International Journal of Pharmaceutics, 94 (1993) 143–152

IJP 03168

Polyamino acid microspheres: Preparation, characterization and distribution after intravenous injection in rats [1]

Chun Li, David J. Yang, Li-Ren Kuang and Sidney Wallace

Department of Diagnostic Radiology, The University of Texas, M. D. Anderson Cancer Center, Houston, TX (USA)

(Received 10 August 1992)
(Modified version received 29 October 1992)
(Accepted 11 December 1992)

Key words: Tissue distribution; Microsphere; Targeted delivery; Injectable drug carrier; Biodegradable polymer; Polyamino acid

Summary

A series of poly(hydroxypropyl L-glutamine) (PHPG) microspheres (mean diameter 2–3 μm) modified from poly(benzyl L-glutamate) (PBLG) were prepared and characterized. The microspheres were radiolabeled with ^{131}I for in vivo evaluation. Two different labeling methods were used for these particles. For PHPG, microspheres were directly labeled with ^{131}I via Iodogen. For PBLG, microcapsules loaded with a radiotracer ([^{131}I]ethyliopanoate) were prepared. The tissue distribution of these particles was studied after intravenous injection in rats. The liver uptake of PHPG microspheres at 20 minutes postadministration was reduced from 74 to 19% of injected dose when hydrophilicity of PHPG microspheres increased. Such reduction was also noted by increasing blood activity and decreasing spleen activity. Moderate clearance of PHPG microspheres from the liver and the spleen was observed in the experimental period (2 days), suggesting that the polymer was degraded. The hydroxy functional groups present on the surface of poly(hydroxypropyl L-glutamine) microspheres allow attachment of targeting devices or drugs. Thus they may find potential applications as targetable drug carriers.

Introduction

During the past decade, several potential site-specific drug delivery systems for parenteral administration, such as liposomes and microspheres have been designed (Davis and Illum, 1988; Gregoriadis, 1988). Their purpose is to change the body distribution of carried drugs, targeting them to specific organs or cells, thereby protecting normal tissue from adverse toxic effects. One of the drawbacks of using particulate delivery systems is the rapid clearance of small particles by the reticuloendothelial system (RES). These particles are taken up mainly by the Kupffer cells of the liver and to a lesser degree by the macrophages of the spleen and the bone marrow (Illum et al., 1982).

Two approaches to deliver particulate carrier

Correspondence to: D.J. Yang, The University of Texas, M.D. Anderson Cancer Center, Box 59, 1515 Holcombe Boulevard, Houston, TX 77030, U.S.A.

[1] Presented in part at the 19th International Symposium on Controlled Release of Bioactive Materials, July 26–31, 1992.

systems to specific sites other than the liver and the spleen have been evaluated. Attempts have been made to block the RES by injecting massive doses of particles (Bradfield, 1980). Although suppression of the RES is possible, this approach is hardly applicable for clinical practice. An alternative solution is to modify the surface characteristics of microspheres so that they will be less recognizable by the RES. Previous work has established that uptake of microspheres by macrophages is a function of their size and of surface properties such as charge and hydrophilicity (Wilkins and Myers, 1966; Arturson et al., 1983). In vitro phagocytosis studies by mouse peritoneal macrophages showed that nonionic hydrophilic microspheres tend to cause the least phagocytosis activities (Tabata and Ikata, 1988). Recently, it has been demonstrated that when coated with two block polyoxyethylene-polyoxypropylene copolymers, poloxamer-338 and poloxamine-908, the circulation time of latex particles in the blood stream was significantly improved as a result of reduced RES uptake (Illum and Davis, 1984; Illum et al., 1987). These results were interpreted in terms of the steric repulsive barriers, generated by the hydroxyethylene segment of the polymer, both to protein adsorption and to particle-cell interaction. Similarly, liposomes containing polyethylene glycol conjugates (Klibanov, 1990; Maruyama, 1992), as well as ganglioside GM_1 (Allen and Chonn, 1987; Gabizon and Papahadjopoulos, 1988) were demonstrated to exhibit a prolonged circulation time in the blood.

Synthetic polyamino acids have been investigated for possible use in a variety of biomedical applications (Anderson et al., 1974). One of the interesting properties of polyamino acids is their ability to degrade into physiological molecules. The present study evaluates the potential of poly(hydroxypropyl L-glutamine) (PHPG) microspheres, derived from poly(benzyl L-glutamate) (PBLG) microspheres, as drug carriers. PBLG was chosen as our model polymer system because of its good solubility and easily modifiable side chain. The possibility of altering the tissue distribution of surface modified microspheres was investigated.

Materials and Methods

Materials

PBLG (M_w 50 000), 3-amino-1-propanol, iopanoic acid and polyvinyl alcohol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 1,6-Hexanediamine and tyramine were obtained from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Iodogen was obtained from Pierce (Rockford, IL, U.S.A.). Sodium [^{131}I]iodide (specific activity 7.75 Ci/mg, 680 mCi/ml) was supplied by DuPont New England Nuclear (Boston, MA, U.S.A.).

Preparation of PBLG microspheres

PBLG microspheres were prepared by the solvent evaporation method. A solution of PBLG (0.33 g) in 15 ml methylene chloride was added to a 200 ml aqueous solution of polyvinyl alcohol (1% w/v). The mixture was stirred at 2000 rpm for 30 min and then at 1000 rpm for an additional 12 h. The emulsion was centrifuged, washed with water, and finally filtered through a 5 μm nylon filter. The microspheres were stored as a wet pellet at 4°C.

Surface modification of PBLG microspheres

PHPG microspheres were derived from PBLG microspheres using the method of Marchant et al. (1983) with modifications. PBLG microspheres (150 mg) were resuspended under sonication in 2 ml aminopropanol containing 5% hexanediamine (w/v) as a crosslinker. The reaction was conducted at 70°C with reciprocal rotation for 2 h (PHPG-1), 4 h (PHPG-2) and 5 h (PHPG-3). To terminate the reaction, the microsphere suspension was diluted with water and centrifuged at 10000 rpm for 15 min. The supernatant was discarded while the microsphere pellet was resuspended in distilled water. This process was repeated five times.

Characterization of PHPG microspheres

To determine the extent of conversion, PHPG microspheres were completely hydrolyzed in 2 N NaOH overnight at 37°C and then neutralized with 2 N HCl. The hydrolytes were analyzed for the amount of remaining benzyl groups by high-

performance liquid chromatography. The HPLC system has an RP-18 column, a Perkin-Elmer isocratic LC pump (Model 250), a PE Nelson 900 series interface, a Spectra-Physics UV/Vis detector (Model SP 8540) and a data station. The elutant (20% acetonitrile in 0.2 M phosphate-buffered solution, pH 4.6) was run at 1 ml/min with UV detection at 254 nm. The amount of unsubstituted benzyl groups, defined as percent mol of polymer repeating units, for commercial PHPG and PBLG was determined to be 1.5 and $100 \pm 4\%$, respectively.

The swelling ratio of the prepared microspheres was determined by weighing the wet microsphere pellets. These pellets were lyophilized to a constant mass and weighed again. The swelling ratio is defined as (wet weight − dry weight) × 100/dry weight

Microscopic examination

For scanning electron microscopic examination (SEM), the microspheres were diluted to varying concentrations in water. Each sample (100 ml) was placed onto a 0.1 μm Nuclepore membrane and air dried. The dried filters were mounted onto stubs and sputter-coated with 200 Å gold-palladium, 80:20, in a Hummer VI (Technics, Springfield, VA, U.S.A.) and examined in a Hitachi Model S520 scanning electron microscope.

Preparation of [^{131}I]ethyliopanoate loaded microcapsules

Iopanoic acid was converted to its ethyl ester using thionyl chloride and ethanol. Radioisotope exchange reaction on the ester yielded ^{131}I-labeled ethyliopanoate (radiochemical yield: 34%) (Yang et al., 1989). Radiolabeled PBLG microcapsules were prepared in the same way as plain PBLG microspheres with a slight modification. Into the methylene chloride solution of PBLG polymer was dissolved unlabeled ethyliopanoate (1% w/v) and [^{131}I]ethyliopanoate (320 μCi). The solution was mixed with a polyvinyl alcohol solution with vigorous stirring. The resulting emulsion was centrifuged and purified as described above (under *Preparation of PBLG microspheres*).

Radiolabeling of PHPG microspheres

^{131}I was directly labeled to the microspheres via covalent bonding (Fig. 1). This was achieved by treating PBLG microspheres with aminopropyl alcohol in the presence of tyramine (1% w/w) and hexanediamine (5% w/w). The reaction was conducted at 70°C for 2, 4 and 5 h. After centrifugation and washing steps, the microspheres

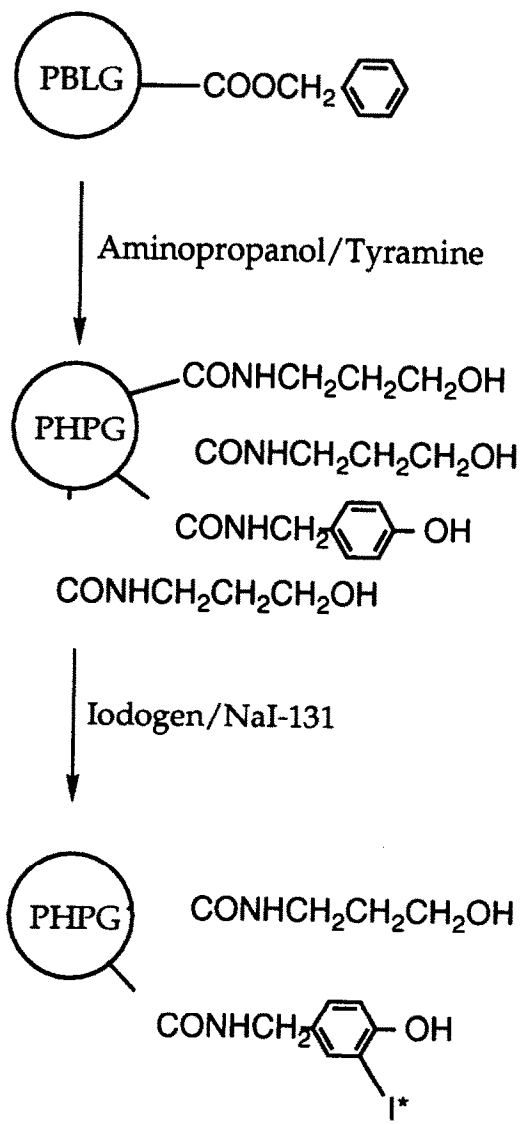

Fig. 1. Reaction scheme for ^{131}I radiolabeling of poly(hydroxypropyl L-glutamine) (PHPG) microspheres. Traces of tyramine unit were introduced to the surface of microspheres to facilitate iodination. PBLG, poly(benzyl L-glutamate).

were resuspended in phosphate-buffered saline (PBS) solution. The suspension (0.2 ml) was then reacted with $Na^{131}I$/0.1 M PBS (0.2 ml, 600 μCi) in a vial containing Iodogen (Fraker and Speck, 1978).

In vitro stability of [^{131}I]ethyliopanoate loaded microcapsules and radiolabeled PHPG microspheres

Radiolabeled PBLG or PHPG particles (20 μCi/ml) were incubated in 50% serum at 37°C. At various time intervals, aliquots of serum were removed and centrifuged. The radioactivity of supernatant was measured with a γ-counter (Packard, Model B5002, Downers Grove, IL, U.S.A.).

Determination of particle size

The size distribution of the microspheres was estimated with a Coulter Counter and Coulter Channelyzer (Coulter Electronics, Hialeah, FL, U.S.A.).

Animals

Female Sprague-Dawley rats (150–175 g) were purchased from Harlan, Inc. (Indianapolis, IN, U.S.A.). All rats were quarantined for 48 h prior to studies.

Tissue distribution of PBLG and PHPG microspheres after i.v. injection

The rats were anesthetized with ketamine (10 mg, i.p.). The microcapsules loaded with [^{131}I]ethyliopanoate or radiolabeled PHPG microspheres were given i.v. through the tail vein at a total volume of 0.4 ml. The dose corresponded to 12 mg of dry microspheres containing ~ 10^9 particles with total activity of about 6 ± 2 μCi. The animals were killed at 20 min, and 3, 6, 24 and 48 h by cervical dislocation. The organs were removed and weighed for radioactivity measurements. The percent of injected dose per g of tissue was determined.

Results

Preparation and characterization of microspheres

PBLG microsphere particles were irregular in shape, with a smooth surface (Fig. 2). The microspheres had a mean diameter of 2–3 μm; more than 97% of the particles were less than 5 μm in diameter. Modification of PBLG microspheres resulted in PHPG particles with hydrophilic hydroxyl functional groups on the microsphere surface. Table 1 summarizes the properties of three preparations of PHPG microspheres. The swelling ratio increased from 3% of PBLG to 36% of PHPG-2, which had been reacted with aminopropanol for 4 h. A scanning electron micrograph revealed that as the reaction time increased, PHPG microspheres became more hydrophilic. Microspheres tended to become flat and sticky after air drying (Fig. 3). Extended treatment (> 5 h) with aminopropanol, however, eventually caused microspheres to be solubilized in aminopropanol solution.

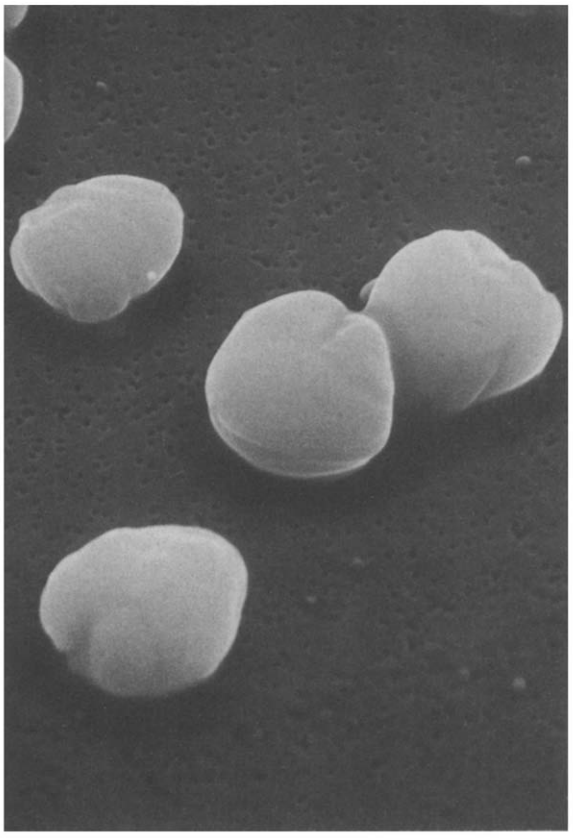

Fig. 2. Scanning electron micrograph of poly(benzyl L-glutamate) microspheres prepared by the solvent evaporation method. (×5000)

TABLE 1

Preparation and properties of PHPG microspheres

Microsphere code	Reaction time (h)	% of conversion [a]	Swelling ratio (%)
PBLG	0	0	3
PHPG-1	2	12	14
PHPG-2	4	86	36
PHPG-3	5	95	n/a

[a] mol% of unsubstituted benzyl group, as determined by HPLC. PBLG, poly(benzyl L-glutamate); PHPG, poly(hydroxypropyl L-glutamine).

Radiolabeling of microspheres

For PBLG, direct labeling with ^{131}I is not possible. Thus, labeling was achieved by encapsulation of a radiotracer [^{131}I]ethyliopanoate (radiochemical yield: 48%). The stability of [^{131}I]ethyliopanoate loaded PBLG microcapsules was determined in 50% rat serum at 37°C. 6% of radioactivity was released after 1 h of incubation; 28% was released after 24 h.

For PHPG, direct labeling with ^{131}I was accomplished by incorporating tyramine units into the microspheres. The phenol units of tyramine act as anchoring sites for ^{131}I (Fig. 1). The radiochemical yield was 65%. Radiolabeling of PHPG microspheres was very stable. Only 1% of radioactivity was found dissociated from PHPG microspheres after 2 h of incubation in 50% serum. Even after 2 days, 96% of radioactivity was still bound to PHPG microspheres.

Tissue distribution of PHPG microspheres

The quantitative distribution of the microspheres in different organs was studied over a period of 24 h after i.v. administration. The radioactivity in the blood, lung, liver, kidney and spleen was determined (Table 2. The data reported represent a percentage of the injected dose per gram of tissue weight. Since blood (usually 7% of body weight, 10–11 g) and liver (7–8 g) weigh the most among the tissues studied, they were the main organs responsible for the observed radioactivity. Including the spleen and the lung, more than 90% of the injected dose was taken up by these tissues in the first 20 min. The activity in the kidney was low. Values obtained

TABLE 2

Organ distribution of poly(hydroxylpropyl L-glutamine) microspheres after intravenous injection to rats (n = 3) [a]

Organ	Microsphere distribution (mean ± SD) after treatment				
	20 min	3 h	6 h	1 days	2 days
(A) PHPG-1: 2 h of treatment with aminopropanol					
Blood	0.21 ± 0.01	0.28 ± 0.04	0.12 ± 0.01	0.07 ± 0.01	0.03 ± 0.01
Lung	4.99 ± 0.58	4.21 ± 0.69	2.49 ± 0.15	1.31 ± 0.09	0.92 ± 0.09
Liver	9.37 ± 0.22	10.20 ± 0.89	7.12 ± 0.80	5.90 ± 0.90	4.31 ± 0.31
Kidney	0.70 ± 0.12	0.44 ± 0.08	0.30 ± 0.04	0.15 ± 0.01	0.11 ± 0.02
Spleen	17.25 ± 4.49	16.22 ± 3.23	15.71 ± 5.83	13.91 ± 2.02	8.93 ± 2.29
(B) PHPG-2: 4 h of treatment with aminopropanol					
Blood	2.03 ± 0.49	0.44 ± 0.06	0.25 ± 0.02	0.06 ± 0.01	0.02 ± 0.01
Lung	20.8 ± 4.88	5.75 ± 0.36	1.62 ± 0.59	0.25 ± 0.10	0.13 ± 0.03
Liver	5.54 ± 0.52	5.15 ± 0.72	2.90 ± 0.13	1.55 ± 0.24	0.90 ± 0.13
Kidney	0.99 ± 0.21	0.67 ± 0.21	0.34 ± 0.03	0.07 ± 0.01	0.04 ± 0.01
Spleen	30.2 ± 4.4	19.6 ± 5.8	17.4 ± 2.3	7.77 ± 1.51	7.53 ± 1.85
(C) PHPG-3: 5 h of treatment with aminopropanol					
Blood	4.41 ± 0.59	2.06 ± 0.10		0.61 ± 0.05	
Lung	3.83 ± 0.40	2.34 ± 0.55		0.48 ± 0.20	
Liver	2.09 ± 0.33	1.78 ± 0.70		0.74 ± 0.20	
Kidney	0.44 ± 0.22	0.57 ± 0.25		0.20 ± 0.03	
Spleen	31.43 ± 5.3	26.02 ± 6.2		14.66 ± 3.2	

[a] Data are presented as percentage uptake/g of tissue.

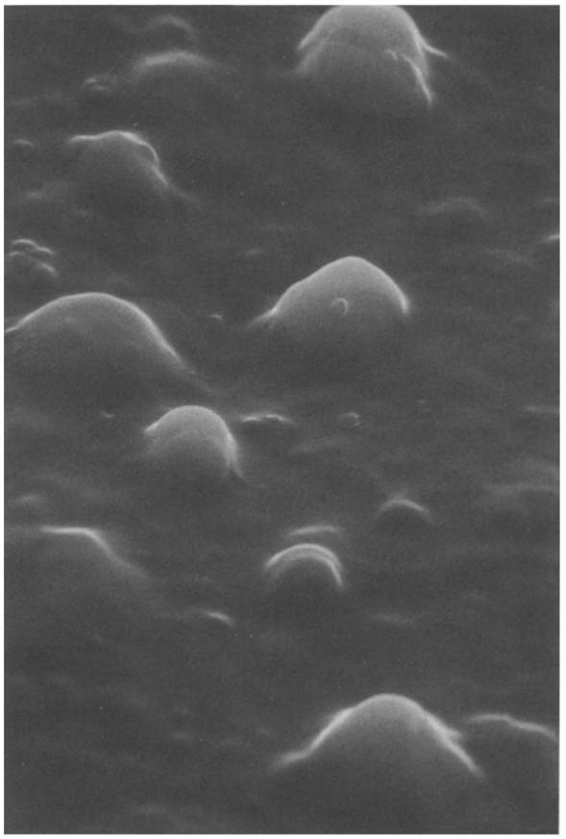

Fig. 3. Scanning electron micrograph of poly(hydroxypropyl L-glutamine) microspheres prepared by reacting poly(benzyl L-glutamate) microspheres with aminopropanol at 70°C for 4 h. (×5000)

from the thyroid were very low (< 0.2% of injected dose per g of tissue, data not shown). PHPG-2 microspheres were localized in the lung in a large quantity, probably because of trapping of aggregated particles by the lung capillary bed. The particles that localized in the lung, however, were redistributed. The activity in the lung of PHPG-2-treated rats redistributed to the levels of PHPG-1 and PHPG-3 3 h after injection (Table 2).

The radioactivity was gradually excreted from the organs. A significant amount of activity was found only in the liver and spleen 2 days after injection. During this period, activity was found in the rat urine and intestinal content.

Distribution data at 20 min after administration are presented in Fig. 4. The data indicate

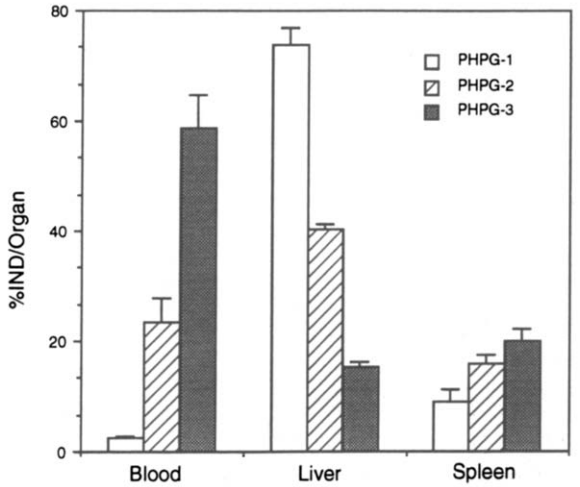

Fig. 4. Distribution of poly(hydroxypropyl L-glutamine) microspheres in various organs 20 min after i.v. administration. PHPG-1 had a reaction time of 2 h; PHPG-2, 4 h; and PHPG-3, 5 h.

that PHPG-3 (see Table 1), the most hydrophilic in the series, had the least liver uptake and the highest radioactivity in the blood. From PHPG-1 to PHPG-2 to PHPG-3, there was a substantial reduction in liver uptake and an increase in the concentration of microspheres circulating in the

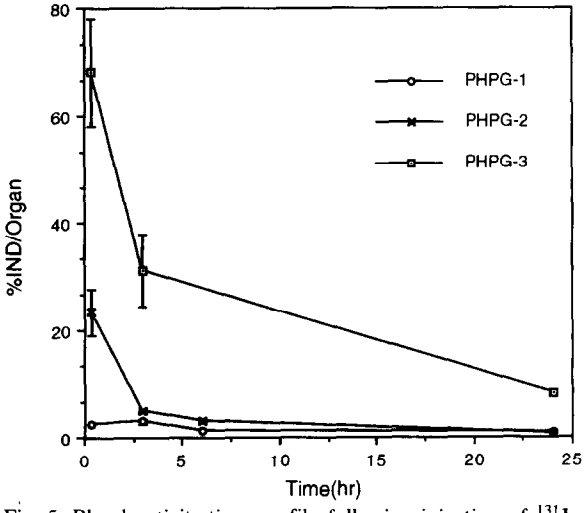

Fig. 5. Blood activity-time profile following injection of ^{131}I-labeled poly(hydroxypropyl L-glutamine) microspheres to rats. PHPG-1 had a reaction time of 2 h; PHPG-2, 4 h; and PHPG-3, 5 h.

blood. Surprisingly, the levels in the spleen increased at the same time.

Fig. 5 shows the kinetic profile of blood clearances. Most blood level activities for PHPG-1 and PHPG-2 microspheres were cleared at 20 min and 180 min, respectively. However, it took 24 h for PHPG-3 to reduce the blood activities to the same level. The estimated half-lives for blood clearance were < 20 min for PHPG-1; 100 min for PHPG-2 and 180 min for PHPG-3.

Tissue distribution of PBLG microspheres

To compare tissue distribution of PHPG microspheres with their precursor-PBLG microspheres, PBLG microcapsules loaded with [^{131}I] ethyliopanoate were prepared and their distribution determined. The results are summarized in Table 3. 20 min after injection, 25% of the injected dose of PBLG microcapsules was found deposited in the liver, a value lower than that of PHPG-1 and PHPG-2. In contrast to PHPG microspheres, total activities of PBLG microcapsules in major organs including liver, blood, spleen and lung can account for only 60% of the injected dose. Activity in all organs was quickly cleared from the body. Because PBLG microspheres are not expected to be degradable in such a short time, the data can possibly be attributed to leakage of radiotracer from the microcapsules.

Discussion

Crosslinked PHPG hydrogel in the form of small particles is an attractive alternative as a carrier for targeting. The hydrogel microspheres were prepared by treating PBLG microspheres with aminopropyl alcohol containing 5% (w/v) hexanediamine as a crosslinker. As reaction time increased, more phenyl groups were substituted by hydroxylpropyl groups. As a result, the microspheres became increasingly hydrophilic, as indicated by the changes of swelling ratio (Table 1). PBLG particles with a mean diameter of 2–3 μm were prepared; more than 95% of the population had diameters less than 5 μm. This is smaller than the diameter of capillaries. For PHPG particles, swelling in contact with biological fluids will increase the particle size, but only to a limited extend (not exceed 50% of PBLG in diameter as compared to PBLG). Furthermore, as the particles became more hydrophilic, they became less rigid, making it possible for them to squeeze through the vascular tube.

Because slow leaching of the ^{131}I label from PHPG microspheres would interfere with results of distribution experiments, we tested the stability of radiolabling. Direct iodination of PHPG microspheres using our reaction scheme (Fig. 1) resulted in stable radiolabeling as shown in in vitro stability tests. The presence of free iodine in vivo is unlikely, since no significant levels of activity were detected in the thyroid. In addition, the radioactivity levels were low in the kidney ($< 1\%$ of injected dose per g). Others have reported a relatively high kidney uptake in mice injected with polymethacrylic nanoparticles ($\sim 18\%$ of injected dose per g), which was attributed to the slow release of ^{125}I from the labeled particles (Rolland et al., 1989).

An attempt to label PBLG microspheres using the iodogen method was unsuccessful because of

TABLE 3

Organ distribution of [^{131}I]ethyliopanoate loaded poly(benzyl L-glutamate) microspheres after intravenous injection to rats (n = 3) [a]

Organ	Microsphere distribution (mean ± SD) after treatment				
	20 min	1 h	3 h	6 h	24 h
Blood	2.60 ± 0.15	1.53 ± 0.71	1.46 ± 0.21	1.30 ± 0.26	0.29 ± 0.16
Lung	7.21 ± 1.81	1.86 ± 0.40	1.06 ± 0.16	1.02 ± 0.19	0.28 ± 0.03
Liver	3.38 ± 0.54	1.80 ± 0.78	1.20 ± 0.15	1.16 ± 0.14	0.54 ± 0.16
Kidney	1.53 ± 0.18	0.74 ± 0.28	0.58 ± 0.09	0.58 ± 0.05	0.24 ± 0.08

[a] Data are presented as percentage uptake/g of tissue.

the lacking of anchoring phenol units in the microspheres. Therefore, PBLG microspheres were labeled by entrapping a radiotracer, [^{131}I]ethyliopanoate. Unfortunately, this approach resulted in a gradual loss of the loaded materials. For this reason, the tissue distribution data reported with PBLG microspheres should be used with caution (Table 3). The data, however, may be regarded as an in vivo release profile. The long-lasting blood level of radioactivity could be of interest if the microcapsules were used for modifying the pharmocokinetics of drugs.

As indicated in Table 2, most PHPG microspheres were cleared from the blood, lung and kidney in two days. Clearance from the liver and spleen was relatively moderate (Table 2). Previously published results suggested that proteolytic enzymes played an important role in the in vitro degradation of structurally similar poly(hydroxyethyl L-glutamine) (Dichinson and Hiltner, 1981). The same enzymes may be responsible for the degradation of PHPG microspheres in the liver and the spleen. The fact that radioactivity was found in the gut contents of rats (data not shown) further supports the degradation of PHPG in the liver. Others have observed the same phenomenon and have suggested that microspheres are secreted by the hepatic cells into the bile and then find their way to the gut (Sjoholm and Edman, 1979). This interpretation suggests that the microspheres are degraded in the lysosomes, yielding fragments small enough to be secreted. To avoid long-lasting accumulation in tissues, the ability of microspheres to degrade is an important consideration in designing drug carriers. Work on long-term degradability and biocompatibility of PBLG and PHPG microspheres in the liver of rats is in progress in this laboratory and will be reported elsewhere.

The RES and particularly the phagocytic cells of the liver and spleen have a major role in removing small foreign particles from the circulation. Thus, it is not surprising that more than 70% of injected PHPG-1 microspheres were taken up by the liver in the initial 20 min (Fig. 4). However, by introducing more hydroxy groups on the surface of PBLG microspheres and making them more hydrophilic, the liver uptake of surface-modified microspheres reduced substantially (Tables 1 and 2). Our observation suggests that hydrophilicity and probably the steric barrier on the surface of PHPG particles as well are responsible for the reduction in liver uptake. Porter et al have previously shown that while liver uptake of small colloidal particles ($d < 150$ nm) was greatly suppressed by coating with poloxamer-407, coating on the surface of particles greater than 250 nm was not very efficient (Porter et al., 1992). This is explained in term of the steric barrier. For larger colloidal particles coated with poloxamer, the surfactant was adsorbed in a more crowed fashion, resulting in reduction in polymer chain mobility and the effectiveness of the steric barrier (Porter et al., 1992). On the other hand, such a correlation between steric barrier and particle size does not exist for PHPG microspheres. Our data showed that the liver is unable to remove PHPG-3 particles, although their diameters were much greater than 250 nm. In fact, when PHPG particles became more hydrophilic, they became less rigid (increase in mobility) (Fig. 3), thus imposing more steric barrier to particle-cell interaction.

One would expect that with decreased liver uptake, more microspheres would remain in the vascular compartment. Fig. 4 indicates that blood activity increased more than 20-fold 20 min after injection, when the liver uptake was reduced from 75 to 20% (Fig. 4). Blood levels measured in a period of one day suggest that extensive treatment of PBLG microspheres with aminopropanol (PHPG-3) leads to a higher blood activity in a longer period of time (Fig. 5). These results clearly demonstrat that the uptake of microspheres by Kupffer cells can be avoided and that targeting to other tissue is possible. It is worth noting that the functional hydroxy group allows attachment of targeting device such as receptor ligands or monoclonal antibodies.

Interestingly, the spleen was able to taken up a higher amount of the injected dose, accompanying a reduction in liver uptake (Fig. 4). The spleen uptake of PHPG-3 microspheres can be approx. 15 times greater than that of the liver on a per gram basis. Similar high spleen accumulation has been observed with PEG-PE-containing

PE liposomes (Litzinger and Huang, 1992), as well as GM_1-containing PC liposomes (Liu et al., 1991). A decrease in liver uptake and the diversion of the particles to the spleen and lung were previously reported when the phagocytic activity of the liver was depressed by means of prior administration of microspheres (Illum et al., 1986). One possible explanation for the observed high spleen uptake in this study is the spleen filtration mechanism. The spleen contains a filtration mechanism to remove particles or liposomes with diameters larger than 250 nm (Liu et al., 1991; Moghimi et al., 1991). Normally, particles injected into the blood stream will be removed efficiently by the liver, and to a lesser degree by the spleen. Modification of PHPG microspheres resulted in a substantial reduction in liver uptake, leaving these particles with better access to spleen filtration. Another possibility is the splenic macrophage uptake of PHPG microspheres. However, this is unlikely because both hydophilicity and the steric barrier will prevent the interaction between PHPG particles and the splenic cells. Further study of the distribution of PHPG microspheres in the spleen, as well as the role of opsonins on particle clearance by the spleen, is needed to clarify this point.

The ability to suppress liver uptake and redirect microspheres to the spleen has two potential implications for the design of targeted delivery systems (Moghimi et al., 1991): (1) to minimize the uptake of both liver and spleen, one should not only consider the surface properties (hydrophilicity) of microspheres but also other microsphere characteristics such as size and mechanical properties (flexibility) that may eventually affect spleen uptake; (2) targeting to the spleen may be achieved by applying the same principles. PHPG microspheres offer a new possibility of achieving these goals.

Conclusion

Poly(benzyl L-glutamate) and poly(hydroxypropyl L-glutamine) microspheres were prepared and their in vivo tissue distribution determined. We demonstrated that with increasing hydrophilicity, the liver uptake of the PHPG microspheres was greatly reduced. Unlike blood activity, spleen uptake increased as a result of surface modification. PHPG microspheres cleared from the liver and spleen in a moderate rate, implying that they are degradable in the liver. PHPG microspheres offer advantages in that drugs may be adsorbed on the microspheres or covalently attached via hydroxy functional groups. Further studies to elucidate the long-term degradability and biocompatibility of these microspheres in the liver are necessary to warrant their use as injectable drug carriers.

Acknowledgement

The authors wish to thank Kim Dulski for her assistance in obtaining SEM photographs. We would also like to thank Dr Kenneth Wright for kind suggestions. This study was supported in part by George and Cleo Cook Fund and the John S. Dunn Foundation. The SEM facility and the animal research facilities are supported by NIH core grant CA16672.

References

Allen, T.M. and Chonn, A., Large unilamellar liposomes with low uptake into the reticuloendothelial system. *FEBS Lett.*, 223 (1987) 42–46.

Anderson, J.M., Gibbons, D.F., Martin, R.L., Hiltner, A. and Woods, R., The potential for poly-α-amino acids as biomaterials. *J. Biomed. Mater. Res. Symp.*, 5 (1974) 197–207.

Arturson, P., Laakso, T. and Edman, P., Acrylic microspheres in vivo: blood elimination, kinetics and organ distribution of microspheres with different surface characteristics. *J. Pharm. Sci.*, 72 (1983) 1415–1420.

Bradfield, J.W.B., A new look at reticuloendothelial blockade. *Br. J. Exp. Pathol.*, 61 (1980) 617–623.

Davis, S.S. and Illum, L., Polymeric microspheres as drug carriers. *Biomaterials*, 9 (1988) 111–115.

Dichinson, H.R. and Hiltner, A., Biodegradation of a poly(α-amino acid) hydrogel: II. In vitro. *J. Biomed. Mater. Res.*, 15 (1981) 591–603.

Fraker, P.J. and Speck, J.C., Protein and cell membrane iodinations with a sparingly soluble chloramide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. *Biochem. Biophys. Res. Commun.*, 80 (1978) 849–857.

Gabizon, A. and Papahadjopoulos, D., Liposome formulations with prolonged circulation time in blood and en-

hanced uptake by tumors. *Proc. Natl. Acad. Sci. USA,* 85 (1988) 6949–6953.

Gregoriadis, G., *Liposomes as Drug Carriers: Recent Trends and Progress,* Wiley, New York, 1988.

Illum, L., Davis, S.S., Wilson, C.G., Thomas, N.W., Frier, M. and Hardy, J.G., Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape. *Int. J. Pharm.,* 12 (1982) 135–146.

Illum, L. and Davis, S.S., The organ uptake of intravenously administered colloidal particles can be altered using a nonionic surfactant (poloxamer-338). *FEBS Lett.,* 167 (1984) 79–82.

Illum, L., Thomas, N.W. and Davis, S.S., Effect of a selected suppression of the reticuloendothelial system on the distribution of model carrier particles. *J. Pharm. Sci.,* 75 (1986) 16–22.

Illum, L., Davis, S.S., Muller, R.H., Mak, E. and West, P., The organ distribution and circulation time of intravenously injected colloidal carriers sterically stabilized with a block copolymer-poloxamine 908. *Life Sci.,* 40 (1987) 367–374.

Klibanov, A.L., Maruyama, K., Torchilin, V.P. and Huang, L., Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett.,* 286 (1990) 235–237.

Litzinger, D.C. and Huang, L., Amphipathic poly(ethylene glycol) 5000-stabilized dioleoylphosphatidylethanolamine liposomes accumulate in spleen. *Biochim. Biophys. Acta,* 1127 (1992) 249–254.

Liu, D., Mori, A. and Huang, L., Large liposomes containing ganglioside GM_1 accumulated effectively in spleen. *Biochim. Biophys. Acta,* 1066 (1991) 159–165.

Marchant, R., Hiltner, A., Hamlin, C., Rabinovitch, A. Slobodkin, R. and Anderson, J.M., In vivo biocompatibility studies: I. The cage implant system and a biodegradable hydrogel. *J. Biomed. Mater. Res.,* 17 (1983) 301–325.

Maruyama, K., Yuda, T., Okamoto, A., Kojima, S., Suginaka, A. and Iwatsuru, M., Prolonged circulation time in vivo of large unilamellar liposomes composed of distearoyl phosphatidylcholine and cholesterol containing amphipathic pol(ethylene glycol). *Biochim. Biophys. Acta,* 1128 (1992) 44–49.

Moghimi, S.M., Porter, C.J., Muir, I.S., Illum, L. and Davis, S.S., Non-phagocytic uptake of intravenously injected microspheres in rat spleen: influence of particle size and hydrophilic coating. *Biochem. Biophys. Res. Commun.,* 177 (1991) 861–866.

Porter, C.J.H., Moghimi, S.M., Illum, L. and Davis, S.S., The polyethylene/polyoxypropylene block copolymer poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow. *FEBS Lett.,* 305 (1992) 62–66.

Rolland, A., Collet, B., LeVerge, R. and Toujas, L., Blood clearance and organ distribution of intravenously administered polymethacrylic nanoparticles in mice. *J. Pharm. Sci.,* 78 (1989) 481–484.

Sjoholm, I. and Edman, P., Acrylic microspheres in vivo: I. Distribution and elimination of polyacrylamide microspheres after intravenous and intraperitoneal injection in mouse and rat. *J. Pharmacol. Exp. Ther.,* 211 (1979) 656–662.

Tabata, Y. and Ikata, Y., Effect of the size and surface charge of polymer microspheres on their phagocytosis by macrophage. *Biomaterials,* 9 (1988) 356–362.

Wilkins, D.J. and Myers, P.A., Studies on the relationship between the electrophoretic properties of colloids and their blood clearance and organ distribution in the rat. *Br. J. Exp. Pathol.,* 47 (1966) 568–576.

Yang, D.J., Ciliax, B.J., Van Dort, M.E., Gildersleev, D.L., Pirat, J.-L, Young, A.B. and Wieland, D.M., Synthesis and receptor binding studies of (±)1-Iodo-MK-801. *Pharm. Res.,* 6 (1989) 474–476.