saline for 4 hr at 37°.

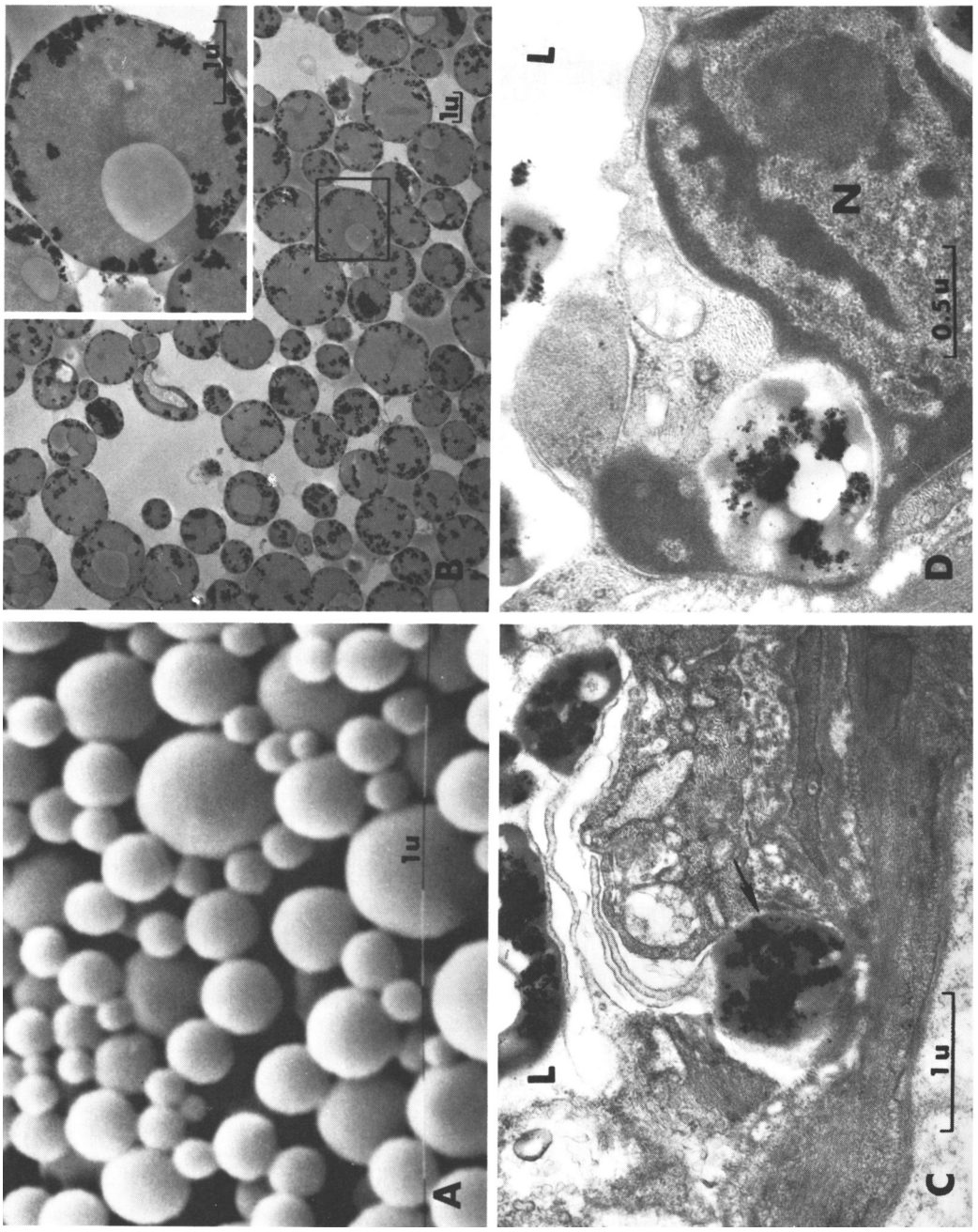


FIG. 1A. Scanning electron micrograph of an evaporated ether suspension of albumin microspheres showing variation in particle size. 1B. Transmission electron micrograph of microspheres. The inset shows electron dense $Fe_3O_4$ particles in the sphere located at the periphery, and a central cavity. 1C. An arteriole in the tail of a rat perfused with microspheres, aggregated microspheres are present in the lumen L. Arrow points to a microsphere which is lodged between two endothelial cells. 1D. A microsphere has been internalized into the cytoplasm of an endothelial cell indenting the nucleus N.

TABLE II. IN VIVO LOCALIZATION OF $^{125}$-I-LABELED ALBUMIN MICROSPHERES CONTAINING $Fe_3O_4$.

| Magnetic field strength in oersteds (Oe) | Number of animals | Ranges of percent distribution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Tail segment number | | | | organ | | | | |
| | | 1 | 2 | 3 | 4 | Liver | Spleen | Kidney | Lung | Heart |
| 0 (Control) | 10 | 0 | 0 | 0 | 0 | 76–85 | 3–9 | <1 | 6–19 | <1 |
| 4000 | 5 | 0 | 0 | 0–3 | 0 | 72–85 | 3–7 | <1 | 5–18 | <1 |
| 6000 | 5 | 0 | 0–4 | 10–25 | 0 | 57–70 | 2–8 | <1 | 6–18 | <1 |
| 8000 | 10 | 0 | 0–3 | 37–65 | 0–2 | 30–48 | 2–6 | <1 | 5–17 | <1 |

perfused with microspheres in a magnetic field of 30-min duration showed that microspheres were internalized by endothelial cells (Fig. 1C) and trapped between the plasma membranes of two adjacent endothelial cells (Fig. 1D).

Drug distribution studies showed that no detectable adriamycin was present in the skin of any tail segment in the absence of a magnetic field as contrasted to localization of 3.9 μg of adriamycin per g skin (wet weight) limited strictly to tail segment 3 in the presence of a magnetic field. Infusion of free adriamycin (0.05 mg/kg) into the caudal artery resulted in no detectable adriamycin in any tail segment. Intravenous injection of free adriamycin at a dose of 5.0 mg/kg showed a uniform concentration of 3.3 μg per g skin (wet weight) in all tail segments. Thus we have achieved a therapeutic drug level at the target site with the infusion of carrier bound adriamycin at a dose 100-fold less than that achieved when the drug is administered in the free form. Despite the fact that 30–48% of the carrier was localized to liver due to shunting from the target site, it should be pointed out that this amount of carrier represents local delivery of less than 1 μg of adriamycin per g of liver (wet weight). The foregoing is in contrast to 15 μg of adriamycin per gram of liver (wet weight) delivered when free adriamycin (5 mg/kg) is administered iv.

These experiments suggest that magnetically guided albumin microspheres are capable of delivering significant concentrations of a drug to a desired site.

*Summary.* A novel carrier system for the delivery of chemotherapeutic agents by magnetic means to desired sites has been developed. Results indicate that the carrier, albumin microspheres with entrapped $Fe_3O_4$, and adriamycin HCl, can be concentrated at a predetermined site *in vivo* by a magnetic field. Carrier delivery of adriamycin is supported by the presence of a significant concentration of the drug at the site of carrier localization. This delivery system allows for the accumulation of local adriamycin which is comparable to that achieved by administration of a 100-fold higher dose of the free drug.

We thank Drs. Nathaniel Berlin, Samuel Taylor, IV, Oscar Hechter, Steven D. Reich, David Ranney, Paul Hollenberg and Mr. Tom Minick for their invaluable support and advice.

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Received September 19, 1977. P.S.E.B.M. 1978, Vol. 158.