Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 25 (2004) 1919–1927
Biomaterials
www.elsevier.com/locate/biomaterials

Mechanistic evaluation of the glucose-induced reduction in initial burst release of octreotide acetate from poly(D,L-lactide-*co*-glycolide) microspheres

Juan Wang[a,1], Barbara M. Wang[a,2], Steven P. Schwendeman[b,*]

[a] *Pharmaceutical Analytical and Development Department, Novartis Pharmaceuticals Corp., 59 Route 10, East Hanover, NJ 07936, USA*
[b] *Department of Pharmaceutical Sciences, University of Michigan, 428 Church Street, Ann Arbor, MI 48109-1065, USA*

Received 29 January 2003; accepted 11 August 2003

Abstract

One major obstacle for development of injectable biodegradable microspheres for controlled peptide and protein delivery is the high initial burst of drug release occurring over the first day of incubation. We describe here the significant reduction in initial burst release of a highly water-soluble model peptide, octreotide acetate, from poly(D,L-lactide-*co*-glycolide) microspheres by the co-encapsulation of a small amount of glucose (e.g., 0.2% w/w), i.e., from 30±20% burst − glucose to 8±3% + glucose (mean±SD, $n = 4$). This reduction is unexpected since hydrophilic additives are known to increase porosity of microspheres, causing an increase in permeability to mass transport and a higher burst. Using the double emulsion-solvent evaporation method of encapsulation, the effect of glucose on initial burst in an acetate buffer pH 4 was found to depend on polymer concentration, discontinuous phase/continuous phase ratio, and glucose content. Extensive characterization studies were performed on two microsphere batches, ±0.2% glucose, to elucidate the mechanism of this effect. However, no significant difference was observed with respect to specific surface area, porosity, internal and external morphology and drug distribution. Continuous monitoring of the first 24-h release of octreotide acetate from these two batches disclosed that even though their starting release rates were close, the microspheres + glucose exhibited a much lower release rate between 0.2 and 24 h compared to those − glucose. The microspheres + glucose showed a denser periphery and a reduced water uptake at the end of 24-h release, indicating decreased permeability. However, this effect at times was offset as glucose content was further increased to 1%, causing an increase in surface area and porosity. In summary, we conclude that the effect of glucose on initial burst are determined by two factors: (1) increased initial burst due to increased osmotic pressure during encapsulation and drug release, and (2) decreased initial burst due to decreased permeability of microspheres.

Keywords: Biodegradable microspheres; Initial burst; Glucose; Continuous monitoring; Permeability

1. Introduction

Injectable biodegradable microspheres have been widely studied in recent years and have become well-established controlled drug delivery systems [1–4]. With one single injection, microspheres allow long-term delivery of the encapsulated drug over a period of weeks to months. However, long-term delivery also raises the challenge of minimizing initial burst of drug release, which is usually not an issue for short-term delivery. Hydrophilic drugs, e.g., peptides or proteins, pose greater challenges than hydrophobic drugs due to their high aqueous solubility. Significant initial burst could result in dangerously high drug concentrations in the body as well as depletion of drug for long-term release. Currently, burst release values between 10% and 80% relative to encapsulated drug load are commonly seen in publications and marketed products.

Several approaches reported to reduce the initial burst include (1) raising polymer concentration [5,6], and reducing drug particle size (for solid in oil in water process) or droplet size of the inner emulsion (for water-in-oil-in-water or oil-in-oil-in-water processes) during

*Corresponding author. Tel.: +1-734-615-6574; fax: +1-734-615-6162.
E-mail address: schwende@umich.edu (S.P. Schwendeman).
[1] Current address: Alkermes, Inc., 64 Sidney Street, Cambridge, MA 02139, USA.
[2] Current address: HGB International Ltd., P.O. Box 588, Berkeley Heights, NJ 07922, USA.

0142-9612/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2003.08.019

encapsulation [7,8]; (2) decreasing the content of low molecular weight water-soluble impurities in the polymer material [9,10]; (3) heating the microsphere product [11]; (4) coating the microsphere product with high viscosity polymer [12]; and (5) incorporation of additives in the microsphere formulation [13,14]. Among these approaches, polymer concentration, the size of drug particle and of inner emulsion droplet are limited either by the physical properties of the material or by the capability of the manufacturing facility. Decreasing the oligomer content and heating the microsphere product has only resulted in partial burst reduction [9–11]. Coating the microsphere product with a high viscosity polymer involves many parameters during manufacturing and raises other issues such as reproducibility. The incorporation of additives has been found to be an effective approach [13,14] and warrants further investigation.

We encapsulated a highly water-soluble peptide drug, octreotide acetate, into poly(D,L-lactide-*co*-glycolide) (PLGA) microspheres. Described here is the finding that under certain conditions the addition of a very small amount (0.2% w/w) of glucose into the formulation significantly reduces the 24-h initial burst. This result is unexpected because hydrophilic additives are known to increase the initial burst by increasing microsphere porosity [15]. Therefore, the objective of this paper was to investigate the mechanism of the glucose effect on the initial burst reduction of octreotide acetate-loaded PLGA microspheres.

2. Materials and methods

2.1. Materials

Octreotide acetate (batch 92072) was provided by Novartis Pharmaceutical Corp. Poly(D,L-lactide-*co*-glycolide, 50:50) (PLGA) (molecular weight, Mw, 53,600) was purchased from Birmingham Polymers, Inc. (Birmingham, AL, lot number D98083). Methylene chloride, acetonitrile, and tetrahydrofuran (THF) were purchased from Aldrich Chemical (Milwaukee, WI). Polyvinyl alcohol (PVA, 88 mol% hydrolyzed, MW 25000) was obtained from Polysciences, Inc. (Warrington, PA). Filter paper (Nylon, 0.45 μm) was purchased from Micron Separations Inc. (Westboro, MA). Glacial acetic acid (USP grade), sodium hydroxide (NaOH) and sodium azide were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium bicarbonate ($NaHCO_3$) was obtained from Mallinckrodt, Inc. (Paris, Kentucky). Ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) was obtained from Sigma (St. Louis, MO). All chemicals were used as received. Dialysis membrane (cut-off MW: 12-14,000) was obtained from Spectrum Laboratories, Inc. (Lagune Hills, CA).

2.2. Preparation of microspheres

Microspheres were prepared using a solvent evaporation technique, which involved formation of a double emulsion [16]. Octreotide acetate (50 mg) was dissolved in 100 μl of water. The solution was mixed with 1–2 ml of PLGA methylene chloride solution (200–400 mg/ml) and emulsified by homogenization using a Virtis "23" homogenizer (The Virtis Company, Gardiner, New York) at maximum speed for 1 min. The emulsion stabilizer (1–2 ml of 1% PVA solution) was then added and the mixture was vortex mixed with a Vortex Genie 2™ (Scientific Industries Inc., Bohemia, NY) at maximum speed for 30 s. The formed emulsion was immediately transferred into a 100 ml 0.3% PVA aqueous solution stirred at 600 rpm [17]. After evaporation of methylene chloride (3 h), the microspheres were collected by filtration through 0.45 μm nylon filter paper, washed with 100 ml of water for three times and vacuum dried for 48 h. Microspheres were stored at 4°C before further investigation. When glucose was encapsulated, it was dissolved in the inner water phase before octreotide acetate was added. The glucose content was calculated as w/w% according to the amount of polymer material used.

2.3. Microsphere characterization

2.3.1. Particle size distribution

The microsphere size distribution was analyzed by a MasterSizer X (Malvern Instruments Ltd., Southborough, MA). The microspheres were suspended in 0.1% aqueous Tween 80 solution, sonicated for 30 s and stirred for 2 min before the analysis. Results are reported as volumetric mean diameter.

2.3.2. Specific surface area

The specific surface area was determined using a Gemini 2360 Analyzer (Micromeritics Instrument Corp., Norcross, GA) by BET adsorption–desorption isotherms of N_2 and Kr gases on the microsphere surface. The area values were normalized by the sample weight.

2.3.3. Porosity analysis

The porosity of microspheres was analyzed by a Mercury Porosimeter AutoPore II 9220 (Micromeritics Instrument Corp., Norcross, GA). Pressure was increased from ambient pressure to 60,000 psi (414 MPa). For this pressure operation, pores greater than 0.003 μm in diameter were measured.

2.3.4. External and internal morphology

The external and internal morphology of microspheres was analyzed by scanning electron microscopy (SEM). Microspheres samples were first freeze-dried for

2 days to remove water. The samples were mounted onto aluminum specimen stubs using double-sided adhesive tape and fractured with a razor blade. The samples were then sputter coated with gold/palladium for analysis by secondary electron emissive SEM (Jeol Ltd., Tokyo, Japan).

2.3.5. Thermal analysis

The glass transition temperature was analyzed by differential scanning calorimetry (DSC) 2920 (TA Instruments, New Castle, DE) using the modulated DSC mode. The temperature was modulated at $\pm 1^{\circ}C/60\,s$ with a ramping rate of $1^{\circ}C/min$ from $0^{\circ}C$ to $70^{\circ}C$.

2.4. Rheological analysis of peptide/glucose solutions

The viscosity measurement was carried out by a Bohlin Rheometer CS (Bohling Instrument Inc., Cranbury, NJ). A cone and plate were used as the measuring unit (cone diameter 20 mm, angle 4°). A constant shear rate of $0.076\,s^{-1}$ was used to evaluate the viscosity of the drug-containing solution at or close to the static state. Constant shear rate ($200\,s^{-1}$) and ramping shear rate (from 200 to $1000\,s^{-1}$) were used to evaluate viscosity of the drug-containing solution under high shear conditions (e.g., during homogenization). Acetate buffer (contained 0.36% w/v glacial acetic acid and 0.02% w/v sodium azide, adjusted to pH 4.0 using 5 N NaOH) was used as the medium to prepare samples. For samples containing glucose, both octreotide acetate and glucose were dissolved in sufficient amount of water, lyophilized for 48 h to remove water, and then dissolved again in known amount of acetate buffer. The weight ratio of glucose to octreotide acetate was maintained at 2.4/100, which is the same ratio used during microsphere formulation for 0.2% w/w glucose content.

2.5. Freezing point depression analysis of peptide/glucose solutions

Freezing point of saturated octreotide acetate aqueous solution was determined with or without presence of glucose. As one of the colligative properties, the relative freezing point depression is a direct indication of relative osmotic pressure of drug aqueous solution. Octreotide acetate (100 mg) and glucose (0 or 2.4 mg) were dissolved in 200-μl pure water. Samples were freeze dried for 48 h to remove water so that drug and glucose molecules were molecularly dispersed. Trace amount of acetate buffer (50 μl) was then added and the mixture was shaken using an Eppendorf Thermomixer 5436 (Brinkmann Instrument, Inc., Westbury, NY) at 500 rpm for 4 h. Mixtures were then weighed in a DSC aluminum pan and then the freezing points of the samples were analyzed using modulated DSC (DSC 2920, TA Instruments, New Castle, DE). The temperature was modulated at $\pm 0.8^{\circ}C/60\,s$ with a ramping rate of $1^{\circ}C/min$ from $-60^{\circ}C$ to $40^{\circ}C$.

2.6. Determination of drug loading inside PLGA microspheres

About 15 mg of microspheres were dissolved in 2 ml of THF and then shaken and sonicated. After a clear solution was obtained, 8 ml of diluent containing 0.2% w/v glacial acetic acid, 0.2% w/v sodium acetate, and 0.7% w/v sodium chloride was added. The mixture was allowed to settle for at least 20 min while the polymer precipitated. The solution was filtered through a 0.45-μm Teflon filter and the filtrate was injected directly into a high performance liquid chromatography (HPLC). Conditions of the HPLC assay was previously described [16].

2.7. Single point determination of initial burst release (24 h burst) of octreotide acetate from PLGA microspheres

About 20 mg of microspheres were weighed and placed into a dialysis bag. The bag was then put into a polyethylene tube containing 10 ml of a 0.05 M sodium acetate buffer solution (pH 4) as the release medium. The lower pH was used because the peptide is unstable at neutral pH [16]. Samples were shaken on an orbital shaker (VWR Scientific, West Chester, PA) at 100 rpm under 37°C. After 24 h, aliquots of the release medium were withdrawn and injected into the HPLC.

Before drug release studies, the dialysis membrane was boiled in 2% $NaHCO_3$ with 1-mM EDTA at pH 8 for 10 min to remove residual metals in the membrane. The membrane was rinsed with distilled water and boiled again in 1 M EDTA solution for another 10 min for sterilization. Sodium azide (0.02%) was added to the solution and the membrane was kept in the solution at 4°C until further use.

2.8. Continuous monitoring of octreotide acetate release from PLGA microspheres

The continuous release rate profiles of octreotide acetate-loaded microspheres with or without 0.2% glucose were obtained using the custom designed continuous monitoring system. Pre-weighed microspheres were placed into a dialysis bag and then into the drug release reservoir (0.05 M sodium acetate, pH 4). The details for the instrument set-up of continuous monitoring and data processing are discussed in our previous publication [16].

3. Results and discussion

3.1. Glucose induced reduction of the initial burst from octreotide acetate-loaded PLGA microspheres

The effect of 0.2% w/w glucose on the initial burst of octreotide acetate-loaded PLGA microspheres is listed in Table 1. In the absence of glucose in the formulation, the 24-h initial burst was high and variable over a wide range (10–60%). However, when 0.2% w/w glucose was included in the formulation, the average initial burst was reduced to 8% and the reproducibility was greatly improved (ranging from 8% to 11%).

3.2. Effect of formulation variables on the effect of glucose to reduce initial burst

In order to evaluate the influence of formulation variables on the glucose-induced reduction in initial burst, microspheres were prepared at various polymer concentrations, DP/CP ratios, and glucose contents. The selected encapsulation conditions are separated into four formulation groups and listed in Table 2.

In formulation group 1, when DP/CP ratio and polymer concentration were fixed at 2% and 200 mg/ml, respectively, the initial burst at different glucose contents were all very high. The 24-h initial burst was reduced from 88% (at 0% and 0.2% glucose content) to 60% (at 1% glucose content).

At 300 mg/ml polymer concentration (group 2), the 24-h initial burst was very high (58%) in the absence of glucose in microspheres. However, at 0.2% glucose content, the 24-h initial burst fell to 11%. At 1% glucose content, the burst was further reduced to 3%.

When the polymer concentration was increased to 400 mg/ml (group 3), the 24-h initial burst was only 5% in the absence of glucose. Increasing the glucose content to 0.2% had little effect on 24-h initial burst (6%). At 1% glucose content, the 24-h initial burst actually increased to 17%.

If the polymer concentration was maintained at 400 mg/ml as in group 3, while the DP/CP ratio was decreased to 1% (group 4), the initial burst in the absence of glucose was 29%. In the presence of 0.2% glucose, the initial burst dropped to 4%. However, with further increase in glucose content, the initial burst slightly increased.

Thus, to summarize, we found that when the initial burst was neither very low nor very high (i.e., between 5% and 60%) in the absence of glucose, adding a small

Table 1
The effect of 0.2% w/w glucose on the initial burst of octreotide acetate-loaded PLGA microspheres

Formulation	24-h initial burst
Without glucose	30±20%[a]
With 0.2% w/w glucose	8±3%

[a] Mean±SD, $n = 4$.

Table 2
Formulation conditions for effect of polymer concentration and DP/CP ratio on the "glucose effect" and properties of microspheres prepared

Group #	Batch #	Polymer conc. (mg/ml)	DP/CP[a] (o/w_2)[b] %	Glucose (w/w %)	L_T[c] (w/w %)	L_A[d] (w/w %)	EE[e] (%)	24 h burst (%)	Particle size
1	990423S2A	200	2	0	11	2.3	21	88	69±26
	990423S2B	200	2	0.2	11	2.1	19	88	70±25
	990423S2C	200	2	1	11	0.9	8	63	71±28
2	990423S3A	300	2	0	8	3.1	39	58	100±57
	990423S3B	300	2	0.2	8	3.4	42	11	118±62
	990423S3C	300	2	1	8	2.4	30	3	118±55
3	990423S4A	400	2	0	6	3.4	57	5	146±74
	990423S4B	400	2	0.2	6	3.5	58	6	154±90
	990423S4C	400	2	1	6	3.0	50	17	164±92
4	990420SA	400	1	0	11	5.2	47	29	94±43
	990423S4D	400	1	0.2	11	5.7	52	4	ND[f]
	990420SB	400	1	1	11	4.2	38	7	98±41
	990420SC	400	1	5	11	1.9	17	13	105±51

[a] The volume of CP was fixed to be 100 ml for all the encapsulation experiments.
[b] "o" is the oil phase, or the methylene chloride phase (same as DP here). "w_2" is the outer aqueous phase (same as CP here). "w_1" is the inner aqueous phase. The multiple emulsion formed during encapsulation is usually abbreviated as $w_1/o/w_2$.
[c] L_T is the theoretical loading of octreotide acetate, which is calculated by charged drug amount divided by sum of the weight of polymer and drug.
[d] L_A is the actual loading of octreotide acetate determined experimentally.
[e] EE is the encapsulation efficiency, which equals to the ratio of experimentally determined loading to the theoretical loading.
[f] Not determined.

amount of glucose (0.2%) would reduce the initial burst. After the initial burst was reduced to certain extent (i.e., $<5\%$), further increase in glucose content only resulted in an increase of initial burst. On the other hand, if the initial burst was already low (i.e., $<5\%$), adding 0.2% glucose did not reduce the initial burst further.

Apart from the initial burst, the microspheres were also tested for the encapsulation efficiency and particle size distribution (Table 2). Increasing the glucose content from 0% to 0.2% had little effect on the encapsulation efficiency. Therefore, the drug loading has little effect on the reduced burst with the presence of glucose. However, the encapsulation efficiency dropped significantly when the glucose content was increased to 1% or even higher. The dropped loading may contribute to the reduced burst in this case. On the other hand, at fixed polymer concentration and DP/CP ratio, increasing glucose content up to 5% had little effect on the particle size distribution.

3.3. Characterization of microsphere properties

Since glucose reduced the initial burst most significantly in group 2 (Table 2), microspheres in this group were selected for a series of characterization studies. Properties that may influence initial burst (i.e., particle size distribution, specific surface area, porosity, internal and external morphology, drug distribution, and glass transition temperature) were examined and the results are listed in Tables 2 and 3. No significant difference was observed between the two batches (i.e., with 0% and 0.2% glucose) with regard to all these properties.

3.4. Possible mechanisms for the effect of glucose on the initial burst

Considering the complexity of the effect of glucose on drug release, we used the following approach in order to elucidate the involved mechanism. First, we envisioned the underlying physical events during entire time course starting from encapsulating glucose with octreotide acetate until the drug release from PLGA microspheres. We then identified specific factors that glucose might have influenced. Finally, we tested and verified whether such factors were influenced by glucose.

3.4.1. Description of underlying physical events during preparation of microspheres and initial drug release period

During encapsulation, both glucose and octreotide acetate are first dissolved in water. This water phase is broken up into small aqueous droplets (inner water phase) and dispersed in the polymer/methylene chloride solution (oil phase) during the emulsification step. The oil phase is further broken up by emulsification to form a w/o/w emulsion and embryonic particles are transferred to a large volume of aqueous continuous phase for hardening. During hardening, methylene chloride is gradually transferred from the oil phase into the surrounding aqueous solution (outer water phase) and the microspheres solidify. Due to the higher osmotic pressure in the inner aqueous phase inside microspheres, water is transported into, and drug and glucose are transported out of, the inner aqueous phase [18]. Both the emulsification efficiency and water influx caused by the osmotic pressure in the inner aqueous phase could affect the size of the inner aqueous droplets. In addition, the instability of the inner aqueous emulsion may cause the inner aqueous droplets to coalesce with each other before the polymer phase solidifies. The size distribution of the inner aqueous droplets is expected to impact the porosity of microspheres, and thus, drug release.

During drying of microspheres, water and residual organic solvent are gradually removed from the polymer due to a pressure gradient induced by vacuum. At the same time, drug and glucose precipitate out of solution inside small pockets surrounded by the polymer matrix.

Table 3
Results from characterization studies of octreotide acetate-loaded microspheres in formulation group 2

Properties	Instrumentation	Glucose content			Conclusion[a]
		0%	0.2%	1%	
Specific surface area	Surface area analyzer	$4.4\pm0.3\,m^2/g$	$4.7\pm0.3\,m^2/g$	$4.9\pm0.2\,m^2/g$	No significant difference
Porosity	Mercury porosimetry	$68.7\pm0.9\%$	$66.0\pm0.9\%$	$73.9\pm0.9\%$	No significant difference
External morphology	SEM	Smooth surface with small pinholes	Smooth surface with small pinholes	ND	No significant difference
Internal morphology	SEM	Porous throughout	Porous throughout	ND	No significant difference
Drug distribution	CM	Relatively homogenous	Relatively homogenous	Relatively homogenous	No significant difference
Glass transition temperature	Modulated DSC	$47.3\pm0.6^\circ C$	$47.5\pm0.6^\circ C$	$46.4\pm0.6^\circ C$	No significant difference
24-h water up-take	Gravimetric method	$140\pm20\%$	$90\pm10\%$	ND[b]	Significant difference

[a] The conclusion for particle size distribution, specific surface area, and porosity are made based on t-test. ($P = 95\%$) All the comparison were made between two batches of microspheres containing 0% and 0.2% glucose content, respectively.
[b] ND, not determined.

These small pockets form aqueous pores or channels (when pores are connected) which could become a diffusion pathway for hydrophilic molecules in microspheres upon hydration.

During initial drug release, water diffuses into the microspheres and begins to dissolve the highly water-soluble glucose and peptide drug. Once dissolved, these small molecules may either diffuse out of the microsphere through aqueous channels (new surface pores and connecting channels may be formed upon they leave the microspheres) or remain inside the microsphere, which would lead to an elevated osmotic pressure. The polymer material is hydrated and gradually gains mobility. The hydrated polymer chains rearrange themselves, causing pores to close particularly at the polymer surface, leading to decreased permeability [16]. The reduced permeability then suppresses the initial burst. Therefore, the release rate of drug is expected to be determined by the concentration gradient of drug molecules, the osmotic pressure across microsphere surface, and the hydraulic and diffusive permeability of microspheres, which includes an effect of the viscosity on the pore-liquid.

3.4.2. *Testing of potential mechanisms*

The emulsification efficiency directly influences the inner aqueous droplet size. Reduced emulsification efficiency was reported to cause an increase in inner aqueous droplet size, porosity, initial burst, and a decrease in encapsulation efficiency [8,19]. In addition, the emulsification efficiency is affected by the viscosity of the inner aqueous phase [20,21]. Therefore, we tested the effect of glucose on the viscosity of octreotide acetate aqueous solution at high shear rates. At higher shear rate (200 1/s), the addition of glucose to the octreotide acetate solution had little effect on viscosity (Table 4). Ramping shear rate from 200 to 1000 1/s did not change the measured viscosity (data not shown). Therefore, the measured viscosity at 200 1/s shear rate should be representative of the viscosity at a much higher shear rate [22], as occurred during homogenization (10^4–$10^5\,s^{-1}$) [23]. These results indicated that glucose had little effect on the viscosity of inner aqueous phase during emulsification. Therefore, the emulsification efficiency does not appear to be affected by the presence of low amounts of glucose. This is consistent with our observation that the two microsphere batches with 0% and 0.2% glucose had similar porosity and encapsulation efficiency.

Table 4
Viscosity determination for octreotide acetate solutions at different drug content and glucose content

Octreotide acetate content (mg/ml)	Glucose content (mg/ml)	Shear rate (s^{-1})	Viscosity (mPa s)
500	0	200	17.9 ± 0.2
500	12	200	17.9 ± 0.7
500	0	0.076	$3.6 \pm 0.2 \times 10^6$
500	12	0.076	$4.8 \pm 0.5 \times 10^6$

During microsphere hardening, water may diffuse into microspheres driven by the osmotic pressure of the inner aqueous phase [18]. Therefore, if the osmotic pressure were changed significantly due to glucose, the water intake could also increase significantly. When 0.2% w/w glucose was added, there was a 13% increase in molarity assuming a theoretical drug loading of 8% and a similar encapsulation efficiency for both small molecules. When 1% w/w glucose was added, this molarity increase was about 70%. Therefore, depending on the amount of glucose added, the porosity is expected to increase significantly due to this factor. Actually, a porosity increase was observed when glucose was increased from 0.2% to 1% w/w (Table 3). Therefore, this factor has an effect of increasing initial burst.

Also, the osmotic pressure inside microspheres during drug release influences the initial burst. For the same drug loading for microspheres with 0% and 0.2% glucose, the presence of glucose is expected to increase the osmotic pressure (since all the drug and glucose inside microspheres is expected to dissolve). During the very initial stage of the release period, the limited water uptake may saturate the inner pores inside microspheres. We compared the freezing point depression of the saturated drug/glucose solution with that of saturated drug solution. The one that had the lower freezing point should have a higher osmotic pressure. The freezing point of saturated drug/glucose solution

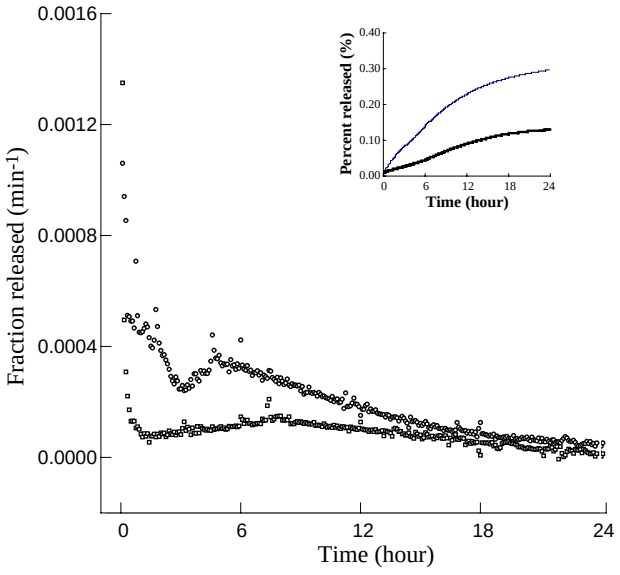

Fig. 1. Continuous release rate profiles for octreotide acetate-loaded PLGA microspheres with 0% (○) or 0.2% (□) glucose during the first 24 h. The inset shows cumulative release profiles for microspheres with 0% (*thin upper curve*) or 0.2% (*thick lower curve*) glucose determined during the same experiment.

was determined to be −6.22°C versus −3.46°C for saturated drug solution. These results indicated that a higher osmotic pressure existed inside microspheres containing 0.2% glucose and could potentially increase the initial burst.

The local viscosity in the aqueous channels inside microspheres affects the diffusion rate of drug and thus drug release. Therefore, we studied the effect of glucose on the viscosity of octreotide acetate aqueous solution at low shear rate (0.076 1/s). No significant difference was observed (Table 4).

In our previous studies, we found that the permeability of microspheres was a primary factor for controlling initial drug release [16]. The initial permeability is determined by the microsphere structure, which includes the microsphere porosity, the structure of the pore network and location of the drug particles inside the microspheres. In addition, the permeability could change as the polymer chains hydrate and rearrange themselves. Therefore, we determined the 24-h water uptake for microspheres containing 0% or 0.2% glucose, which is an indicator of microsphere permeability. The microspheres containing 0.2% glucose took up much less water as compared to those that did not contain glucose (90% with 0.2% glucose versus 140% without glucose, see Table 3), even though the osmotic pressure for the latter was higher. This result suggested that the microspheres containing 0.2% glucose had a lower permeability than those without glucose by the end of the first day release.

The continuous release rate profiles (Fig. 1) also showed that the overall release rate is reduced with

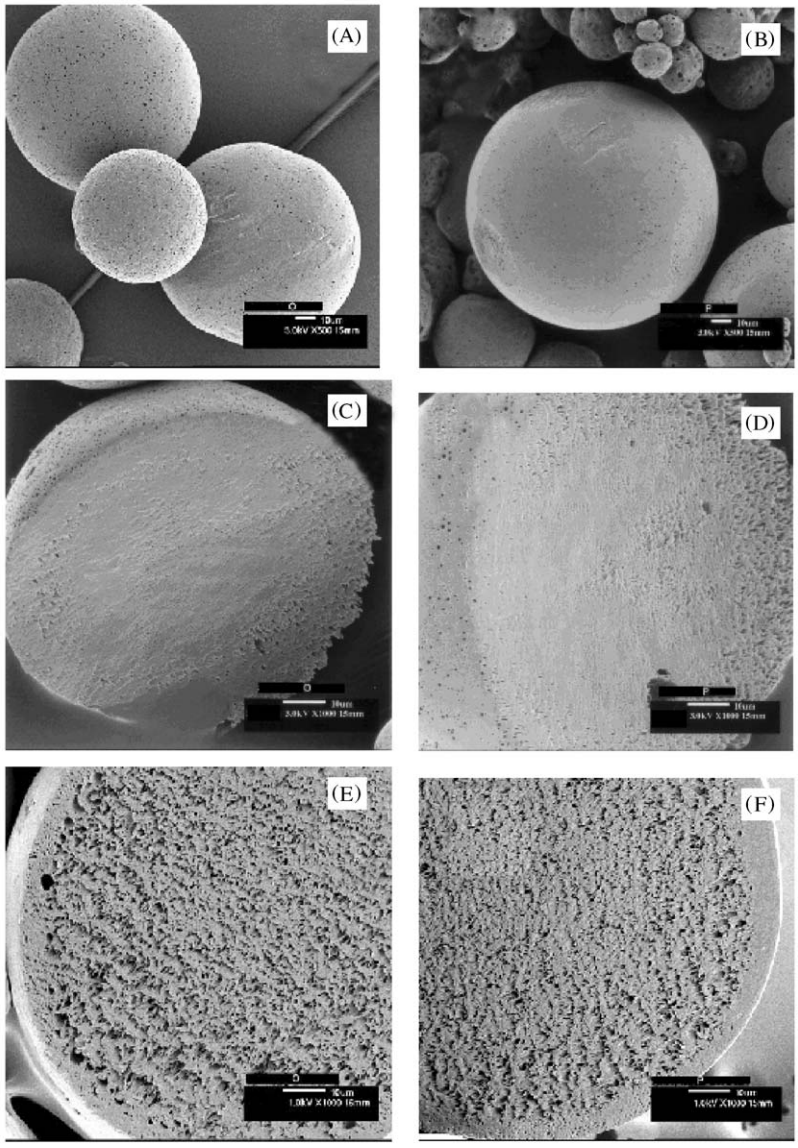

Fig. 2. External (A and B) and internal (C, D, E, and F) morphology of octreotide acetate-loaded PLGA microspheres with 0% (A, C, and E) or 0.2% (B, D, and F) glucose before hydration (A, B, C, and D) and after 24 h (E and F) in release medium.

microspheres containing 0.2% glucose as compared to the microspheres without glucose. Initially, the two batches had similar release rates, which is likely due to drug leaching out from the microsphere surface. However, as time proceeds, the release rate for microspheres with 0.2% glucose dropped much faster and was maintained at a lower rate thereafter. This result indicated that microspheres with 0.2% glucose had lower permeability than the microspheres containing no glucose. The SEM results also revealed that a skin layer was formed for both batches of microspheres after 24-h of drug release (Fig. 2). Even though the external and internal morphology of these two types of microspheres appeared similar initially (Figs. 2A and B or C and D), after 24 h of incubation, the skin of the microspheres containing 0.2% glucose appeared to be thicker and denser (Figs. 2E and F). This result also indicated that the microspheres containing 0.2% glucose had a lower permeability during the first 24 h of drug release due to more rapid and pronounced spontaneous polymer rearrangements induced by glucose [17]. The cause of this effect is not clear but may be due to the changes in interfacial tension. For example, it is well known that polyols increase surface tension of water [24].

Okada et al. has reported that the interaction between leuprorelin and PLGA raised the glass transition temperature (T_g) of the polymer material significantly and this interaction resulted in low initial burst of their microspheres [9,25,26]. We tested the effect of glucose on the T_g of PLGA in microspheres in our experiments. No significant effect was observed (see Table 3).

In summary, for octreotide acetate-loaded PLGA microspheres, glucose had two types of effects. Firstly, glucose increased the osmotic pressure in the inner aqueous phase during encapsulation as well as the osmotic pressure in microspheres during drug release. Both of these effects favored an increase in initial burst. Secondly, glucose decreased the permeability of microspheres as a result of more rapid and pronounced spontaneous rearrangement of polymer microstructure, which in turn reduced the initial burst of octreotide acetate. The physical-chemical basis for such rearrangement is currently not well understood. This effect seems to be most dominant at 300-mg/ml polymer concentration and 2% DP/CP ratio condition. The overall effect of glucose on octreotide acetate release from microspheres was a combination of the above two effects.

Acknowledgements

The authors acknowledge Tox-Pathology Department at Novartis and Mr. Paul Grosenstein for the help with the SEM study. This work was supported in part by a gift from Novartis Pharmaceutical Corp and a grant from NIH (HL 68345).

References

[1] Ogawa Y. Injectable microcapsules prepared with biodegradable poly(alpha-hydroxyl) acids for prolonged release of drugs. J Biomater Sci (Polym Edn) 1997;8:391–409.
[2] Okada H, Toguchi H. Biodegradable microspheres in drug delivery. Crit Rev Ther Drug Carrier Systems 1995;12:1–99.
[3] Thies C. Formation of degradable drug-loaded microparticles by in-liquid drying processes. In: Microcapsules and nanoparticles in medicine and pharmacy. Ann Arbor: CRC Press; 1991, p. 47–71.
[4] Benita S. Microencapsulation-methods, industrial applications. New York: Marcel Dekker Inc.; 1996.
[5] Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm Res 1991;8:713–20.
[6] Blanco-Prieto M, Leo E, Delie F, Gulik A, Couvreur P, Fattal E. Study of influence of several stabilizing agents on the entrapment and in vitro release of pBC 264 from poly(lactide-co-glycolide) microspheres prepared by a w/o/w solvent evaporation method. Pharm Res 1996;13:1127–9.
[7] Bodmer D, Fong JW, Kissel T, Maulding HV, Nagele O, Pearson JE. Sustained release formulations of water soluble peptides. US Patent No. 5688530, 1997.
[8] Sah HK, Toddywala R, Chien YW. Biodegradable microcapsules prepared by a w/o/w technique: effects of shear force to make a primary w/o emulsion on their morphology and protein release. J Microencapsul 1995;12:59–69.
[9] Okada H, Doken Y, Ogawa Y, Toguchi H. Preparation of three-month depot injectable microspheres of leuprorelin acetate using biodegradable polymers. Pharm Res 1994;11:1143–7.
[10] Yamamoto M, Okada H, Ogawa Y, Miyagawa T. Polymer, production and use thereof. US Patent No. 4728721, 1988.
[11] Kamei S, Yamada M, Ogawa Y. Method of producing sustained-release microcapsules. US Patent No. 5575987, 1996.
[12] Okumu FW, Darling S, Dao LN, Fielder PJ, Sullivan SA, Tipton AJ, Cleland JL. SABER provides burst free release of growth hormone from PLGA microspheres. In Millennial World Congress Meeting, 2000.
[13] Takada S, Kurokawa T, Miyazaki K, Iwasa S, Ogawa Y. Utilization of an amorphous form of a water-soluble GPIIb/IIIa antagonist for controlled release from biodegradable microspheres. Pharm Res 1997;14:1146–50.
[14] Toshio Y, Hiroaki O. Sustained release microcapsule for water soluble drug. US Patent No. 5271945, 1993.
[15] Mallard C, Coudane J, Rault I, Vert M. The use of additives to modulate the release of a sparingly water soluble drug entrapped in PLA50 microparticles: in vivo investigation. J Microencapsulation 2000;17:95–110.
[16] Wang J, Wang BM, Schwendeman SP. Characterization of the initial burst release of a model peptide from poly(D,L-lactide-*co*-glycolide) microspheres. J Controlled Release 2002;82:289–307.
[17] Wang J, Schwendeman SP. Mechanisms of solvent evaporation encapsulation processes: prediction of solvent evaporation rate. J Pharm Sci 1999;88:1090–9.
[18] Herrmann J, Bodmeier R. The effect of particle microstructure on the somatostatin release from poly(lactide) microspheres prepared by a w/o/w solvent evaporation method. J Controlled Release 1995;36:63–71.
[19] Schugens C, Laruelle N, Nihant N, Grandfils C, Jerome R, Teyssie P. Effect of the emulsion stability on the morphology, porosity of semicrystalline poly L-Lactide microparticles prepared by w/o/w double emulsion–evaporation. J Controlled Release 1994;32:161–76.
[20] Walstra P. Formation of emulsions. In: Becher P, editor. Encyclopedia of emulsion technology. New York: Marcel Deker Inc.; 1983. p. 57–128.

[21] Maa Y-F, Hsu C. Liquid–liquid emulsification by rotor/stator homogenization. J Controlled Release 1996;38:219–28.
[22] Martin A, Swarbrick J, Cammarata A. Rheology. In: Martin A, editor. Physical pharmacy. Philadelphia: Lea & Febiger, 1983; p. 453–76.
[23] User Manual for Bohlin Rheometersin. Bohlin Instruments 1999.
[24] Timasheff SN. Stabilization of protein structure by solvent additives. In: Ahern TJ, Manning MC, editors. Stabilization of protein pharmaceutics Part B: In vivo pathways of degradation and strategies for protein stabilization. New York: Plenum Press; 1992.
[25] Okada H, Yamanoto M, Heya T, Inoue Y, Kamei S, Ogawa Y, Toguchi H. Drug delivery using biodegradable microspheres. J Controlled Release 1994;28:121–9.
[26] Heya T, Okada H, Ogawa Y, Toguchi H. Factors influencing the profiles of TRH release from copoly(D,L-lactic/glucolic acid) microspheres. Int J Pharm 1991;72:199–205.