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Size and morphological analysis of albumin microspheres in the lungs and liver of mice

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Summary

Albumin microspheres containing magnetite (mean diameter 1.10 ± 0.85 μm; range 0.1–6.0 μm when hydrated in the injection medium) were injected into the tail vein of male mice. Mice were killed at intervals and microspheres, collected from their lungs and liver by magnet, were examined for the microsphere size and also used for morphological observation. No significant differences were found between microspheres lodged in the lungs and liver in the mean diameter and size distribution, showing 1.85 ± 1.55 and 1.24 ± 0.90 μm at 1 h post-injection, respectively. The mean diameters of microspheres lodged in the lungs and liver were dependent on their residence time. Temporary increases in the mean diameter were observed between 1 h and 1 day in both organs, in the lungs by 31.7% and in the liver by 44.4%, respectively. During the following period of 6 days, the size of the microspheres in the lungs decreased gradually, and, in contrast, in the liver the size decreased rapidly. The cavity formation and surface damage probably from the hydration and attack of enzyme in the living tissue could be observed on the surface of microspheres collected from mice.

Introduction

Microspheres have been suggested as new drug delivery systems to direct drugs to specific organs and cells, thereby inducing either improvement in efficacy or reduction in the side-effects of drugs (Davis et al., 1984; Guiot and Couvreur, 1986).

It has been demonstrated that for the targeting of drugs with microspheres, the size plays a great role in controlling drug delivery to the target organs and the subsequent uptake of drugs in tissue (Kanke et al., 1980; Illum et al., 1982; Burger et al., 1985) and it is also critical to estimate the degradation process of microspheres in vivo for the drug release studies.

Therefore, the problem of estimating the exact fate of microspheres in the body has been a current topic of research. Current methods for the observation of residence and degradation of microspheres in organs involve either light and fluorescence microscopic (Willmott et al., 1985; Fujimoto et al., 1985; Lee and Koh, 1987) or transmittance electron microscopic observation (Edman et al.,. 1983a; Willmott et al., 1984) followed by section and staining of the organs examined. The mean diameter and size distribution were only measured on presenting the microspheres to be injected.

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Since no attempt has been made to observe the actual size and morphological change of microspheres lodged in the organs directly, the present report is concerned with the particle size and morphological characteristics of microspheres injected intravenously and lodged in the lungs and liver of male mice using chemically stabilized albumin microspheres (AMS) as model biodegradable microspheres. AMS have already been used as diagnostic agents (Scheffel et al., 1972; Hagan et al., 1978) and drug carriers for both targeting of drugs (Willmott et al., 1985; Burger et al., 1985) and chemoembolization therapy (Fujimoto et al., 1985; Natsume et al., 1985). In this study, magnetite incorporated in AMS was adopted as a tool to collect the AMS by magnet from mice after injection.

Materials and Methods

Preparation of AMS

AMS containing magnetite were prepared by sonication (US-300, Nihonseiki, Japan) of a mixture of 200 mg of bovine serum albumin (Sigma, U.S.A.) in 1 ml of distilled water and 0.2 ml of magnetic fluids (W-40, Taiho Ind., Japan) in 30 ml of iso-octane (Tokyo Kasei, Japan) containing 0.1 ml of sorbitan monooleate (Sigma, U.S.A.) on an ice bath. The resultant emulsion was treated with 1 ml of 5% glutaraldehyde solution (25%, Sigma, U.S.A.) at 1000 rpm for 30 min at room temperature to cross-link the matrix. The mixture was centrifuged (Minor, MSE, U.K.) at 3000 rpm for 20 min. The supernatant was decanted and washing with acetone repeated thrice. The finally washed AMS were vacuum-dried and were stored at 4 °C until needed. The final product was in the form of discrete spherical units and recovered as a free-flowing dark-brown powder.

Swelling experiments

To measure the effect of hydration on the microsphere size in organs and in the injection medium (Zolle et al., 1970; Tomlinson and Burger, 1985), AMS were placed into mouse liver homogenate and physiological saline solution (PSS, pH 7.4, Perrin and Dempsey, 1974) at 37 °C for 1 h, respectively. Then the AMS was collected from the mediums by a constant-flow magnet separation apparatus and the size of AMS measured as described below.

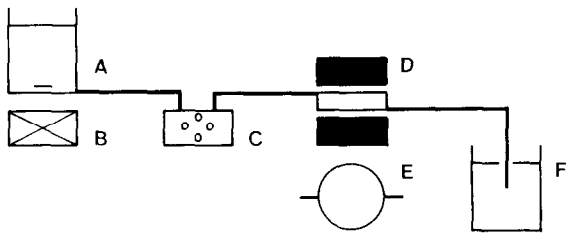

Fig. 1. A constant-flow magnet separation apparatus. A = homogenate; B = stirrer; C = peristaltic pump; D = electric magnet; E = collection chamber; F = waste.

Animal experiments

Each male mouse (ICR, 20–24 g) was rapidly injected via a tail vein with 3 mg of AMS dispersed in 0.3 ml of PSS containing 0.2% Tween 80 (Sigma, U.S.A.). To avoid cluster formation (Leu et al., 1984), sonication was performed prior to injection. Three mice were sacrificed at given intervals following injection and the lungs and liver were isolated. PSS was added to facilitate homogenation, which was accomplished with a minihomogenizer (Ultra-turrax, F.R.G.).

Recollection of AMS from mouse organs

AMS injected and lodged in the mice lungs and liver where recollected from the homogenates of the respective organ by a constant-flow magnet separation apparatus as shown in Fig. 1. Applied magnetic strength and flow rate were fixed on 10 K gauss (Cenco, U.S.A.) and 1.5 ml/min (MP-3, Tokyo Rikakikai, Japan), respectively. Microspheres recollected were washed with PSS and dehydrated with absolute ethanol for scanning electron microscopy.

Size measurement and morphological analysis

AMS prepared or collected from mice were sputter-coated (JFC-1100, Jeol, Japan) with a 30 nm layer of gold and got the microphotographs from a scanning electron microscope (JCM-35C, Jeol, Japan) for the size measurement and morphological observation. Size measurement was car-

ried out from the resultant microphotographs; the diameters of at least 200 AMS were manually measured and their mean diameter was calculated according to the method by Allen (1981). Microspheres under 0.1 μm were not counted throughout experiments.

Results

Characteristics of AMS

By varying time and strength of sonication, AMS could be produced in the mean size of 0.69 ± 0.45 μm (mean $\pm$ S.D.).

Microspheres placed into mouse liver homogenate and PSS for 1 h at 37 °C increased in mean diameter, showing 1.08 ± 0.83 and 1.10 ± 0.85 μm, respectively, as shown in Table 1. No significant differences in mean diameter and size distribution were observed between AMS hydrated in both mediums.

Size distribution of AMS lodged in organs

Following sacrifice of 3 mice at every interval, the lungs and liver were removed. With the constant-flow magnet separation apparatus, AMS injected and lodged in the lungs and liver could be collected from the homogenates of respective organs without any disruption of their morphological structure which we then examined on a scanning electron microscope as shown in Fig. 2.

TABLE 1

Characteristics of microspheres manufactured and hydrated in mouse liver homogenate and physiological saline solution

Diameter (μm)	Percent		
	Manufactured	Liver homogenate	PSS [a]
0.1–0.5	38.8	15.1	16.4
0.5–1.0	49.2	53.4	49.0
1.0–2.0	10.4	22.3	25.0
2.0–3.0	1.2	4.4	5.8
3.0–4.0	0.4	2.8	1.9
4.0–5.0	–	1.6	1.0
5.0–6.0	–	0.4	1.0
Mean $\pm$ S.D.	0.69 ± 0.45	1.08 ± 0.83	1.10 ± 0.85

[a] Physiological saline solution.

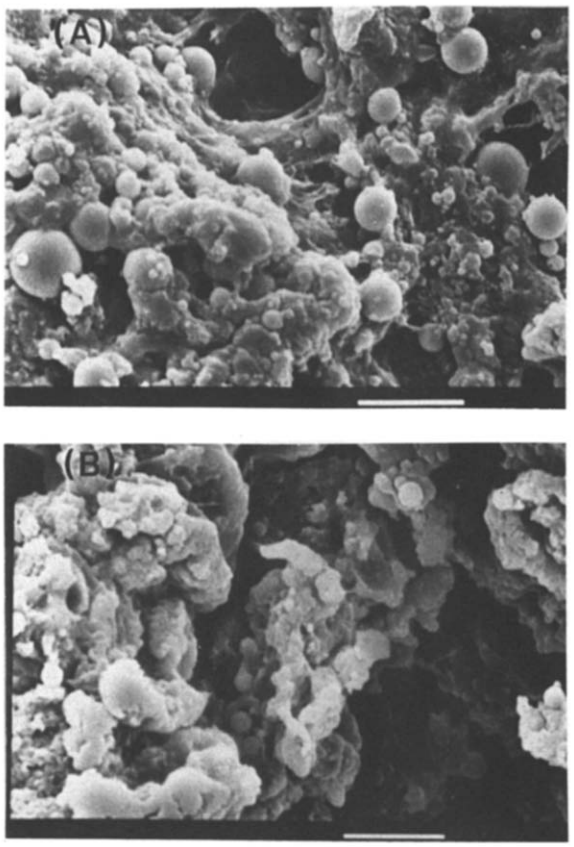

Fig. 2. Scanning electron microphotographs of microspheres collected from the lungs and liver of mice at 1 h post-injection Scale bar 10 μm. A: lung, B: liver.

But it was difficult to get free-flowing AMS because of adhesion of AMS on the membrane of organ capillaries.

TABLE 2

Characteristics of microspheres lodged in the lungs and liver of mice at 1 h post-injection

Diameter (μm)	Percent	
	Lung	Liver
0.1–0.5	22.6	22.3
0.5–1.0	21.2	30.3
1.0–2.0	19.2	29.5
2.0–3.0	15.5	14.0
3.0–4.0	9.1	2.3
4.0–5.0	6.1	1.3
5.0–6.0	6.4	0.3
Mean $\pm$ S.D.	1.85 ± 1.55	1.24 ± 0.90

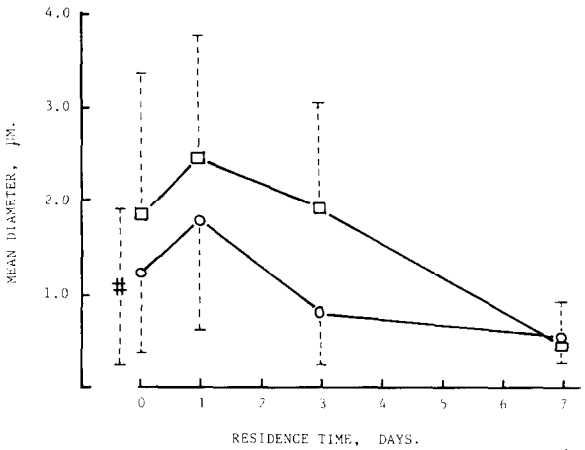

Fig. 3. Influence of residence time in mouse organs on the size of albumin microspheres. #, microspheres dispersed in the injection medium; □, lung; ○, liver. The starting points of both lines stand for the 1 h post-injection time and dotted vertical lines indicate S.D.

Mean diameters of AMS lodged in the lungs and liver of mice at 1 h post-injection were larger than that of AMS dispersed in the injection medium, showing 1.85 ± 1.55 and 1.24 ± 0.90 μm, respectively, as shown in Table 2. The difference of mean diameter of AMS entrapped in both organs at 1 h post-injection appeared not to be in excess of 50%. And the AMS lodged in the lungs distributed more broadly than those in the liver. Among the AMS entrapped in the lungs and liver, the submicron size (< 1 μm) were 43.8 and 52.6% and the larger than 3 μm were 21.6 and 3.9%, respectively.

Effect of residence time on the size of AMS lodged in organs

It can be seen from Fig. 3 that the mean diameter of AMS lodged in the lungs and liver is dependent on their residence time in the organs. A temporary increase was observed between 1 h and 1 day; in the lungs by 31.7% and in the liver by 44.4%, respectively. The rapid decrease nearly to the mean diameter of 7 days following injection was observed in the liver during the next 2 days, on the contrary, the mean diameter of AMS lodged in the lungs decreased gradually with a relatively constant rate throughout the next experimental time. And about 90% of AMS lodged in both organs after 1 week injection were of submicron size.

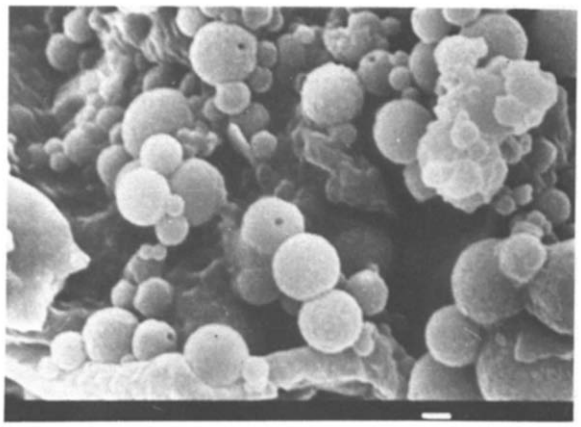

Fig. 4. Cavities observed on the surface of the albumin microspheres subjected to 1 day of residence in mouse liver. SEM. Scale bar = 1.0 μm.

Actually, great care must be taken to measure the size of collected AMS using scanning electron microscopy since submicron AMS, which were damaged or degraded to a large extent in organs, are easily subject to be destroyed during the sample preparation.

Morphological characteristics

Fig. 4 shows that cavities were formed on the surface of AMS upon lodgement in the liver of mice for 1 day. The same kinds of cavities were also found on the AMS subjected to incubation in

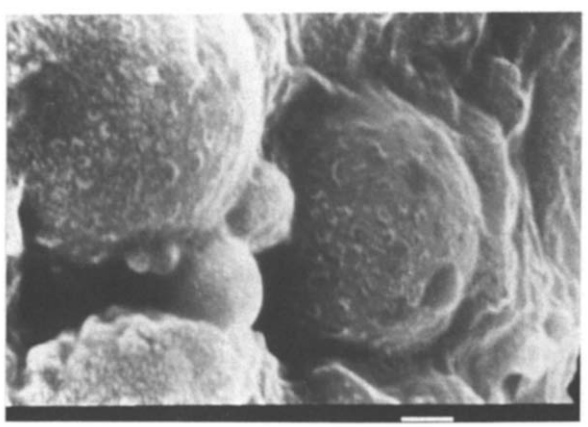

Fig. 5. Biodegradation of the surface of the albumin microspheres subjected to 12 h of residence in mouse lungs. SEM. Scale bar = 1.0 μm.

the PSS for 1 h at 37 °C, but this phenomenon appeared on a limited portion of AMS in both cases.

Fig. 5 shows a scanning electron microphotograph of the AMS lodged in the lungs of mice for 12 h. Many damaged signs, like small craters, appeared on the whole surface of AMS, and as also shown in Figs. 2 and 4, some AMS were strongly stuck to or engulfed into the membrane of organ capillaries.

Discussion

It is generally accepted that, following intravenous injection, microspheres of 7 μm or more in diameter are rapidly entrapped in the lungs by mechanical filtration, whereas microspheres with a diameter of 5 μm or less are mainly taken up by cells of the reticuloendothelial system predominantly in the liver and to a lesser extent in the spleen (Kanke et al., 1980; Illum et al., 1982; Burger et al., 1985). However, a few reports have dealt with the actual size of microspheres lodged in organs after injection.

From this aspect, we injected the AMS of 0.1–6.0 μm in diameter (mean diameter 1.10 ± 0.85 μm) when swelled by hydration in the injection medium into mice via tail vein.

The most obvious conclusion which can be drawn from the results presented herein is that we failed to demonstrate much difference between AMS entrapped in the lungs and liver of mice as regards their mean diameter and size distribution. Moreover, it is of interest to find out the dependence of the mean diameter of microsphere lodged in the lungs and liver of mice on their residence time. During residence in both organs, the AMS showed a temporary increase in size and also showed a difference in the decreasing patterns of size as a function of residence time in the lungs and liver.

These phenomena are not consistent with the previous findings described above considering only the size of AMS injected.

The possible explanations for the temporary increase in the period of 1 h and 1 day of post-injection, in spite of the relatively rapid elimination of AMS in the same period from organs (Scheffel et al., 1972; Sugibayashi et al., 1979; Willomott et al., 1985), would be the redistribution of AMS from the lungs to liver followed by dissociation of clustered AMS which were formed in the bloodstream just after injection, then entrapped initially in the lungs by mechanical filtration due to their enlarged diameter (Kanke et al., 1980; Illum and Davis, 1983; Leu et al., 1984), and the rapid disappearance of smallest microspheres in both organs probably due to biodegradation may play some role. A similar redistribution phenomenon has been observed in the previous articles on the biodegradable polymeric microspheres in mice (Edman and Sjöholm, 1983b; Bissery et al., 1984; Laakso et al., 1986; Gipps et al., 1986).

On the contrary, the decrease of size in both organs during the afterward experimental time may be explained mainly by a simultaneous degradation of AMS with residance time but more rapidly in the liver than in the lungs. And the increased mean diameter of AMS entrapped in the lungs and liver at 1 h post-injection compared with that of microspheres dispersed in the injection medium might be accounted for by clearance of relatively small AMS by other reticuloendothelial system including other organs.

In fact, it is difficult to compare directly the results of this study with those of previous findings only in terms of size range because the distribution and its kinetics of microspheres injected into a vein has been reported to be affected also by other conditions, in which surface charge and surface affinity (Wilkins and Myers, 1966; Illum et al., 1986), coating materials on the surface (Illum and Davis, 1984), dosage levels, detection techniques and species of experimental animal used (Douglas et al., 1986) are included. Furthermore, much differences in the distribution patterns has been reported between 3 types of heat-stabilized AMS having similar size but stabilized at different temperatures (Sugibayashi et al., 1979) and a recent report (Krause et al., 1987) has demonstrated the case of high deposition of non-polymerized unilamellar liposomes of 29.4 ± 15 nm in mean diameter in the lungs. Consequently, it is assumed that the size of the microspheres cannot be a true factor for the biodistribution of

microspheres when their mean diameter is about 1–2 μm or submicron.

It is important to determine the degradation process of microspheres in the body inasmuch as it plays a great role on the microsphere distribution kinetics and drug release from the microspheres. Until recently, morphological observation of microsphere degradation in organs has been performed by light, fluorescence and transmittance electron microscopy in terms of shape deformation of initial round microspheres injected. In this study, we tried to observe the surface of AMS lodged ion the lungs and liver from the recollected AMS from organs directly.

As shown in Fig. 4, cavities were found on the surface of AMS lodged in the liver. The same kinds of cavities have been also observed on the adriamycin-loaded albumin microspheres subjected to dissolution and Gupta et al. (1986) proposed that this is probably caused by the dissolution of entrapped adriamycin in the microspheres. But from the results of this study in which cavities were found where the AMS loaded no drug and which were subjected to in vivo and in vitro tests, it is assumed that these cavities are probably due to the release of inner non-cross-linked albumin followed by erosion or breaking off of not sufficiently cross-linked weak areas on the surface of AMS by hydration and degradation under the in vitro or in vivo conditions.

As shown in Fig 5, surface damage appeared on the AMS and offers clear-cut evidence of an initial degradation process of AMS probably by attack of enzyme in living tissue along with the phenomenon of cavity formation described above.

The collection process of microspheres from organs after i.v. injection made it possible to measure the size of the microspheres lodged in the organs directly and also to observe their surface damage in living tissue. These studies also show the possibility of using magnetite incorporated in the AMS as a tracer for the biodistribution and biodegradation test of microspheres themselves.

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