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Biodegradability of Poly(γ-benzyl L-glutamate)/poly(ethylene oxide) /poly(γ-benzyl L-glutamate) Block Copolymer in Mice

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Biodegradability of poly(γ-benzyl L-glutamate)/poly(ethylene oxide)/poly(γ-benzyl L-glutamate) block copolymer (GEG) having different content of poly(ethylene oxide) (PEO) were examined using magnetite as a tracer in mice. GEG microspheres containing magnetite were injected into mice through tail vein. Biodegradability and tissue distribution of microspheres were examined by analyzing the amount of magnetite in the microspheres recollected from mice organs after specific time interval. The results showed that GEG microsphere of high content of PEO was degraded more rapidly than those of low content of PEO in the mice organs.

Key words: Biodegradability, Magnetite, Microspheres, Poly(γ-benzyl L-glutamate), Poly(ethylene oxide), Block copolymers

INTRODUCTION

Biodegradable synthetic polymers had been developed and evaluated as drug carriers for their noble delivery system. Recently, synthetic biodegradable polymers, e.g. poly(lactic acid) (Juni *et al.*, 1985), poly(glycolic acid)/poly(lactic acid) copolymer (Iwata and McGinity, 1993), poly(3-hydroxybutyrate) (Wang *et al.*, 1990), poly(glutamic acid), poly(glutamtic acid)/leucine copolymer and polycarbonate (Kojima *et al.*, 1985) have been applied for controlled-release drug delivery systems. Especially, poly(γ-benzyl L-glutamate) (PBLG) polymer is a kind of polypeptide which has advantages of biodegradability, biocompatibility, ease control of hydrophilic/lipophilic balance and introducing of spacer group.

Using PBLG as a basic block, Cho *et al.* synthesized a series of the biodegradable block copolymers of poly(γ-benzyl L-Glutamate)/poly(ethylene oxide)/poly (γ-benzyl L-glutamate) (GEG) (Cho *et al.*, 1990), poly(ε-carbobenzoxy L-lysine)/poly(ethylene oxide)/poly(ε-carbobenzoxy L-lysine) (Cho *et al.*, 1992) and poly(γ-benzyl L-glutamate)/poly(ethylene oxide)/lactoselactone) (Kim *et al.*, 1992).

Synthetic biopolymeric drug-carriers must be estimated the tissue compatibility and biodegradability in the body. In general, radiolabelled method (Yoshioka *et al.*, 1981), fluorescence microscopic method (Lee and Koh, 1987), scanning electron microscopic method (Sanders *et al.*, 1986) and gel permeation chromatography (Spenlehauer *et al.*, 1989) have been adopted for this purpose. Recently the magnetite was examined as a new tracer to evaluate biopolymers especially for their biodegradability in the body (Lee *et al.*, 1988a; Lee, 1988b; Oh *et al.*, 1993).

In this study, to evaluate the effect of the contents of PEO block on the biodegradability of GEG block copolymers, the magnetite containing in the microspheres was adopted as a tracer followed by injection of GEG microspheres into mice.

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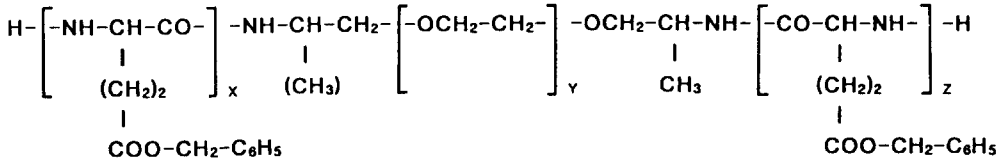
MATERIALS AND METHODS

Materials

GEG were prepared and characterized by Cho *et al.* (1990) as shown in Scheme 1. Magnetic fluids (Ferrocolloid, HC-50) which are hydrophobic colloidal solution of magnetite was obtained from Taiho Industry (Tokyo, Japan). All other reagents were of reagent grade and used as received.

Preparation of microspheres

Polymer microspheres containing magnetite were



Scheme 1. Chemical structure of poly(γ-benzyl L-glutamate)/poly(ethylene oxide)/poly(γ-benzyl L-glutamate) [PBLG/PEO/PBLG]

prepared by a solvent-extraction process (Paventto *et al.*, 1992). In brief, 50 milligrams of polymer and 5 μl of magnetic fluids were dissolved in 5 ml of methylene chloride. And the polymer solution with magnetite was added to a vessel containing 15 ml of 1% aqueous solution of poly(vinyl alcohol). The mixture was then emulsified by sonication (Ultrasonic generator, Nihonseiki Kaisha, Japan) at 100 W for 3 minutes. The o/w dispersion was rapidly added to a 10% isopropyl alcohol and stirred for another 15 min to extract methylene chloride. The microspheres were collected by centrifugation and filtration. The microspheres were then washed with 10% isopropyl alcohol and vacuum dried in a desiccator for at least 24 hours.

Characterization of Microspheres

The average particle diameter of microspheres was measured by the laser scattering particle size analyzer (Mastersizer/E, Malvem, England) and the microspheres were subjected to scanning electron microscopy observation (JSM 840A, Jeol, Japan).

To determine the magnetite contents of microsphere, a weighed amount of microspheres was dissolved in hydrochloric acid, and the concentration of Fe was measured by atomic absorption spectrophotometry (PU9200X, Pye Unicam, U.K.) at 248.3 nm.

Biodegradability Test

The biodegradability of microspheres was tested using male ICR mice (5 to 6 weeks of age, weighing 20-25 grams) which were fed with animal chows and tap water *ad libitum*. Each mouse was injected via tail vein with 3 mg of microspheres dispersed in 0.3 ml of phosphate-buffered saline solution (pH 7.4) containing 0.2% Tween 80. To avoid cluster formation, sonication was performed prior to injection.

Five mice were sacrificed at given intervals and each organ was isolated and homogenized with phosphate-buffered saline solution (pH 7.4). Magnetic microspheres were recollected from the homogenates by a constant-flow magnet separation apparatus (Lee *et al.*, 1988a). Microspheres in the chamber were separated by centrifugation at 1500 rpm for 5 minutes. Recovered magnetic microspheres recollected was dissolved with hydrochloric acid and assayed by atomic absorption spectrophotometer at 248.3 nm.

RESULTS AND DISCUSSION

GEG block copolymers having different contents of PEO were synthesized and characterized as perviously described (Cho *et al.*, 1991).

The solvent extraction technique was adequate to make microspheres of the GEG block copolymer over solvent evaporation and freeze drying technique exa-

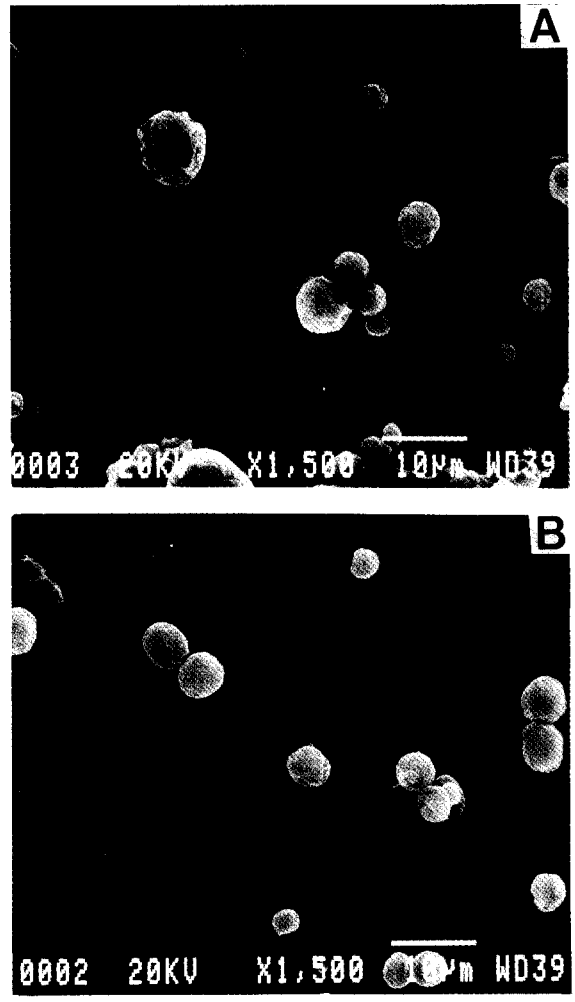


Fig. 1. Scanning electron micrographs of magnetic microspheres prepared by solvent extraction method (×1500). A. GEG-L microspheres, B. GEG-H microspheres

Table I. Mean particle size of microspheres and content of magnetite

Name	Mole % of PEO	Mean diameter, μm	Magnetite content, %
GEG-L	16.2	4.65± 1.02	4.2
GEG-H	53.4	4.86± 0.95	5.1

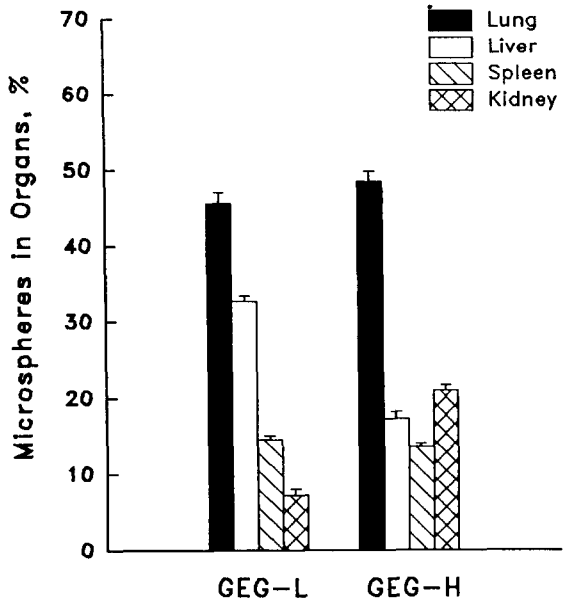


Fig. 2. Initial biodistribution of GEG-L and GEG-H microspheres in mice organs

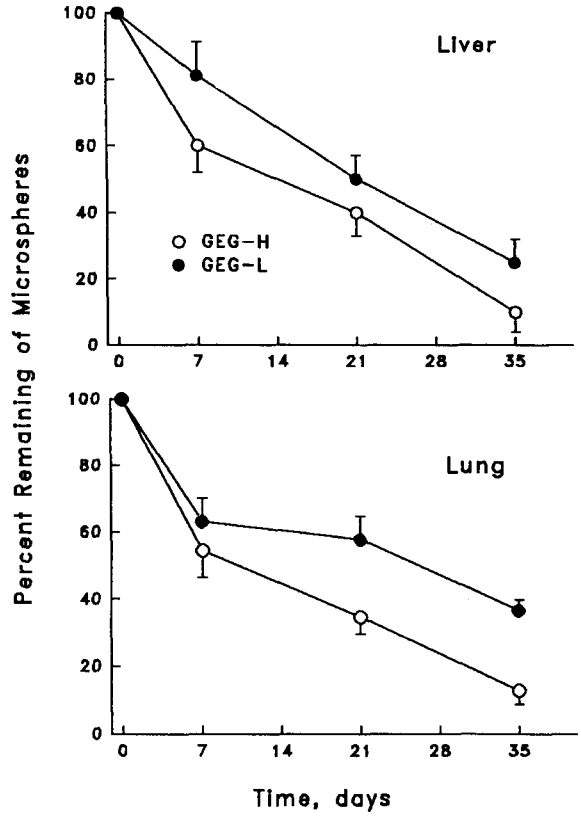


Fig. 3. Biodegradation of GEG-L and GEG-H microspheres in mice liver and lung

mined. The shape of microspheres prepared by solvent extraction method was spherical as shown in Fig. 1. Here, L and H in the GEG block copolymer represent the low and high content of PEO in the GEG block copolymers, respectively. Their size was ranged from 0.5 to 10 μm in diameter, which is suitable for injecdtion. Average particle size of microspheres was summarized in Table I. Mean diameter was similar regardless polymers used. Magnetite can be easily introduced into microspheres physically without altering the chemical and physical properties of the polymer. The contents of magnetite in the microspheres prepared with GEG-L and GEG-H were 4.2 and 5.1%, respectively.

The biodistribution of the microspheres in the tissue of mice in 1 hour after injection was shown in Fig. 2. The use of magnetite as a tracer brings about many simplification in handing as well as in the recovery and detection of microspheres in living tissue. Generally microspheres of 7 μm or more in diameter are rapidly entrapped in the lungs, whereas microspheres with a diameter of 5 μm or less are mainly taken up by cells of the reticuloendotherial system predominantly in the liver and to a lesser extent in the spleen (Burger et al., 1985). In this study, about half portion of microspheres injected was localized in the lungs.

The recovery 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. The degree of *in vivo* degradation of microspheres was evaluated from the remaining magnetite in microspheres. The amount of magnetite in microspheres recollected from the mice lungs and liver were shown in Fig. 3. Magnetite content in GEG-H microspheres was decreased faster than that of GEG-L microspheres. The reason of faster degradability of GEG-H microsphere may be the increase of hydrophilicity and swelling by introduction of hydrophilic PEO group in GEG block copolymer (Cho *et al.*, 1988, 1992).

In conclusion, the biodegradability of GEG block copolymer could be controlled by adjusting their PEO contents and the advantage of using magnetite as a tracer for the estimation of *in vivo* properties including degradation of polymers was confimed again.

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