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RESEARCH PAPER

Encapsulation of Hydrophilic and Lipophilic Drugs in PLGA Nanoparticles by the Nanoprecipitation Method

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ABSTRACT

The purpose of this study was to assess the relative advantages and drawbacks of the nanoprecipitation–solvent displacement method for a range of drugs with respect to the particle size and drug encapsulation in polylactic-co-glycolic acid (PLGA) nanoparticles. The particle size analysis indicated a unimodal particle size distribution in all systems, with a mean diameter of 160–170 nm, except for insulin nanoparticles, which showed a smaller particle size. The results of the encapsulation efficiency analysis demonstrated that more lipophilic drugs, such as cyclosporin and indomethacin, do not suffer from the problems of drug leakage to the external medium, resulting in improved drug content in the nanoparticles. In spite of the fact that valproic acid is a liquid that is very sparingly soluble in water, very low encapsulation efficiency was obtained. Ketoprofen, a drug sparingly soluble in water, demonstrated intermediate values of encapsulation that were well correlated with its intermediate lipophilicity. More hydrophilic drugs, such as vancomycin and phenobarbital, were poorly encapsulated in PLGA nanoparticles. Insulin was preferentially surface bound on the PLGA nanoparticles. However, a strong hypoglycemic effect of the insulin was observed after administration of the suspension of PLGA nanoparticles with surface-bound insulin to the ileum loop of male Wistar rats.

INTRODUCTION

The use of biodegradable polymers as drug carriers has long been of interest in controlled-release technology because of the ability of these polymers to be reabsorbed by the body. The field of biodegradable polymers is progressing rapidly, so that researchers now have at their disposal a substantial number of degradable polymers with

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a range of degradation rates (1). The most widely used and studied class of biodegradable polymers is the polyesters, including polylactic acid (PLA) and polylactic-*co*-glycolic acid (PLGA). Micro- and nanoparticulate systems formulated with these polymers have shown wide applicability to oral delivery (2,3), subcutaneous injection (4–6), and sustained delivery of lipophilic (7) and some hydrophilic drugs (2,6).

Efficient delivery of bioactive agents and peptides or proteins to the systemic circulation and then to a target cell or organ has received considerable attention in medicine due to recent advances in biotechnology. Nevertheless, the methods available for preparing biodegradable micro- and nanoparticles are basically applicable to lipophilic drugs, and methods to encapsulate water-soluble drugs are still scarce. A method very often used to prepare colloidal carriers of lipophilic drugs of a matricial type (nanospheres) or a vesicular type (nanocapsules) was described by Fessi et al. (8). This method involves the nanoprecipitation of a preformed polymer from an organic solution in which it is held by the diffusion of the organic solvent in the aqueous medium in the presence of a surfactant. This method is basically applicable to lipophilic drugs due to the miscibility of the solvent with the aqueous phase. Taking into consideration the lack of information about more hydrophilic drugs, we attempted to assess the relative advantages and drawbacks of the method described above for a range of drugs with respect to the particle size and drug encapsulation. Cyclosporin A, indomethacin, and valproic acid were used as more lipophilic drugs, insulin as an amphiphilic polypeptide drug, ketoprofen as a sparingly water-soluble drug, and vancomycin and phenobarbital as more hydrophilic drugs (9).

EXPERIMENTAL

Materials

Polylactic-*co*-glycolic acid with a molecular weight of 10 kD and a lactide-glycolide ratio of 75:25 was purchased from Wako Pure Chemicals (Osaka, Japan). Pluronic F-68 was a gift from Adeka Chemicals (Tokyo, Japan). Bovine insulin was purchased from Sigma Chemical Company (St. Louis, MO). Vancomycin hydrochloride, phenobarbital, valproic acid, cyclosporin A, indomethacin, and ketoprofen were purchased from Wako Pure Chemicals. All references to water imply the use of MilliQ water previously filtered through a 0.2-μm cellulose nitrate membrane. All other chemicals were at least reagent grade and were used without further purification.

Preparation of the Nanoparticles

The PLGA nanoparticles were prepared by a precipitation–solvent evaporation method similar to that employed by Fessi et al. (8). Briefly, 75 mg of PLGA and 2.5 mg of a drug were dissolved or suspended (vancomycin and insulin) in 5 ml of acetone. This organic phase was poured into 15 ml of water containing 75 mg of Pluronic F-68 with moderate stirring at room temperature. Nanoparticles were immediately formed, and acetone was then removed from the colloidal suspension by rotoevaporation under reduced pressure. The resulting particle suspension was filtered through a 1.0-μm cellulose nitrate membrane filter and concentrated to a final volume of 10 ml by removal of water under the same conditions. Before they were added to the organic phase, insulin and vancomycin were dissolved in 150 μl of 0.1 N hydrochloride acid (HCl) adjusted with 0.1 N sodium hydroxide solution and water, respectively, to pH 4.0. For insulin, various buffer solutions were used as indicated in the aqueous phase. The compositions and pH's of the buffer solutions are shown in Table 1.

Determination of Particle Size

The mean particle size and the particle size distribution of the nanoparticles were determined by a dynamic

Table 1

Composition of the Buffers Used in the Preparation of Insulin-Loaded PLGA Nanoparticles

Buffer (pH 25°C)	Composition of 1000 ml (g)				Ionic Strength
	KH_2PO_4	NaCl	$Na_2HPO_4 \cdot 12H_2O$	Citric Acid	
1 (5.5)	—	4.675	2.113	0.480	0.101
2 (7.0)	2.368	—	9.777	—	0.099
3 (5.5)	—	—	10.529	2.363	0.100

light scattering method at 25°C (Otsuka Electronics Co., Osaka, Japan).

Determination of Drug Loading

The loading efficiency of all drugs in PLGA nanoparticles was determined as described below. Nanoparticles were separated from the aqueous medium by ultracentrifugation at 20,000 rpm for 30 min. The amount of drug present in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium. The aqueous medium was directly injected into a high-performance liquid chromatography (HPLC) system composed of a pump (LC-5A, Shimadzu Co., Kyoto, Japan), an ultraviolet (UV) detector (SPD-6A, Shimadzu Co.), an integrator (C-R3A, Shimadzu Co.), a syringe-loading sample injector (Model 7125, Rheodyne, CA) and a GL-PACK Nucleosil 100-5C18 column (150 × 4.6 mm i.d.). The HPLC conditions used for each drug are described in Table 2.

Before determination of drug encapsulation efficiency, insulin-loaded PLGA nanoparticles were purified from the free insulin by gel filtration through Sephadex (Sephadex G-50, Pharmacia Biotech, Sweden) in the same buffer as used for the preparation of the nanoparticles. The final nanoparticle suspension was then concentrated to 10 ml and divided into two portions. The amount of insulin present in the first portion was immediately assayed by the method described above. The second portion was diluted to 20 ml with the second fluid (pH 6.8) of the JP 13 (17) and stirred at a moderate speed at 37°C for 10 min. The amount of insulin was then assayed using the method described above.

In Situ Absorption of Insulin-Loaded Polylactic-*co*-Glycolic Acid Nanoparticles

Male Wistar rats weighing 200–230 g that had fasted for 24 hr prior to the experiments were anesthetized by an i.p. injection of 60 mg/kg sodium pentobarbital. The rats were restrained in a supine position on a board kept at 37°C. A small midline incision was made in the abdomen, and 10 cm loops of the ileum were identified and ligated at both ends. This ileum loop was made at the end of the small intestine, just proximal to the ileo-cecal junction. The rats were stabilized for 30 min after the operation and then injected as follows.

Either 0.5 ml or 1 ml of an insulin-loaded PLGA nanoparticle suspension (containing 4 U/ml of insulin) prepared in phosphate buffered saline (PBS) (pH 5.6) was administered directly into the ileum loop. As a control, 1 ml of a PBS (pH 7.4) solution containing 4 U/ml of insulin was used. The dose of insulin was 10 or 20 U/kg body weight. Approximately 5 min before administration, a 0.2-ml sample of blood was taken from the jugular vein. Subsequent blood samples (0.2 ml) were taken at 15, 30, 60, 120, and 240 min after dosing. Serum was separated by centrifugation at 13,000 rpm for 1 min and kept frozen until analysis. The relative efficacy was calculated according to the method described by Morishita et al. (18).

Table 2

Chromatographic Conditions for the Determination of Drug Loading

Drug	Mobile Phase	Fr (ml/min)	WL (nm)	Ref. No.
Insulin	Acetonitrile–0.1% trifluoroacetic acid–sodium chloride (31:69:0.58 v/v/w)	1.2	220	10
Vancomycin	Acetonitrile–0.005 M potassium dihydrogenphosphate (10:90 v/v), pH 2.8 with phosphoric acid	1.2	282	11
Phenobarbital	0.01 M potassium phosphate buffer (pH 7.0)–acetonitrile–methanol (110:50:30 v/v/v)	0.5	210	12
Valproic acid	0.01 M sodium dihydrogenphosphate–acetonitrile (60:40 v/v), pH 2.3 with phosphoric acid	2.0	210	13
Indomethacin	Acetonitrile–0.25 × 10^{-3} M sodium acetate (55:45 v/v), pH 3.5 with acetic acid	1.0	280	14
Cyclosporin A	Acetonitrile-water (75:25 v/v), column heated at 65°C	1.5	210	15
Ketoprofen	Methanol-water (80:20 v/v)	0.6	280	16

Fr, flow rate; WL, wavelength.

RESULTS AND DISCUSSION

It has already been shown that nanoparticles are useful carriers for the parenteral and oral administration of drugs having moderate lipophilicity. The encapsulation of hydrophilic drugs into hydrophobic polymers is still a limiting factor in the improved delivery of proteins and peptides through nanoparticulate carriers. Assessing the advantages and drawbacks of the existent methodology is the first step in designing appropriate formulations for proteins and peptides.

Particle Size

The mean particle sizes and the polydispersities of PLGA nanoparticles loaded with different drugs are shown in Table 3. As estimated by laser light scattering, the mean particle size of the PLGA nanoparticles was in the range 160–170 nm, with a relatively narrow particle size distribution for all formulations (polydispersity values).

For insulin nanoparticles, the mean particle size was related to the composition and pH of the buffer used. The use of a buffer solution that contains a salt that induces polymer ''salting-out'' (e.g., NaCl) typically results in polymer-polymer complex aggregation. On the other hand, other salts provide an opportunity to tailor the degrees of both polymer-polymer complex aggregation and interparticle aggregation, thus regulating the stability and other properties of hydrogen-bonded interpolymer complexes (19) and, consequently, the size of the final particles.

Drug Loading

The loading efficiencies of various drugs in PLGA nanoparticles is shown in Table 4. Vancomycin and phenobarbital had low encapsulation efficiencies in PLGA nanoparticles. It has been documented by a number of investigators that highly hydrophilic drugs suffer from problems of low affinity with the polymer, leading to an unsatisfactory loading efficiency (4). If there is poor interaction between the drug and the polymer, the drug will tend to diffuse from the organic phase to the external aqueous medium during the spontaneous emulsification process of the polymer. Although phenobarbital was completely dissolved in the organic phase, the drug might have leaked out during diffusion of the remaining acetone from the organic phase droplets into the aqueous dispersing medium.

Valproic acid is an organic acid originally used as a solvent that was serendipitously found to have an anticonvulsant effect (20). In spite of the fact that valproic acid is a liquid that is very sparingly soluble in water, very low encapsulation efficiency was obtained. Very low affinity between valproic acid and PLGA or a solvent action on the polymer are suggested as possible reasons for the low encapsulation of valproic acid. On the other hand, indomethacin and cyclosporin A demonstrated the highest encapsulation efficiencies. Cyclosporin A is an atypical peptide that possesses very high hydrophobicity and a significant oral activity as an immunosuppressant. The encapsulation efficiency of ketoprofen, a drug sparingly soluble in water, correlated well with its intermediate lipophilicity. These results demonstrated that more lipophilic drugs do not suffer from the problems of leakage of drug to the external medium, resulting in improved drug content in the nanoparticles. In any case, no clear relationship has been established between particle size and the amount of drug loaded in the PLGA nanoparticles.

Insulin is a polypeptide sparingly soluble in water and insoluble in organic solvents. It was reported that insulin

Table 3

Characteristics of Size and Polydispersity of PLGA Nanoparticles Loaded with Various Drugs

Drug	Size (nm)	Polydispersity
Insulin (buffer 1)	169.5 (2.54)	0.199 (0.021)
Insulin (buffer 2)	104.7 (2.20)	0.059 (0.012)
Insulin (buffer 3)	118.6 (1.46)	0.159 (0.048)
Vancomycin	186.9 (1.05)	0.201 (0.072)
Phenobarbital	157.4 (1.30)	0.187 (0.066)
Valproic acid	166.2 (3.40)	0.185 (0.008)
Indomethacin	167.6 (1.15)	0.127 (0.017)
Cyclosporin A	169.2 (6.09)	0.135 (0.017)
Ketoprofen	167.4 (5.22)	0.112 (0.006)

Each value represents the mean. SD is shown in parentheses.

Table 4

Encapsulation Efficiency of Various Drugs in PLGA Nanoparticles

Drug	Loading Amount (%)
Vancomycin	12.1 (1.3)
Phenobarbital	9.4 (1.3)
Valproic acid	5.6 (1.0)
Indomethacin	93.9 (1.3)
Cyclosporin A	83.7 (3.4)
Ketoprofen	46.2 (1.2)

Each value represents the mean. SD is shown in parentheses.

Table 5

Encapsulation Efficiency of Insulin in PLGA Nanoparticles

	Insulin Recovered (%)	Insulin Encapsulated (%)	Insulin Surface Bound (%)
Buffer 1 (5.5) (NaCl)	47.0 (3.1)	8.2 (0.4)	38.8 (1.7)
Buffer 2 (7.0)	17.3 (2.7)	6.8 (0.7)	10.5 (0.8)
Buffer 3 (5.5)	61.4 (2.6)	12.1 (0.9)	49.3 (1.4)

Each value represents the mean. SD is shown in parentheses.

exhibits a special affinity for lipophilic surfaces, and it was suggested that insulin might adsorb onto hydrophobic surfaces by a mechanism similar to that inducing its self-aggregation (21). Soluble proteins and peptides can easily become insoluble under a wide variety of conditions, such as solvent change (22). The presence of some neutral salts in the solution was shown to accelerate the aggregation of insulin, with the efficacy of insulin aggregation increasing as the salting-out potency increased (23). These facts were taken into consideration and applied here to the preparation of the nanoparticles. The loading efficiency of insulin in PLGA nanoparticles is shown in Table 5. The results demonstrated that almost 80% of the recovered insulin was preferentially surface bound on PLGA nanoparticles. Just 20% of the insulin was encapsulated into the nanoparticles. No clear relationship could be established between the particle size and the amount of drug loaded or surface bound in the PLGA nanoparticles. The amount of insulin adsorbed or encapsulated into the nanoparticles was related to the composition and pH of the buffer solution used. The best result was obtained with a buffer solution free of salting-out salts, with a pH close to the isoelectric point of insulin.

These results closely agree with the results reported for albumin, a protein that shows a high conformational flexibility in changing environmental conditions. In the case of albumin, the maximum protein adsorption was usually observed on hydrophobic surfaces and under pH conditions close to its isoelectric point (4.6–5.6) (24–26).

In Situ Absorption Experiments

Studies aimed at maximizing the bioavailability of orally administered protein and peptide drugs have been ongoing for many years. Poor absorption and low stability in the gastrointestinal fluid are the biggest problems to be overcome. To overcome such problems, many pharmaceutical approaches have been taken (27).

In a previously reported study, insulin adsorbed to hydrolyzable nanoparticles was effective in reducing the blood glucose levels of diabetic rats after subcutaneous administration, but no change of the glucose level was observed after oral administration of the same hydrolyzable nanoparticles (28). Two explanations were postulated: nanoparticles do not protect the protein against proteolytic degradation, or they are not absorbed by the gastrointestinal tract (28). Later, it was demonstrated that nanocapsules obtained from hydrolyzable polymers loaded with insulin were able to reduce, for a prolonged period, the plasma glucose levels in diabetic rats when administered orally (29). More recently, it was shown that absorption of insulin associated with nanocapsules occurs in all parts of the gut to various degrees, but the most effective site of absorption seems to be the ileum (30).

It is known that the particle surface properties, as well as particle size, govern accumulation in Peyer's patches (lymphoid follicles distributed throughout the small intestine), with penetration being favored by particles of small size (<1 μm) with hydrophobic surfaces (31). Peyer's patches are most prominent in the ileum and are characterized by the presence of specialized cells called M cells (31). Figure 1 shows the changes in the serum glucose level following the ileum loop injection of an insulin-loaded PLGA nanoparticle suspension. A consider-

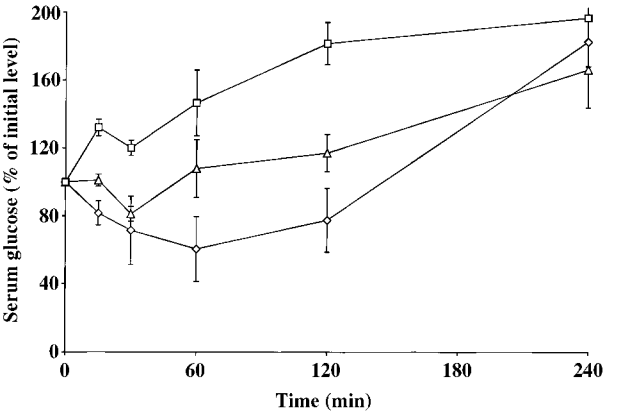

Figure 1. Effect of intraileal administration of insulin-loaded PLGA nanoparticles on serum glucose levels: □, insulin solution (20 U/kg); ◇, insulin-loaded PLGA nanoparticles suspension (20 U/kg); △, insulin-loaded PLGA nanoparticles suspension (10 U/kg). Values represent the mean for three rats ± SD.

able hypoglycemic effect was observed for the higher dose (20 U/kg), indicating that insulin was absorbed from the ileum loop. On the other hand, administration of an insulin solution (20 U/kg) into the loops did not decrease the serum glucose level.

CONCLUSION

The above findings demonstrated that the nanoprecipitation method is most applicable for encapsulation of lipophilic drugs. However, the possibility of using this method in formulations for proteins and peptides is appealing. The use of a biodegradable polymer, PLGA, to produce surface-bound peptides, thereby improving their bioavailability, was demonstrated. In other words, it was shown here that an appropriate delivery system can be designed based on the physical properties of the protein or peptide drug and using the existing delivery system.

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