International Journal of Pharmaceutics, 52 (1989) 87–89
Elsevier

IJP 01812

Magnetite as a tracer for the estimation of biodistribution of microspheres: a critical consideration

P.K. Gupta

College of Pharmacy, University of Kentucky, Lexington, KY 40536 (U.S.A.)

(Received 29 November 1988)
(Modified version received 1 February 1989)
(Accepted 2 February 1989)

A recent report by Lee (1988) has advocated the use of magnetite (Fe_3O_4) as a marker for the estimation of biodistribution of microspheres in mice. The magnetite was incorporated in chemically cross-linked albumin microspheres, and the distribution of microspheres in major tissues of mice, following their i.v. administration, was monitored over a period of 7 days in terms of the tissue levels of magnetite. Although interesting, and relatively simple as discussed by Lee (1988), this technique of evaluation of biodistribution of microspheres, or other colloidal particles, needs some critical consideration overlooked in the reported study.

As already mentioned by Lee (1988), magnetite is an inert chemical, which is mechanically trapped within the microsphere matrix during the emulsification of albumin and its subsequent stabilization accomplished either by heat or chemical reaction. Depending upon factors like the relative amounts of albumin and magnetite used per batch, the size of the microspheres, and the presence of an additional chemical, e.g. drug, all batches of such a microsphere preparation contain a varying proportion of unincorporated Fe_3O_4 (Gupta et al., 1988). Unfortunately the methods reported by Lee (1988) and Lee et al. (1988) do not reveal the exact amount of magnetite employed per batch. However, experiments in our laboratory have revealed that starting with 100 mg albumin and 100 mg magnetite slurry (30–35% w/v Fe_3O_4 in water; Ferrofluidics, Nashua, NH), the yield of microspheres, after discarding unincorporated Fe_3O_4, approximate 80 ± 10 mg. These microspheres contain about 18% w/w of Fe_3O_4 (Gupta et al., 1988), a value close to that reported by Lee (1988). However, these figures also suggest that a fresh batch of magnetic albumin microspheres, from which unincorporated Fe_3O_4 has not been removed, may contain a total of 30–40% w/w of Fe_3O_4. If this preparation is directly administered in vivo, free as well as the microsphere-entrapped Fe_3O_4 will be distributed to the major tissues of the body. Hence the removal of the unincorporated Fe_3O_4 is important. The unincorporated magnetite is usually removed by using a small bar magnet (Gallo et al., 1989; Gupta et al., 1986, 1988), a horse-shoe magnet (Sprandel et al., 1987) or gel chromatography (Kiwada et al., 1986). Unfortunately, the method reported by Lee (1988) and Lee et al. (1988) does not include any step for the efficient removal of the unincorporated fraction of Fe_3O_4. Hence the conclusions regarding the quantitation of biodistribution of microspheres, on the basis of the distribution of Fe_3O_4, are likely to be erroneous.

In addition to the potential source of error discussed above, the use of magnetite for the

Correspondence: P.K. Gupta, College of Pharmacy, University of Kentucky, Rose Street, Lexington, KY 40536–0082, U.S.A.

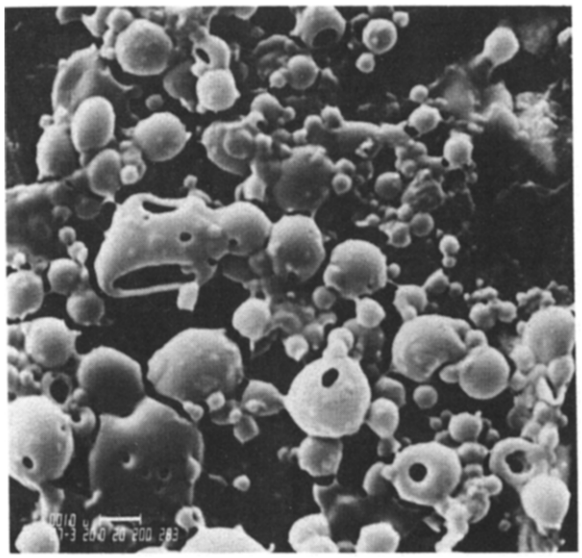

Fig. 1. A representative scanning electron microscope photograph of magnetic albumin microspheres, prepared by heat-stabilization at 120 °C, and subjected to hydration in normal saline at 37 °C for 4 h. Bar = 1.0 μm.

estimation of long term biodistribution of degradable microspheres appears conceptually difficult. This is particularly true for albumin microspheres which readily degrade in vivo (Bernard et al., 1980; Zolle et al., 1970). Figs. 1 and 2 show representative scanning and transmission electron photomicrographs of magnetic albumin microspheres, respectively, following their hydration in normal saline at 37 °C. The formation of pores

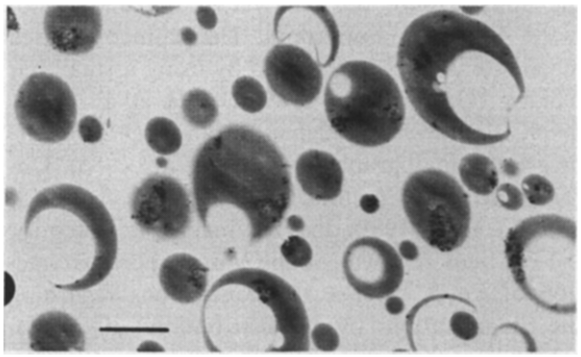

Fig. 2. A representative transmission electron microscope photograph of magnetic albumin microspheres, prepared by heat-stabilization at 120 °C, and subjected to hydration in normal saline at 37 °C for 4 h. Bar = 0.5 μm.

and cavities in the microsphere matrix, as a result of hydration, provides an indication of the ease with which these particles lose their integrity in the body. Although the degradation of microspheres is known to depend upon several factors including their size and the degree of matrix stabilization (Bernard et al., 1980; Zolle et al., 1970), the in vivo half-life of microsphere and magnetite, in a given tissue, cannot be assumed to be similar. Rapid degradation of microspheres will release the unincorporated Fe_3O_4 (Fig. 2), and at any time beyond this point, the quantitation of tissue levels of Fe_3O_4 as an indication of biodistribution of microspheres will again lead to erroneous interpretation of the results.

The above examples have not been cited with a view to criticize the method described by Lee (1988). However, they are intended to be a caveat to the use of magnetite in the quantitation of the biodistribution of microspheres. In fact this method does hold substantial potential for qualitative studies on the biodistribution of microspheres, e.g. determination of the effect of size or the composition of microspheres on their preferential distribution in vivo, and for the rapid screening of the site(s) of localization of microspheres by performing Prussian blue reaction (Gupta et al., 1989; Lillie, 1965). The method can however be extended for quantitative studies only under circumstances where microspheres can be produced with uniform batch to batch magnetite content, and the unincorporated Fe_3O_4 is completely removed before their administration. The extent to which these characteristics can be practically accomplished is again debatable (Gupta and Hung, 1989). Indeed if long-term quantitation of biodistribution of microspheres is intended, it will also be important to consider the relative rate of metabolism of Fe_3O_4 and microspheres in the body.

References

Bernard, N.G., Shaw, S.M., Kessler, W.V., Landott, R.R., Peck, G.E. and Dockerty, G.H., Distribution and degradation of iodine-125 albumin microspheres and technetium-99m sulphur colloid. *J. Pharm. Sci.*, 15 (1980) 30–34.

Gallo, J.M., Gupta, P.K., Hung, C.T. and Perrier, D.G., Evaluation of drug delivery following the administration of magnetic albumin microspheres containing adriamycin to the rat, *J. Pharm. Sci.*, 78 (1989) 190–194.

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

Gupta, P.K., Hung, C.T. and Rao, N.S., A study on the ultrastructural disposition of adriamycin-associated magnetic albumin microspheres in rats. *J. Pharm. Sci.*, in press.

Gupta, P.K., Hung, C.T., Lam, F.C. and Perrier, D.G., Albumin microspheres. III. Synthesis and characterization of microspheres containing adriamycin and magnetite. *Int. J. Pharm.*, 43 (1988) 167–177.

Gupta, P.K., Morris, C. and Hung, C.T., Evaluation of magnetic albumin microspheres for site-specific delivery of adriamycin. *Proc. Univ. Otago Med. Sch.*, 64 (1986) 63–64.

Kiwada, H., Sato, J., Yamada, S. and Kato, Y., Feasibility of magnetic liposomes as a targeting device for drugs. *Chem. Pharm. Bull.*, 34 (1986) 4253–4258.

Lee, K.C., The use of magnetite for estimation of microspheres in mice. *Int. J. Pharm.*, 47 (1988) 265–267.

Lee, K.C., Koh, I.B., Kim, W.B. and Lee, Y.J., Size and morphological analysis of albumin microspheres in the lung and liver of mice. *Int. J. Pharm.*, 44 (1988) 49–55.

Lillie, R.D., *Histopathologic Technique and Practical Histochemistry*. 3rd ed., Mc-Graw Hill, NY, 1965.

Sprandel, U., Lanz, D.-J. and von Horsten, W., Magnetically responsive erythrocyte ghosts. *Methods Enzymol.*, 149 (1987) 301–312.

Zolle, I., Rhodes, B.A. and Wagner, H.N., Jr., Preparation of metabolized radioactive human serum albumin microspheres for studies of the circulation. *Int. J. Appl. Radiat. Isotopes*, 21 (1970) 155–167.