Pergamon

0969-8051(94)00124-3

Nucl. Med. Biol. Vol. 22, No. 2, pp. 147–155, 1995

0969-8051/95 $9.50 + 0.00

Effective Targeting of Magnetic Radioactive ^{90}Y-microspheres to Tumor Cells by an Externally Applied Magnetic Field. Preliminary *In Vitro* and *In Vivo* Results

URS O. HÄFELI,[1]* SIOBHAN M. SWEENEY,[2] BEVERLY A. BERESFORD,[2] JOHN L. HUMM[3] and ROGER M. MACKLIS[1]

[1]Cleveland Clinic Foundation, Radiation Oncology Department, Desk T-18, 9500 Euclid Avenue, Cleveland, OH 44195, U.S.A., [2]Joint Center for Radiation Therapy and Department of Radiation Oncology, Harvard Medical School and Dana Farber Cancer Institute, 50 Binney Street, Boston, MA 02115, U.S.A. and [3]Department of Medical Physics, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, U.S.A.

(Accepted 17 October 1994)

Magnetic biodegradable poly(lactic acid) microspheres that incorporate both magnetite and the β-emitter ^{90}Y were prepared. By applying a directional external magnetic field gradient in excess of 0.02 Tesla/cm across a 96-well plate containing neuroblastoma cells incubated with the ^{90}Y magnetite loaded microspheres, the radiation dose to the cells could be enhanced or reduced relative to the dose from a uniform loading of the well with ^{90}Y–DTPA. Using the MTT assay, cell survival was measured for the magnetic field directed from above (cell sparing) and from below (cell targeting) the well plate, resulting in $65 \pm 8\%$ or $18 \pm 5\%$ survival respectively. This method was then applied to an *in vivo* murine tumor model. The biodistribution of intraperitoneally injected magnetic radioactive microspheres, after 24 h in mice, showed that $73 \pm 32\%$ of the radioactivity was found on the subcutaneous tumor that had a rare earth magnet fixed above it. In contrast, the tumor radioactivity with no attached magnet was $6 \pm 4\%$. Magnetically targeted radiopolymers such as ^{90}Y-microspheres show great promise for regional or intracavitary radiotherapy.

Introduction

Targeted approaches to radiotherapy using long-range β-emitting isotopes linked to biologically selective molecules such as antibodies have shown limited success, primarily due to the relatively small tumor uptake at levels of acute bone marrow toxicity (Leichner *et al.*, 1988; Rosenblum *et al.*, 1991). There is currently a recognized need to improve the specific concentration of the radiotoxic drug in the tumor and to control the localization of the drug more accurately. Microspheres, appropriately sized for the target organ, satisfy both these requirements. Each sphere is able to incorporate up to several million radioactive atoms, and when made magnetic through preparation with magnetite, can be maneuvered within the body to cancerous lesions by externally applied magnets or by the magnetic field generated by an MRI machine.

Magnetic microspheres are not new. Currently their most important application in medicine is their *in vitro* use for the depletion of cancer cells from bone marrow (Kemshead *et al.*, 1986). For this purpose, cancer specific antibodies are chemically bound to the surface of magnetic microspheres. After incubating the microspheres with a patient's bone marrow, the cancer cells are selectively retained by the microspheres and can then be pulled off from the healthy bone marrow with a magnet.

Magnetic microspheres have not yet been used in humans. This is primarily due to the fact that magnetic microspheres, like all microparticulate carriers, do not easily cross membranes thus limiting their systemic application to systemic diseases (Rusetskii *et al.*, 1985; Gallo and Hassan, 1988). This fact, however, makes them attractive candidates for the delivery of high doses of anticancer agents in intracavitary therapy. A review of such magnetic delivery systems made from materials like albumin (Widder *et al.*, 1979), chitosan (Kharkevich *et al.*, 1989) or silicone (Turner *et al.*, 1975) has recently been published (Gupta and Hung, 1989).

Each of the previously described magnetic microspheres contained chemotherapeutic agents which

*Author for correspondence.

had to be released immediately adjacent to the tumor in order to be effective. In contrast, radiolabeled microspheres can deposit dose and produce biological damage over a distance which is determined by the range of the radionuclide emissions. The radiopharmaceutical used, therefore, only needs to be brought near the tumor and immobilized there. Because of this pharmacologic difference, it is preferable to use microspheres which avidly retain the radiopharmaceutical for two or more half-lives, rather than releasing it into the surrounding tissue.

First therapeutic attempts with such systems were done in 1961 (Blanchard *et al.*, 1965). Resin microspheres loaded with ^{90}Y were intraveneously or intratumorally injected into patients who had astrocytomas or liver, prostate, lung or tongue cancers. These attempts were not very successful due in part to the severity of the patients' condition (the patients were selected from a cohort for whom conventional therapy was either not possible or offered no hope of cure or palliation). Additionally, the applied microspheres were not well defined in size, and thus caused complications in 30% of the patients. More successful was the treatment of hepatomas with ^{90}Y-microspheres made out of glass (Mantravadi *et al.*, 1982; Houle *et al.*, 1989). These very stable microspheres are ideal for use in embolization therapy of the liver, because they get stuck in the capillaries and concomitantly irradiate the tumor and occlude the nutrient supply.

A major drawback of glass microspheres is that they cannot always be used in cavities such as the knee joints or intraspinal space, because they sometimes cause anatomical distress. To overcome this problem we have developed biodegradable magnetic microspheres made from poly(lactic acid) (Häfeli *et al.*, 1994). They release less than 5% of the bound radioactivity within 3 weeks, and shortly thereafter begin to physically decay. This makes them an ideal carrier for the β-emitter ^{90}Y, since the microspheres remain intact for a duration much greater than the physical half-life (64.1 h) of the radioisotope.

The purpose of this paper is to demonstrate that these magnetic radioactive microspheres can be used to target tumors in a model system. Their cytotoxicity is analyzed through an *in vitro* cell system in which human tumor cells are either killed or spared depending on how efficiently ^{90}Y-microspheres are targeted by a magnetic field towards or away from the cells. Information about the *in vivo* stability and targetability of this radiopharmaceutical is obtained from the biodistribution of radioactive microspheres in mice with and without magnetic focussing.

Materials and Methods

Preparation of radioactive magnetic microspheres

Magnetic microspheres (=MMS) were prepared by a solvent evaporation method as described earlier (Häfeli *et al.*, 1994). Briefly, 100 mg of poly(lactic acid) (=PLA) (Polysciences) with a mol. wt of 2000 Da was dissolved in methylene chloride and 11 mg of magnetite Fe_3O_4 (Black Iron Oxide, Polysciences) was added and sonicated. The suspension was then injected into a stirred solution of 1.5% polyvinyl alcohol and the MMS filtered through a 40 μm cell strainer (Falcon), washed, and dried. After reconstitution in PBS at pH 5.7, the MMS were directly labeled with ^{90}Y in 0.01 M HCl (NEN). Free radioactivity was washed off by 3 subsequent centrifugations at 12,000 ***g*** for 2 min each. The highest radioactive concentration used was 74 MBq per 20 mg MMS and the labeling efficiencies reached 98%.

Cell culture and survival tests

Two different cell types were used in these experiments. Human neuroblastoma cells were used for *in vitro* studies because of their documented radiosensitivity to low dose rate radiation and their single-plane growth characteristics on plastic. For *in vivo* experiments, EL-4 murine lymphoma cells were selected because of their radiosensitivity (similar to the neuroblastoma cells) and their ease of locally confined subcutaneous growth in the thin abdominal fascial planes of mice. Other subcutaneous tumor models spread within fascial planes, making careful post-mortem analyses of locally adherent radioactivity levels difficult.

Human neuroblastoma SK-N-MC cells, obtained from ATCC, were grown in a controlled atmosphere of 5% CO_2 in 20 mL flasks as monolayers in RPMI supplemented with 10% fetal bovine serum. These cells were selected in part because their radiosensitivity parallels that seen in some potential clinical targets which might be amenable to this therapeutic strategy. The cells were used for the experiments after trypsinization and 2 subsequent washes by centrifugation. Then, 3×10^4 cells each were plated into the wells of a 96-well plate in groups of 8 and allowed to adhere for 24 h prior to the addition of up to 0.4 mg of microspheres labeled with up to 263.0 kBq per well. The microspheres were either attracted toward the cells plated on the bottom of the well, by placing a magnet (Radio Shack) below the dish, or away from the cells, by fixing the magnet above the dish. For this work, iron magnets of $5 \times 2 \times 1$ cm were purchased from Radio Shack, with a field strength of 0.09–0.11 Tesla at the magnet surface. In what follows, the magnet which attracts the magnetite laden microspheres to the bottom of the dish and next to cells shall be referred to as the "killing magnet". The magnet placed to attract the microspheres away from the cells and the bottom of the well is referred to as the "sparing magnet" (Fig. 1). A third experiment in which water soluble ^{90}Y-DTPA was mixed uniformly into a series of wells was performed, in order to serve as a baseline control for which the activity and distribution were *known*. It was prepared by adding

10 μL of 0.01 M DTPA (Sigma) to 50 μL of dH_2O followed by the addition of the ^{90}Y in 0.01 M HCl up to a specific activity of 1963 kBq/g. After 24 h of incubation the radiotoxic agents were removed by 3 subsequent washes. After another 24 h of incubation in 100 μL media, the survival of tumor cells was measured by an MTT assay (Furukawa *et al.*, 1991; Weichert *et al.*, 1991): 10 μL of MTT (5 mg/mL) was added to each well and the plates were incubated for an additional 4 h. The blue formazan crystals which formed inside the live cells were dissolved by the addition of 150 μL of DMSO and the intensity was measured at 540 nm in a microplate reader.

Dose calculations for the in vitro study

A calibration curve to determine the response of the beta counter was performed from serial dilutions of up to 788.9 kBq of the stock ^{90}Y-DTPA solution, using sample aliquots of the same 300 μL vol. A second calibration curve was measured for a fixed activity of ^{90}Y diluted to different volumes, to establish the dependence of count rate with sample size.

From a uniform distribution of the activity within the well, the dose was calculated using the MIRD (medical internal radiation dose committee) protocol (Loevinger *et al.*, 1991). The difficulty in the accurate determination of dose from an internally distributed beta emitter, is in the evaluation of the absorbed fractions ϕ_i. Under conditions of charged particle equilibrium, i.e. when the distribution of emitters is uniform and fully surrounding the target volume up to a distance of the maximum range of the most energetic beta particle, then the absorbed fraction is equal to 1.0. For the case of the target cells lying directly at the boundary of a hot–cold interface, where the cells are irradiated by a 2π geometry (a hemisphere), the absorbed fraction for all energies is equal to 0.5. This is a good approximation for the dose to the cells attached to the bottom of a cell culture well from a well mixed solution of ^{90}Y-DTPA. To satisfy the conditions of charged particle equilibrium for the source distribution above the cells, the medium would need to be at least 1 cm deep, which is the range of the most energetic beta particle of 2.25 MeV. The depth of the medium in the wells, in this work, was 8 mm. Although the cells were not exposed to a full build-up of beta particles, the contribution from the missing 2 mm top layer to the cell layer is small (<5%), and therefore does not seriously depart from the conditions of charged particle equilibrium. The cells at the center of the well approximate 2π geometry and therefore the dose to these cells will be given by the equilibrium dose constant, i.e. ϕ_i's = 1, multiplied by the cumulative activity divided by two. Since the equilibrium dose constant for ^{90}Y is 1.984 rad/h per μCi/g, or 53.6 cGy/MBq · h in SI units, the dose rate to cells at the center of the well is approx 26.8 cGy/MBq · h.

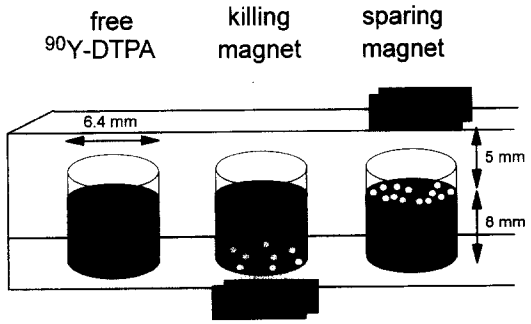

Fig. 1. Schematic diagram of SK-N-MC monolayer neuroblastoma cells (dark ovals) in a 96-well plate in which each well contained 300 μL of media. The first well indicates the ^{90}Y evenly distributed as the DTPA-complex throughout the well. The next two wells show the movement of the microspheres (white spheres) produced by a sparing and killing magnet, respectively (dark rectangles). Not drawn to scale.

To evaluate the dose to the cell layer from the magnetically guided microsphere experiments, two sets of extreme conditions were simulated in the dosimetry calculations. For the "killing magnet", it was assumed that all of the radioactive laden microspheres were instantly attracted to the bottom of the cell culture well. Thus the source layer was modeled as an infinite layer of 20 μm thickness, directly adjacent to the target cell layer. For the "sparing magnet" it was assumed that all of the microspheres were instantly attracted to the top layer of the culture medium, i.e. formed a 20 μm thick infinite layer remaining at 8 mm distance from the cell layer. The absorbed doses were calculated to the layer of cells by a Monte Carlo simulation of the geometry of the source and target layers, where a composite Berger point kernel for the ^{90}Y spectrum (Berger, 1971, 1973) was used to describe the energy absorption as a function of distance.

Biodistribution of radioactivity in mice

The 1×10^7 EL-4 murine lymphoma cells in 100 μL of RPMI were injected subcutaneously in the abdominal midline of female C57BL6/N mice (Taconic) that were 7–10 weeks old. Care was taken not to penetrate the abdominal cavity. Round and very localized tumors of ~0.5 g grew within 14 days. The animals were then separated into two groups:

(a) *Magnet bearing group.* Two groups of 10 animals each were shaved around their abdomen under the short term anesthesia of Metaphane®. A round, 2 mm thick rare earth magnet with a dia of 10 mm (Magnet Sales & Mfg, Culver City, CA, U.S.A.) was then taped directly above the tumor. The magnetic field on the surface of the magnet was 0.12–0.16 Tesla. The positive pole of the magnet faced away from the animal and the tape was cut such that the animal's movement was not hindered. The mice were intraperitoneally (i.p.) injected through a 20 $G\frac{1}{2}$ needle with 2 mg of MMS labeled with 1110 kBq of ^{90}Y.

(b) *No magnet control group.* Two groups of 10 animals each were i.p. injected with 2 mg of MMS labeled with 1110 kBq of ^{90}Y.

Groups of mice with and without magnets were sacrificed after 2 and 24 h, respectively, and the radioactivity of the whole blood, heart, lung, liver, spleen, kidney, femur, small intestine and tumor was measured. The area immediately below the tumor, the tumor bed, was obtained by dabbing the area with a cotton swab and measuring the activity on the swab. Finally, the radioactivity of the carcass was measured. Before analyzing the data, the following assumptions were made: (1) the amount of blood is 9% of the total weight of the mouse, (2) both kidneys are of equal activity and (3) the total murine bone weight is 2.0 g. A calibrated β-scintillation counter (Bioscan) and a dose calibrator (Radcal) were used to determine the radioactivity levels.

Results

Cell toxicity of radioactive ^{90}Y–MMS

The survival of the SK-N-MC cells after exposure to radioactively labeled MMS is shown in Fig. 2. The survival curve is exponential for the tested dose range of the ^{90}Y–MMS which were added to the well and then pulled with the killing magnet into immediate contact with the cancer cells (see Fig. 1). The dose necessary to reach the detection limit of the MTT-assay (between 15 and 20% of cell survival) was reached at 150 kBq of ^{90}Y–MMS per well. The survival of SK-N-MC cells could be increased by adding the same amounts of ^{90}Y–MMS to the wells but placing a magnet in sparing position. Visual inspection revealed that virtually all the magnetic reactive microspheres moved to the top of the media (8 mm away from the cell layer on the bottom of the well) thus decreasing the cytotoxic impact on them.

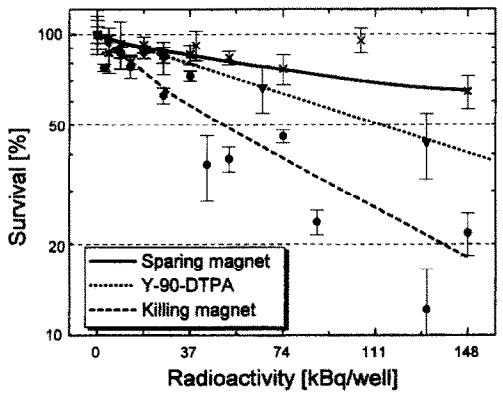

Fig. 2. Survival of SK-N-MC cells after incubation with different forms of ^{90}Y for 24 h, as measured by the MTT assay. The data are expressed as a percentage of non-irradiated control cell survival (= 100%). The error bars represent $\pm$1 SD. *Solid line*, different amounts of ^{90}Y–MMS were incubated with a magnet attached to the top of the well ("sparing magnet"). The results were averaged from 6 different experiments. *Dotted line*, freely distributed ^{90}Y–DTPA was added (average of 3 different experiments). *Dashed line*, ^{90}Y–MMS were incubated with a magnet attached to the bottom of the well ("killing magnet"). The results of 4 different experiments were averaged.

The survival curve of freely distributed ^{90}Y-DTPA is exponential below 259 kBq per well. Above that point it becomes linear, reaching the detection limit of the MTT assay around 600 kBq. Since the dose estimate to the cells from the uniform distribution of ^{90}Y can be assumed to be considerably more accurate than under either magnet geometries, the relative effectiveness, derived from the slope of the respective survival curves, can be used as an indirect indicator of the dose, relative to the ^{90}Y–DTPA control, received by the cells for each magnet configuration.

Under the conditions of the ^{90}Y–DTPA experiment, the dose rate was calculated to vary between 0.2 and 0.4 Gy/h for cells at the edge and center of the well, respectively, giving rise to an average D_0 slope for the survival curve of approx 4 Gy. Note that the survival slopes of all 3 curves exhibit, instead of a shoulder, a slight upward curvature. This is consistent with the measurement of a single average survival level from a non-uniform exposure of the cells (Humm *et al.*, 1990), such as is the case in this irradiation geometry.

The theoretical predictions for the relative dose per unit activity in the middle of the well for the killing and sparing magnet geometries relative to the ^{90}Y–DTPA are 11.36 and 0.03, respectively. These figures suggest, for the two magnet studies, a significantly greater departure from the control ^{90}Y–DTPA experiment, than is experimentally observed (see Fig. 2). Using the experimental data, one can obtain an indirect estimate of the cellular dose by analyzing the slopes of the respective survival curves.

Ignoring differences in dose rate between the two magnet studies and the ^{90}Y–DTPA experiment, the ratio of the slopes for the killing magnet relative to the ^{90}Y–DTPA is approx 2.3 at the D_{50} survival level. For the sparing magnet, a reduction in survival to the 50% level was never attained at the activities used, and it is anticipated that the killing effect per unit well activity in the sparing magnet geometry may be less than one tenth of the ^{90}Y–DTPA control radiotoxicity, due to the concave shape of the survival response. After the 70% survival level, the relative effectiveness of the killing and sparing magnet were approx 2.7 and 0.6 respectively. This is an overall dosimetric improvement due to magnetic manipulation of the activity distribution of 4.5. Thus if the dose differential between malignant and normal tissue can be improved by this amount as a result of magnetic intervention, the current clinical therapeutic ratios for targeted therapy of between 10:1 and 15:1, i.e. 20–30 Gy to the tumor for a bone marrow toxic dose of 2 Gy, might render the administration of traditional therapeutic doses >60 Gy possible.

Biodistribution of ^{90}Y–MMS in mice

Intraperitoneally injected radioactive microspheres were initially found near the injection site (small intestine, liver, tumor) and then slowly dispersed within 24 h throughout the i.p. area as shown in

Fig. 3(a). Despite the earth's gravity, the MMS did not seem to concentrate in the midline of the mice, possibly due to the normal movement of the mice. The biodistribution of the same amount of i.p. injected ^{90}Y–MMS looked completely different after a small, but powerful magnet was placed on top of a subcutaneously grown tumor in the abdominal midline. Figure 3(b) shows that essentially no radioactivity was found in the blood, lung or heart. The small intestine contained 31 kBq/g and the spleen 29 kBq/g after 2 h. The majority of the radioactivity (1226 kBq/g) was found on and immediately adjacent to the tumor.

The biodistribution results were also plotted as pie-graphs to better trace the ultimate fate of the radioactivity. Figure 4(A) shows clearly that after i.p. injection and without the influence of a magnetic force, the ^{90}Y–MMS disperse. A significant amount (7%) of the injected ^{90}Y was microscopically found to be loosely bound to the external surface of the spleen. Figure 4(B) shows the targeting influence of the attached magnets. After 2 h, 61 ± 38% of the ^{90}Y–MMS were associated with the tumor and during the next 22 h the MMS concentrated even more at the tumor site increasing to 73 ± 32%.

Discussion

The data have demonstrated that magnetic poly(lactic acid) microspheres can be targeted *in vitro* and *in vivo* by an external magnetic field. In an *in vitro* system, consisting of a 96-well plate with SK-N-MC neuroblastoma cells growing on the bottom, the radioactively labeled ^{90}Y–MMS were 4.5 times more cytotoxic with a magnet in sparing position than in killing position, as determined from Fig. 2 at the 70% survival level. When added to the wells in the form of the water soluble ^{90}Y–DTPA complex, the same Bequerel amounts were, as expected, less toxic than ^{90}Y–MMS with a killing magnet in position but more toxic than ^{90}Y–MMS with a sparing magnet.

To be better able to compare the different survival curves, we have printed the measured amounts of radioactivity and calculated doses for 20, 50 and 90%

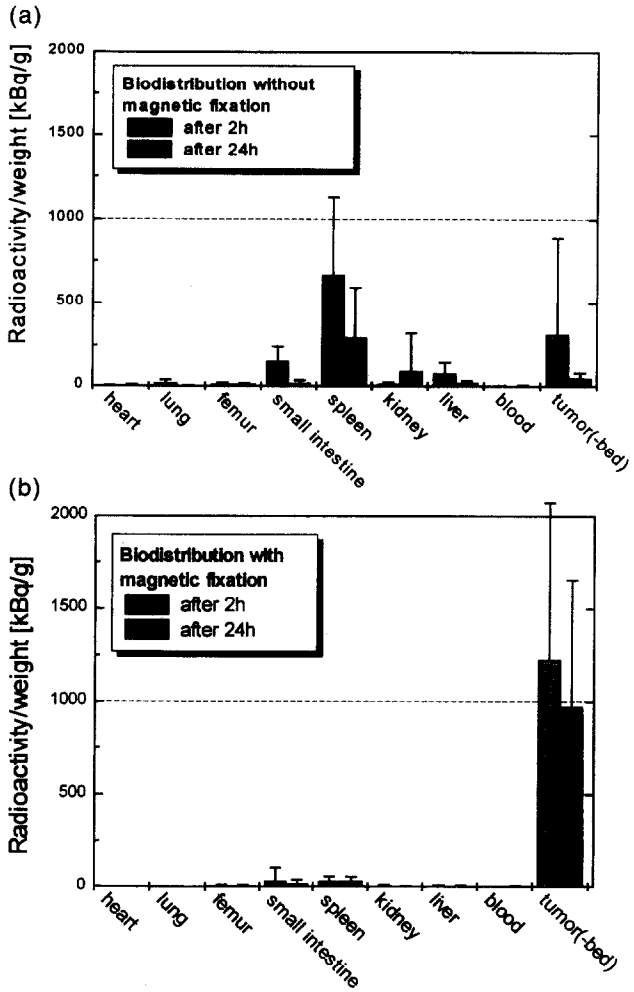

Fig. 3. Biodistribution of ^{90}Y–MMS, after 2 and 24 h, in mice with a subcutaneous EL-4 tumor in the abdominal midline. The tumor(-bed) radioactivity was defined as the radioactivity from and immediately above the tumor. The results from 10 animals were averaged in each group. (a) No external magnetic field was present. (b) A 10 mm dia. rare earth magnet was taped directly above the tumor.

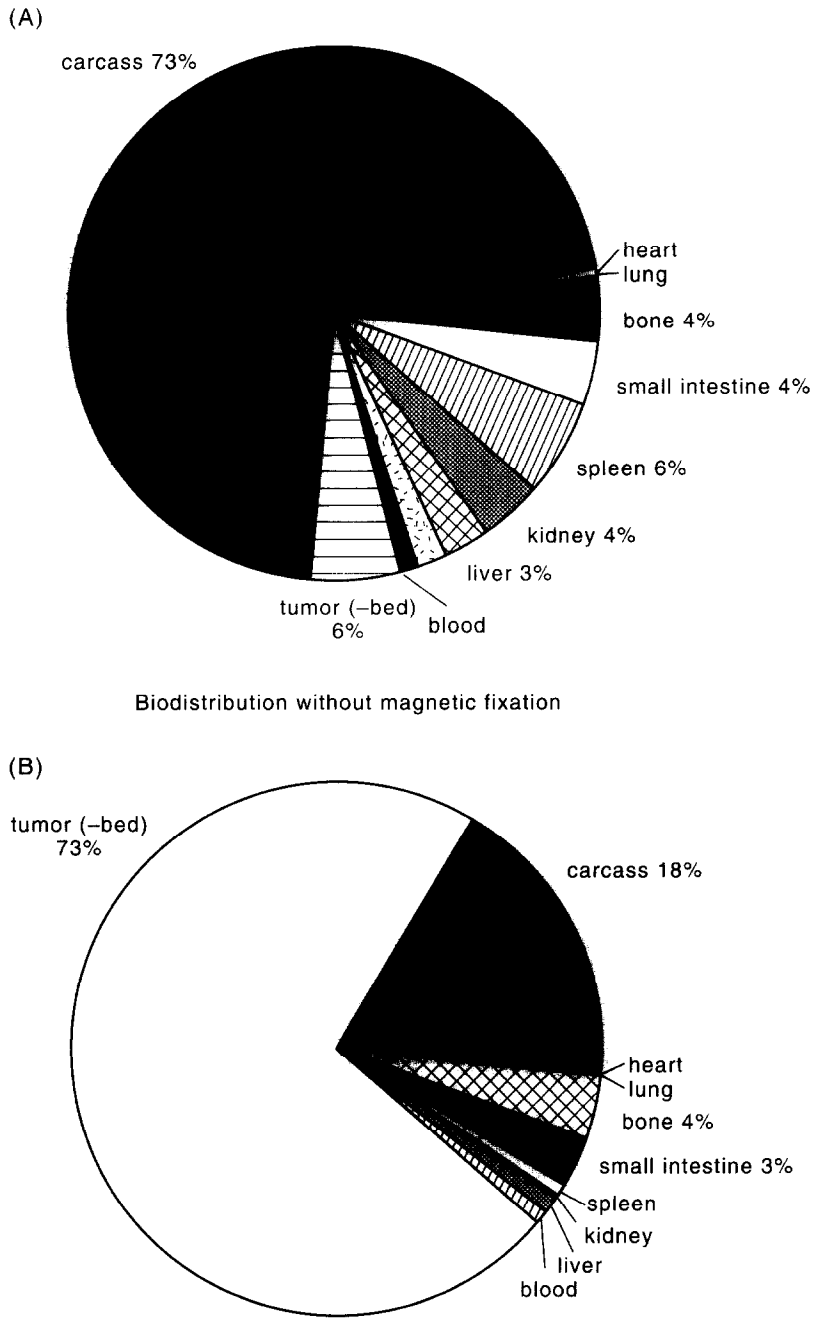

Fig. 4. Total body biodistribution of ^{90}Y–MMS in mice with a subcutaneous EL-4 tumor in the abdominal midline. The biodistribution was determined after 24 h and was calculated as a percentage of the total body radioactivity (average of 10 animals). (A) No external magnetic field was present. (B) A magnet was taped directly above the tumor.

survival in Table 1. At first glance, the calculated doses for the free ^{90}Y seem higher than expected, but the doses necessary to kill a certain fraction of the neuroblastoma cells agree very well with the data from a colony forming assay published by Marchese *et al.* (1987) who tested different human cell lines for radiosensitivity. One of these cell lines was SK-N-SH neuroblastoma cells which are almost identical in radiosensitivity to the SK-N-MC cells. Marchese *et al.* determined a D_0 of 1.6 Gy when they irradiated the tumor cells with a dose rate of 81 Gy/h. This slope changed considerably to a D_0 of 4.0 Gy when the dose rate was decreased to 0.51 Gy/h. The dose rate in our experiments with ^{90}Y–DTPA was similar, ~0.4 Gy/h, resulting in the same D_0 of ~4 Gy. The calculated doses of ^{90}Y–MMS with a killing magnet were

Table 1. Comparison of the necessary amounts of ^{90}Y for cell kill

Survival	Free ^{90}Y-DTPA	Killing magnet	Sparing magnet
90%	27.43 kBq	6.51 kBq	22.52 kBq
	(0.52 Gy)	(1.31 Gy)	(0.00 Gy)
50%	114.71 kBq	51.38 kBq	231.65 kBq
	(2.17 Gy)	(10.32 Gy)	(0.01 Gy)
20%	430.28 kBq	139.61 kBq	453.65 kBq*
	(8.13 Gy)	(28.03 Gy)	(0.02 Gy)

*Extrapolated from the curve in Fig. 2.
( ): Computed doses to the SK-N-MC cells as described under Materials and Methods.

substantially higher than the values from the "standard" ^{90}Y–DTPA survival curve. This may be due to overestimation of the absorbed fraction ϕ in the well geometry, perhaps due to a large number of microspheres floating in the medium at distances $>20\,\mu$m above the cell layer. There is an additional uncertainty in the dose arising from the well edges. This will give rise to a dose gradient from the cells at the edge of the well to those at the center, resulting in a further reduction in the dose rate by two, due to the missing dose contribution from sources above the cells beyond the well edge. This leads to a variation in the cellular dose rate across the bottom of the well from between 13.4 and 26.8 g · cGy/MBq · h with a corresponding variation in the doses, when adjusted for the exposure duration. The theoretical estimates of the dose made for the sparing magnet significantly underestimated the biological response. Possible explanations are that either some MMS escaped the magnetic pulling, or that some nonmagnetic but radioactive parts of microspheres sunk to the bottom of the well near the SK-N-MC cells.

The magnetic microspheres were also tested in mice *in vivo*. A biodistribution study showed that magnetic microspheres can be held near a target organ when under the influence of a magnetic field, and that they become even more concentrated during a 24 h test period. The difference between tumor connected radioactivity of $6 \pm 4\%$ without a magnet and of $73 \pm 32\%$ with a magnet was highly significant.

The 96-well plate *in vitro*-system that we used to measure the radiotoxicity and moveability of ^{90}Y–MMS highlights all the attributes of this magnetic radiopharmaceutical but was not without its problems. The standard deviations were larger than expected, probably due to the relatively small number of cells per well. In addition, a slight decrease in survival was observed even when nonradioactive MMS were added. This was not due to the release of toxic substances from the microsphere preparation or biodegradation processes but instead appeared to be due to the physical properties of the microspheres in the size range of 10–20 μm. An electron microscopic study showed that some of the tumor cells tried to adhere to these microspheres [see Fig. 5(B)]. This resulted in less cell contact with the bottom of the well thus allowing some of the cells to be rinsed off with

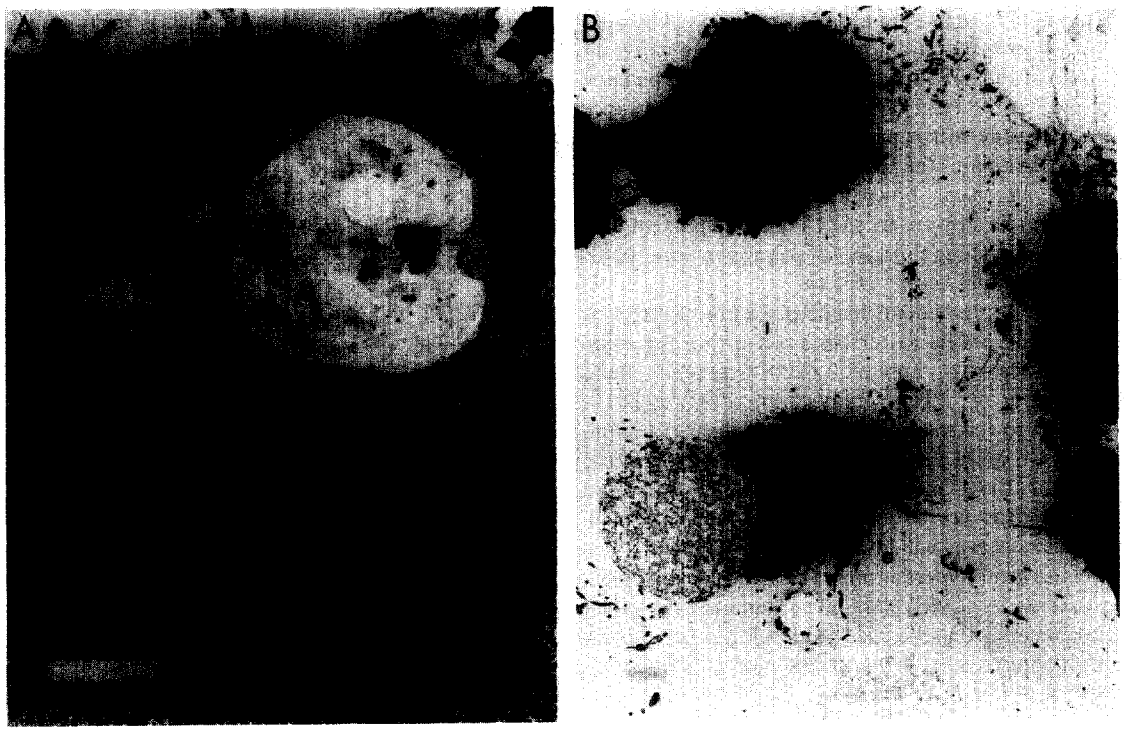

Fig. 5. Transmission electron microscopic picture of 70 nm thick slices of SK-N-MC cells incubated for 24 h with magnetic microspheres. The bars represent 2 μm. (A) Small microsphere ($\sim 4\,\mu$m) after being phagocytosed from the neuroblastoma cell. (B) Microspheres of more than 10 μm diameter that cannot be phagocytosed but can have cells adhere to them, thus decreasing the contact to the well plate and increasing the likelihood of their getting rinsed off in the wash step.

the MMS. Additionally, some of the microspheres smaller than 5 μm were phagocytosed [see Fig. 5(A)]. In order to achieve only the desired effects of the microspheres, it is thus very important to narrowly define their size distribution.

The approach of directing magnetic poly(lactic acid) microspheres from outside the body to lesions near the body's surface resulted in a very high targeting efficiency. This mode of physically targeting a β-emitting radiopharmaceutical could become a useful technique for the treatment of small, inaccessible tumor nodules for which brachytherapy would be impossible. The radiation characteristics of ^{90}Y with its maximum range of 1 cm make it useful for the treatment of cancer lesions of up to that diameter.

One possible therapeutic application that we are considering involves the treatment of neoplastic meningitis in the spine. In this scenario, the radiopharmaceutical ^{90}Y–MMS would be injected intrathecally and then be moved in the nonuniform magnetic field of a strong permanent magnet to tumor lesions which had been previously localized by CT or MRI. The magnetic field of an MRI machine just *outside* the opening can also be used for this targeting. At this location, the main magnetic field is non-homogeneous, producing field gradients of 0.02–0.14 T/cm within 30 cm of the magnet opening (measurements from a 1.7 T MRI machine). This method of targeting works because of the special anatomy of the intrathecal space which constrains the microspheres to motion along a single axis. Once the microspheres start to move intrathecally towards the middle of the MRI magnet, they can be stopped and retained at the target site with a small magnet taped to the outside of the patient's spine and would then locally irradiate the cancerous deposits near the magnet. We have shown in a spine model that the MMS can be stopped by such a local magnet and are currently pursuing this kind of therapy in a rat model with intraspinal tumor growth.

Recent improvements in the precision of directing small metal pellets to certain areas within the brain with externally applied electromagnetic fields (Howard *et al.*, 1989; Grady *et al.*, 1990) could be extended to the magnetic ^{90}Y-microspheres for clinical tests in brain tumors. Furthermore, the cytotoxic effect of these microspheres could be increased through exposure to an alternating magnetic field. The field's energy will be absorbed by magnetite thus inducing local hyperthermia (Sako and Hirota, 1986; Jordan *et al.*, 1993). Magnetic radioactive microspheres could thus be used to develop a new family of targeting agents for use in the intracavitary treatment of a variety of tumors.

Acknowledgements—This work was supported by the American Cancer Society grant No. ACS-PDT-442. We appreciate the assistance that Lydia Cartar gave in editing this manuscript.

References

Berger M. J. (1971) Distribution of absorbed dose around point sources of electrons and beta particles in water and other media. *J. Nucl. Med.* Suppl. **5,** 5–23.

Berger M. J. (1973) Improved point kernels for electron and beta ray dosimetry. *NBSIR* 73–107.

Blanchard R. J., LaFave J. W., Kim Y. S., Frye C. S., Ritchie W. P. and Perry J. F. (1965) Treatment of patients with advanced cancer utilizing ^{90}Y microspheres. *Cancer* **18,** 375–380.

Furukawa T., Kubota T., Suto A., Takahara T., Yamaguchi H., Takeuchi T., Kase S., Kodaira S., Ishibiki K. and Kitajima M. (1991) Clinical usefulness of chemosensitivity testing using the MTT assay. *J. Surg. Oncol.* **48,** 188–193.

Gallo J. M. and Hassan E. E. (1988) Receptor-mediated magnetic carriers: basis for targeting. *Pharmaceut. Res.* **5,** 300–304.

Grady M. S., Howard M. A., Broaddus W. C., Molloy J. A., Ritter R. C., Quate E. G. and Gillies G. T. (1990) Magnetic stereotaxis: a technique to deliver stereotactic hyperthermia. *Neurosurgery* **27,** 1010–1016.

Gupta P. K. and Hung C. T. (1989) Magnetically controlled targeted micro-carrier systems. *Life Sci.* **44,** 175–186.

Häfeli U. O., Sweeney S. M., Beresford B. S. and Macklis R. M. (1994) Biodegradable magnetically directed ^{90}Y-microspheres: novel agents for targeted intracavitary radiotherapy. *J. Biomed. Mat. Res.* **28,** 901–908.

Houle S., Yip T. C. K., Shepherd F. A., Rotstein L. E., Sniderman K. W., Theis E., Cawthorn R. H. and Richmond-Cox K. (1989) Hepatocellular carcinoma: pilot trial of treatment with Y-90 microspheres. *Radiology* **172,** 857–860.

Howard M. A., Grady M. S., Ritter R. C., Gillies G. T., Quate E. G. and Mollo J. A. (1989) Magnetic movement of a brain thermoceptor. *Neurosurgery* **24,** 444–448.

Humm J. L., Chin L. M., Cobb L. and Begent R. (1990) Microdosimetry in radioimmunotherapy. *Rad. Protect. Dosimetry* **31,** 433–436.

Jordan A., Wust P., Fahling H., John W., Hinz A. and Felix R. (1993) Inductive heating of ferrimagnetic particles and magnetic fluids: physical evaluation of their potential for hyperthermia. *Int. J. Hyperthermia* **9,** 51–68.

Kemshead J. T., Heath L., Gibson F. M., Katz F., Richmond F., Treleaven J. and Ugelstad J. (1986) Magnetic microspheres and monoclonal antibodies for the depletion of neuroblastoma cells from bone marrow: experiences, improvements and observations. *Br. J. Cancer* **54,** 771–778.

Kharkevich D. A., Alyautdin R. N. and Filippov V. I. (1989) Employment of magnet-susceptible microparticles for the targeting of drugs. *J. Pharm. Pharmacol.* **41,** 286–288.

Leichner P. K., Yang N., Frenkel T. L., Loudenslager D. M., Hawkins W. G., Klein J. L. and Order S. E. (1988) Dosimetry and treatment planning for Y-90-labeled anti-ferritin in hepatoma. *Int. J. Radiat. Oncol. Biol. Phys.* **14,** 1033–1042.

Loevinger R., Budinger T. F. and Watson E. E. (1990) *MIRD Primer for absorbed dose calculations.* Revised edition. The Society of Nuclear Medicine, New York.

Mantravadi V. P., Spigos D. G., Tan S. and Felix L. (1982) Intraarterial Y-90 in the treatment of hepatic malignancy. *Radiology* **142,** 783–786.

Marchese M. J., Zaider M. and Hall E. J. (1987) Dose-rate effects in normal and malignant cells of human origin. *Br. J. Radiol.* **60,** 573–576.

Rosenblum M. G., Kavanagh J. J., Burke T. W., Wharton J. T., Cunningham J. E., Shanken L. J., Silva E. G., Thompson L., Cheung L., Lamki L. and Murray J. L. (1991) Clinical pharmacology, metabolism, and tissue distribution of ^{90}Y-labeled monoclonal antibody B72.3

after intraperitoneal administration. *J. Natl. Cancer Inst.* **82,** 1629–1636.

Rusetskii A. N., Panisov M. I., Ruuge E. K. and Torchilin V. P. (1985) Substantiation of using magnet-directed localization of drugs for the treatment of thrombosis. *Biulleten Vsesoiuznogo Kardiologicheskogo Nauchnogo Tsentra Amn Sssr* **8,** 100–105.

Sako M. and Hirota S. (1986) Embolotherapy of hepatomas using ferromagnetic microspheres, its clinical evaluation and the prospect of its use as a vehicle in chemoembolo-hyperthermic therapy. *Gan To Kagaku Ryoho* **13,** 1618–1624.

Turner R. D., Rand R. W., Bentson J. R. and Mosso J. A. (1975) Ferromagnetic silicone necrosis of hypernephromas by selective vascular occlusion to the tumor: a new technique. *J. Urology* **113,** 455–459.

Weichert H., Blechschmidt I., Schröder S. and Ambrosius H. (1991) The MTT-assay as a rapid test for cell proliferation and cell killing: application to human peripheral blood lymphocytes (PBL). *Allerg. Immunol.* **37,** 139–144.

Widder K. J., Senyei A. E. and Ranney D. F. (1979) Magnetically responsive microspheres and other carriers for the biophysical targeting of antitumor agents. *Adv. Pharmacol. Chemother.* **16,** 213–271.