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The use of magnetite for estimation of microspheres in mice

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Summary

For the use of magnetite as a tracer to estimate the biological fate of microspheres after injection, albumin microspheres containing magnetite were injected i.v. into mice; then they were examined for their biodistribution along with an in vitro test. Magnetite recollected from the homogenates of mice organs with a magnet was assayed by atomic absorption spectrophotometry.

With the increasing interest on the microspheres as new drug delivery systems to direct drugs as well as diagnostic agents to specific organs and cells (Davis et al., 1984; Davis et al., 1986), the problem of estimating their exact biological fate in the body has become a current topic of research.

Until recently, the labeling of radioactive isotopes on the polymer and drug incorporated into the microspheres has been presented as the top-level method used for the tracing of polymeric microspheres after intravascular injection with the assumption that label and microspheres are to be found together. But in spite of some unquestionable advantages, the use of radioactive isotopes may lead to erroneous conclusions. When ^{14}C is used, its introduction into the polymer backbone brings practically no alteration of chemical and physical properties of the macromolecule, but this is not always true with iodine and tritium (Rypáček et al., 1980). The use of radiolabelled drug incorporated in the microspheres causes the leakage of tracer (Morimoto et al., 1981) and much differences in biodistribution data were observed on the doubly labeled technetium sulfur colloid and also between on the albumin microspheres labeled with different radioisotopes (Ng et al., 1980). Reports of microspheres found in the brain, cerebrospinal fluid, and colloids crossing the gastrointestinal tract in significant quantities need to be questioned in terms of label integrity as mentioned by Davis et al. (1986), and stability of radiolabelled micropheres in vivo have to be considered (Ng et al., 1980; Laakso and Smedsrod, 1987). Furthermore, handling of radioactive isotopes is rather hazardous.

Fluorescent labeling of microspheres has been also adopted for the same purpose (Willmott et al., 1985), but it is a qualitative, not a quantitative method, and it includes a fluorescence microscopic procedure.

The present paper is concerned with a new method which uses magnetite as a tracer of microspheres after injection and its application for the

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biodistribution of chemical-stabilized albumin microspheres as a model biodegradable microsphere. Magnetite, Fe_3O_4, is non-toxic to normal body constituents and also, due to their small size (10–20 nm in diameter) they are not ferromagnetic, but superparamagnetic, which is easily magnetized with a low magnetic field. From these properties, magnetite has been increasingly applied not only for labeling and cell sorting by immunomagnetic microspheres (Muller-Ruchholtz et al., 1987), but also for delivering drugs to a specific site by drug-loaded magnetic microspheres (Sugibayashi and Morimoto, 1986). And Lee et al. (1988) used magnetite to measure the actual size of microspheres entrapped in mice organs after i.v. injection.

The albumin microspheres containing magnetite were prepared and sized as previously described (Lee et al., 1988). In brief, mixture of bovine serum albumin (Sigma, U.S.A.) and magnetic fluids (W-40, Taiho Ind., Japan) was sonicated in iso-octane (Tokyo Kasei, Japan) containing sorbitan mono-oleate (Sigma, U.S.A.) on the ice bath. The resultant emulsion was treated with glutaraldehyde solution (Sigma, U.S.A.), then washed with acetone and vacuum-dried. The microspheres prepared were sized by scanning electron microscopy (JCM-35C, Jeol, Japan) and their mean diameter and size distribution were calculated by the methods of Allen (1981).

Mean diameter of microspheres prepared was 1.08 ± 0.88 μm (mean $\pm$ S.D.) and their content of magnetite was 18.4%. After hydration in the injection medium, mean diameter increased to 1.79 ± 1.24 μm with 65.4% of the microspheres ranging between 0.5 and 3.0 μm.

Three mg of microspheres dispersed in 0.3 ml of saline solution containing 0.2% Tween 80 (Sigma, U.S.A.) was rapidly injected into the tail vein of male mice (ICR, 20–24 g). To avoid cluster formation, sonication was performed prior to injection. Three mice were sacrificed at given intervals following injection, 1 h, 1 d, 3 d and 7 d, respectively, and lungs, liver, spleen and kidneys were isolated. Saline solution was added to facilitate homogenation, which was accomplished with a minihomogenizer (Ultra-turrax, F.R.G.) and then recollected the microspheres in the respective homogenates with a constant-flow magnet separation apparatus (Lee et al., 1988). Applied magnetic strength and flow rate were fixed on 15 kG (Cenco, U.S.A.) and 2 ml/min (MP-3, Tokyo Rikakikai, Japan) through an elastic tube of 0.4 mm diameter. The glass collecting chamber used has a dimension of diameter 15 mm and depth 3 mm. Microspheres recovered with adherent organ tissue were eluted with additional saline solution and separated by centrifugation at 3000 rpm for 10 min and then digested with 1% of a mixture of protease and lipase in pH 8.0 phosphate buffer solution for 1 day in a shaking incubator. Recovered magnetite was dissolved with hydrochloric acid and assayed by atomic absorption spectrophotometry (PU9000, Pye Unicam, U.K.) at 248.3 nm with Fe as a standard.

As a primary study, an in vitro recovery test was performed. The spiked samples of mice lungs, liver, spleen and kidneys with 50 μg of microspheres as magnetite, respectively, and subjected to incubation at 37 °C for 1 h were applied on the constant-flow magnet separation apparatus. The mean recovery rates were within the range of 95.4 to 98.2%, and no significant differences appeared between the organs studied.

Total in vivo recovery rate from mice organs examined 1 h after injection was $75.6 \pm 4.7\%$ ($n = 3$) and Fig. 1 shows the biodistribution of the microspheres in terms of postinjection time. Initially the microspheres were concentrated mainly in the lungs and liver, 68.3 and 28.6% of entrapped microspheres in organs examined, whereas traces of microspheres were found in the spleen and kidneys. It can be seen that between 1 h and 1 d following injection the amount of microspheres decreased in the lungs to a large extent by 52.9%, whereas it remained almost unchanged in the liver. Later on, the amount of microspheres in the lungs decreased relatively rapidly, showing a terminal half-life of about 3 days, but in the liver it had a slower clearance than in the lungs. Those in spleen and kidney showed a slight fluctuation but its values were negligible. No magnetite was found in blood samples at the times studied. Red blood cells were not retained by this method but by high gradient magnetic separation techniques (Graham, 1981; Owen, 1982).

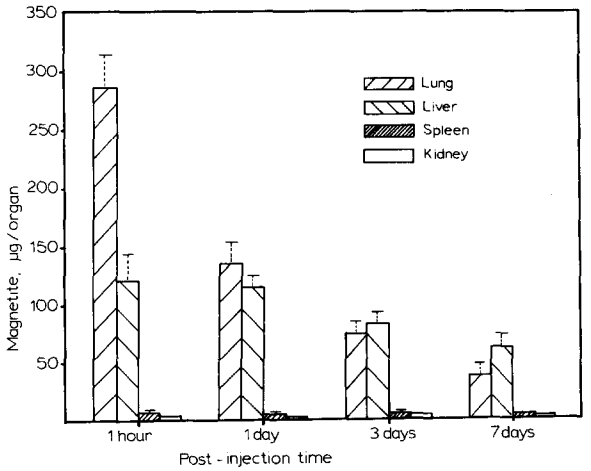

Fig. 1. Organ distribution of magnetite-labeled albumin microspheres after i.v. injection to mice. Error bars are ±S.D.

Other studies to compare this method with those of albumin microspheres labeled with ^{125}I are currently in progress.

In conclusion, the use of magnetite as a tracer brings about many simplifications in handling as well as in the recovery and detection of microspheres in living tissue over the radioisotope method and fluoromicroscopic technique. Magnetite can be easily introduced into microspheres physically without altering the chemical and physical properties of the polymer. The recovery and concentration of microspheres per animal organ after injection can be simply achieved with a magnet and the assay can be done with a conventional atomic absorption spectrophotometer. Furthermore, the leakage of magnetite due to the hydration is assumed unlikely to occur because magnetite is stable and rather large particles which do not easily diffuse out of the microspheres.

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