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Rapid communication

Long acting porous microparticle for pulmonary protein delivery

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Abstract

This study investigated the porous-microparticle (PM) with low mass density and large size for pulmonary drug delivery. PM was prepared by the water-in-oil-in-water ($W_1/O/W_2$) multi-emulsion method with cyclodextrin derivative as a porogen and a stabilizer of peptide drugs. Herein, sucrose ethyl acetate (SAIB) was incorporated in PM for long acting protein release. *In vitro* release studies, the rapid release rate of proteins from PM was reduced due to the high viscosity of the added SAIB. As a result, BSA release from PM continued up to 7 days. This result suggests that PM having sustained release characteristics may be successfully applied for long-term pulmonary administration of protein or peptide drug. In addition, it is expected that these particles arrive at a deep lung epithelium due to low density (high porosity) and limit macrophage recognition because of big particle size (more than 5 μm).

Keywords: Sucrose acetate isobutylate (SAIB); Cyclodextrin derivative; Poly(lactide-*co*-glycolide) microparticle; Pulmonary delivery; Long-term protein release

1. Introduction

In recent year, the lungs have gained much attention as a noninvasive alternative for protein delivery in that the macromolecules are efficiently absorbed in the lung surface area of approximately 100 m^2, crossing thin pulmonary absorption barrier (Bivas-Benita et al., 2005). Furthermore, the lung exhibits characteristic features such as ample blood supply, avoidance of hepatic first-pass metabolism, and low enzymatic metabolism, which facilitates the systemic therapy by the inhalable aerosol of proteins (Coldrons et al., 2003; Kohler, 1990; DeBoer et al., 2001).

It is interesting to note that high deposition of inhaled proteins throughout the lung is shown when proteins constitute particles of aerodynamic diameter between 1 and 5 μm (Agu et al., 2001; Pandey et al., 2003). These particles are accumulated in the deep lungs where provide continuous absorption of proteins (Agu et al., 2001). However, protein particles measuring less than approximately 1 μm are exhaled during normal tidal breathings and particles measuring more than approximately 6 μm are deposited in the upper airways with a vigorously mucocilliary clearance (Agu et al., 2001; Jones, 1984). The novel inhalable insulin, Exubera®, therefore considered 1–5 μm particle size for increasing the bioavailability of insulin (Agu et al., 2001). Nevertheless, it was found that particles measuring 1–5 μm in the deep lungs could be removed by alveolar macrophages that recognize and uptake small foreign particles, which can limit therapeutic activity of proteins (Agu et al., 2001; Musante et al., 2002).

According to Edwards et al. (1997), the large porous microparticular system is ideal. The porous particles of more than 5 μm can be deposited in the deep lungs. The large size and low mass density due to porous structure helped their deep lung deposition, thus avoiding phagocytic clearance (Ben-Jebria et al., 2000; Edwards et al., 1997; Koushik et al., 2004; Okumu et al., 2002; Steckel and Brandes, 2004; Dunbar et al., 2002).

However, unfortunately the protein release behavior of these porous particles was unsatisfactory, despite the utilization of biodegradable polymer for the sustained protein release. The release of recombinant human growth hormone (rhGH) from the porous poly(lactide-*co*-glycolide) (PLGA) microparticles released up to 100 wt.% in only 1 day (Kim et al., 2006).

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The immunoglobulin release from lipid-based hollow-porous microparticles was finished in approximately 18 h (Bot et al., 2000). The porous poly(acrylic acid)–cysteine microparticles completed the drug release after 1 h (Bernkop-Schnurch et al., 2003). In summary, these porous particles were characterized by high initial bursts and short-term release in respiratory tract (Tomoda et al., 2005).

The aim of this study is to investigate long acting PM. We prepared PM using cyclodextrin derivative as a porogen and utilized SAIB as an additive for sustained protein release of PM. In particular, SAIB, a highly viscous hydrogel in water, is soluble in volatile organic solvent (such as dichloromethane and ethanol) used for the preparation of microparticles by $W_1/O/W_2$ multi-emulsion method. Furthermore, the metabolites of SAIB are inactive for labile proteins (Okumu et al., 2002; Lee et al., 2006b). This SAIB is expected to inhibit a rapid protein release from porous surface of microparticles.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), sodium hyaluronate (HA), sodium azide, Tween 80, SAIB, sodium chloride trinitrobenzene sulfonic acid (TNBS) and polyvinyl alcohol (PVA) (Mn 12,000–23,000) were obtained from Sigma (St. Louis, MO, USA). Dichloromethane (DCM) was bought from J.T. Baker (Deventer, Netherlands) Sulfobutyl ether β-cyclodextrin sodium salt (SBE-CD) was kindly provided from CyDex Corp (Calabasas, USA). PLGA, RG 502H (lactide:glycolide = 50:50, Mw 9300) and RG 504H (lactide:glycolide = 50:50, Mw 50,100) were purchased Boehringer-Ingelheim (Petersburg, USA). BCA protein assay Kit was purchased from Pierce (Milwaukee, USA)

2.2. Preparation of protein loaded PM

Microparticles were fabricated by the conventional $W_1/O/W_2$ multi-emulsions. BSA (50 mg) was dissolved in 1.0–1.5 ml of phosphate buffer saline (PBS, pH 5.1) containing 2.5 mg HA and 100–250 mg SBE-CD. Herein, HA was used for improving protein loading efficiency. In our previous research, the viscous property of HA in aqueous solution diminished leakage of proteins in aqueous phase to the outer phase during microparticle formation (Lee et al., 2006a). PLGAs, RG 502H and RG 504H at different weight ratio (300/100, 150/150, 0/300) and SAIB were added to 3 ml of DCM solution for the preparation of organic phase (Table 1). Each solution was then mixed together and emulsified by vigorous vortexing for 30 s and then injected into 0.5 wt.% PVA and 0.9 wt.% NaCl aqueous solution. The emulsification was carried out for 5 min by a homogenizer (manufactured by Tokushu Kika Kogyo Corp.) at 4000 rpm. The resultant mixtures were hardened by gentle stirring for 40 min and then collected by centrifugation at 3000 rpm for 2 min. The particles obtained were washed three times with 0.9 wt.% NaCl aqueous solution and freeze-dried for 3 days.

2.3. Protein loading

The actual protein loading efficiency in the microparticles was measured by the TNBS method described in the literature (Bezemer et al., 2000). The absorbance of each sample was read on a microplate reader at a test wavelength of 450 nm. In addition, the absorbance at 450 nm is proportional to the protein concentration. The actual BSA content in the microparticle was calculated using following equation:

$$\%\,\mathrm{BSA} = \left(\frac{P_t}{M_t}\right) \times 100 \quad (1)$$

where P_t is the total amount of protein embedded in microparticles and M_t is the total amount of microparticle harvested.

In addition, the loading efficiency was determined using following equation:

$$\%\,\text{encapsulation efficiency} = \left(\frac{L_a}{L_t}\right) \times 100 \quad (2)$$

where L_a is the amount of protein embedded in microparticles and L_t is the theoretical amount of protein (obtained from feeding condition) incorporated into microparticles.

2.4. Morphology and particle size distribution

The morphology of microparticles was confirmed with a scanning electron microscopy (SEM, Hitachi S-3000N). Before analyzing samples, the dried microspehres were vacuum-coated with a carbon.

A laser light scattering technique (Mastersizer 2000, Malvern) was employed to confirm the particle size distribution of microparticles. Herein, the compressed air system was utilized to inhibit the aggregation of dried microparticles (Shinha and Trehan, 2005).

Table 1
Compositions used for constituting PM and characterization of PM ($n = 3$)

Sample	Compositions (mg)					% BSA	% loading efficiency	Mean particle size (μm)
	BSA	SBE-CD	RG 502H	RG 504H	SAIB			
PO-1	50	0	300	100	0	7.8 ± 0.5	87.5 ± 1.2	22.6 ± 2.9
PO-2	50	100	300	100	0	5.1 ± 0.4	68.6 ± 2.5	20.1 ± 3.2
PO-3	50	200	300	100	0	3.2 ± 0.6	56.6 ± 2.9	23.7 ± 4.7
PO-4	50	300	300	100	0	1.2 ± 0.6	23.2 ± 5.5	–
PO-5	50	200	300	100	150	9.0 ± 0.3	90.9 ± 0.9	22.5 ± 2.6
PO-6	50	200	300	100	300	9.2 ± 0.6	91.2 ± 0.7	–

2.5. Aerodynamic diameter of PM

Mass median aerodynamic diameter (MMAD) of particles was evaluated using a 7-stage Andersen Cascade Impactor (Andersen Samplers Inc., Atlanta, GA, USA).

2.6. In vitro protein release studies

Protein release in PBS (pH 7.4, 0.01% sodium azide, 0.02% Tween 80) was monitored by BCA protein assay kit (Sandor et al., 2001). Thirty milligrams of microparticles were dispersed in 5 ml of PBS and incubated under mild stirring at 37 °C. Aliquots of supernatant were withdrawn at predetermined time intervals and an equal amount of fresh PBS added to maintain a constant volume of medium. The measurement of protein concentration by a microplate reader was performed at 562 nm.

3. Results and discussion

3.1. Morphology of PM

PM was prepared by the conventional multi-emulsion method with PLGA and SBE-CD (as a porogen) (Table 1). As shown in Fig. 1, general PLGA microparticles have smooth surface and spherical shape. However, adding SBE-CD to these PLGA microparticles led to a porous surface. By increasing SBE-CD content, porosity of microparticles was elevated (Fig. 1(a)–(c)). It is assumed that SBE-CD affected DCM evaporation rate before microparticle formation and modified the surface morphology of particles (Wang and Wang, 2002). Furthermore, as Irie and Uekama (1997) reported, SBE-CD used in this study was evaluated to show a minimum intramuscular irritation. In addition, above 300 mg of SBE-CD (PO-4 batch), constituted microparticles were broken down. This implies that high porosity of more than the threshold may deteriorate structural stability of microparticles.

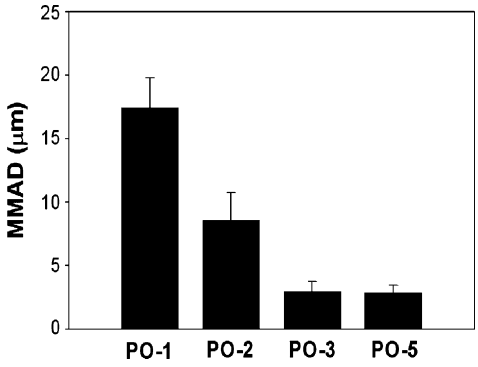

Fig. 2. The MMAD of PM: PO-1, PO-2, PO-3 and PO-5 ($n = 3$).

Fig. 2 shows each MMAD of microparticles. As the content of SBE-CD increased to 200 mg, the MMAD decreased to approximately 3 μm. This PM with the MMAD less than 5 μm is expected to deposit in the deep lung with greater efficiency (Agu et al., 2001; Pandey et al., 2003).

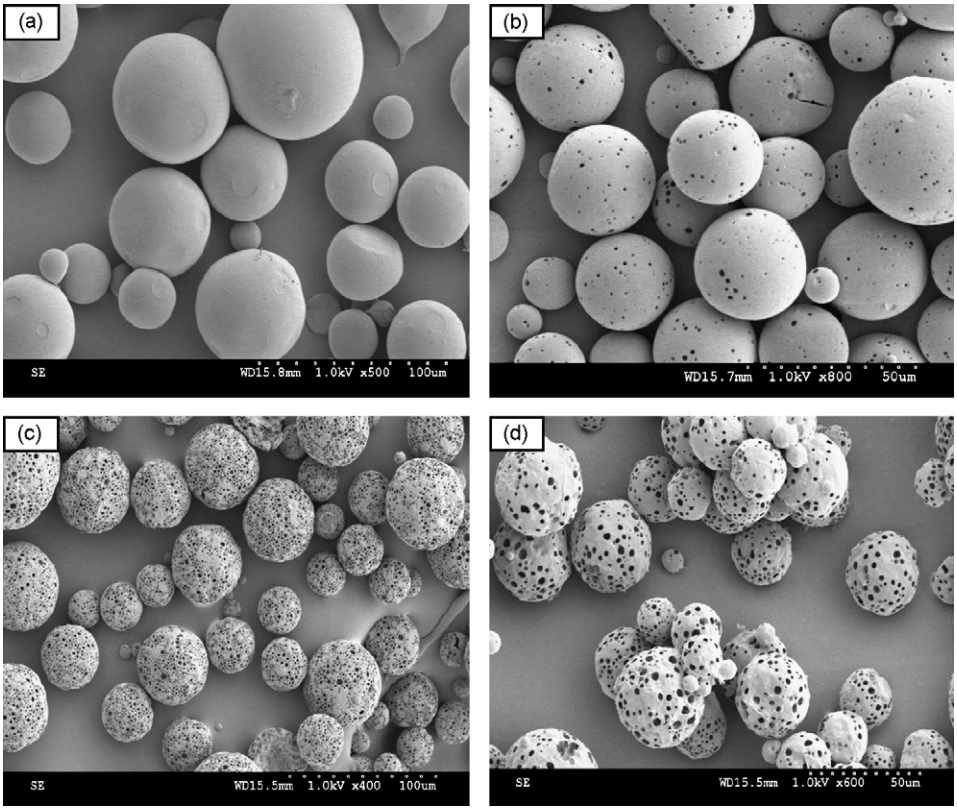

Fig. 1. The SEM images of PM: (a) PO-1, (b) PO-2, (c) PO-3 and (d) PO-5.

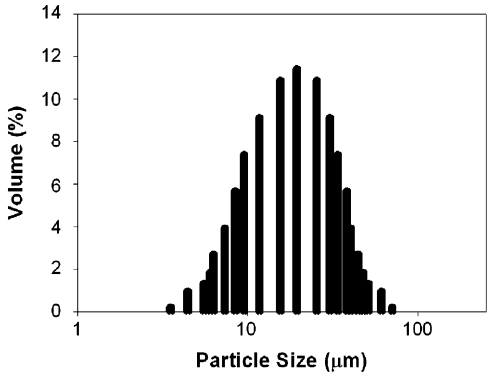

Fig. 3. The particle size distribution of PM (PO-5) determined by a laser light scattering technique.

3.2. BSA loading of PM and addition of SAIB

As shown in Table 1, the presence of porous surface resulted in the decline of BSA loading efficiency. By increasing the amount of SBE-CD (consequently augmentation of surface porosity), BSA loading efficiency (%) and incorporated BSA content (%) decreased to 56.6% and 3.2%, respectively, due to leakage of hydrophilic BSA. However, it is interesting to note that the addition of viscous hydrogel, SAIB improved BSA loading efficiency (%) and incorporated BSA content (%) (PO-5 and PO-6 batch). It appears that during secondary emulsification, high viscosity of SAIB in the organic phase holds a hydrophilic BSA and inhibits leakage of BSA to the outer phase through porous surface of microparticles. This PM from PO-5 batch (Fig. 1(d)) showed particle size of approximately 22.5 μm (Table 1), unimodal particle distribution (Fig. 3), and the MMAD of approximately 2.9 μm (Fig. 2). However, in the PO-6 batch, though they have a high protein loading, surface porosity was lost probably due to an increase of internal density derived from excess SAIB.

In addition, the residual DCM content in the PM was detected at below 500 ppm (by gas chromatography), fulfilling the USP XXIII requirement.

3.3. Drug release kinetics of PM

In vitro BSA release was performed in PBS incubated at 37 °C and the amount of BSA was evaluated using the BCA assay kit.

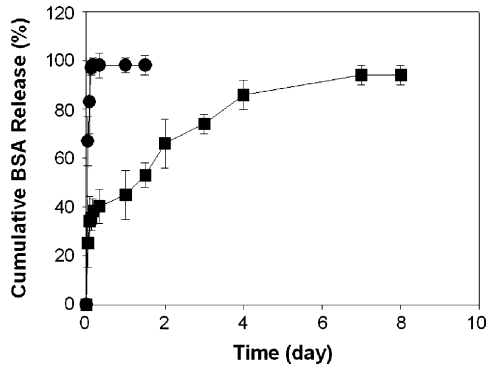

Fig. 4. Cumulative protein release (%) from PM: PO-3 (●) and PO-5 (■) ($n = 3$).

Fig. 4 shows the release time of PM prepared by PO-3 batch or PO-5 batch. In PO-3 batch, BSA release was completed within 4 h, which is attributable to rapid BSA release from porous pathways. Unlike PO-3 batch, the PO-5 batch showed the enhanced BSA release behavior. As shown in Fig. 4, the release of BSA was continued up to 7 days. It is assumed that the viscous hydrogel network of SAIB formed after microparticles were hydrated induced a continuous BSA release. In addition, the period of protein release from PM incorporating SAIB enhanced 42-fold better than PM without SAIB.

4. Conclusion

We have demonstrated that large PM containing protein drug can be successfully prepared using PLGA and SBE-CD. The MMAD of PM were evaluated approximately 3 μm, possibly avoiding macrophage uptake and accumulating the deep lung epithelium. The introduction of SAIB to this PM allowed long-term protein release (up to 7 days), while PM without SAIB showed only 4 h release profile. This finding suggests the feasibility of the long-acting macromolecule drug delivery via respiratory tract.

References

Agu, R.U., Ugwoke, M.I., Armand, M., Kinget, R., Verbeke, N., 2001. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir. Res. 2, 198–209.

Ben-Jebria, A., Eskew, M.L., Edwards, D.A., 2000. Inhalation system for pulmonary aerosol drug delivery in rodents using large porous particles. Aerosol Sci. Technol. 32, 421–433.

Bernkop-Schnurch, A., Egger, C., Iman, M.E., Krauland, A.H., 2003. Preparation and in vitro characterization of poly(acrylic acid)-cystein microparticles. J. Controlled Release 93, 29–38.

Bezemer, J.M., Radersma, R., Grijpma, D.W., Dijkstra, P.J., Feijen, J., van Blitterswijk, C.A., 2000. Zero-order release of lysozyme from poly(ethylene glycol)/poly(butylene terephthalate) matrices. J. Controlled Release 64, 179–192.

Bivas-Benita, M., Zwier, R., Junginger, H.E., Borchard, G., 2005. Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. Eur. J. Pharm. Biopharm. 61, 214–218.

Bot, A.I., Tarara, T.E., Smith, D.J., Bot, S.R., Woods, C.M., Weers, J.G., 2000. Novel lipid-based hollow-porous microparticles as a platform for immunoglobulin delivery to the respiratory tract. Pharm. Res. 17, 275–283.

Coldrons, V., Vanderbist, F., Vanbeeck, R.K., Arras, M., Lison, D., Preat, V., Vanbever, R., 2003. Systemic delivery of parathyroid hormone (1-34) using inhalation dry powders in rats. J. Pharm. Sci. 92, 938–948.

DeBoer, A.H., Molema, G., Frijlink, H.W., 2001. In: Molema, G., Meijer, D.K.F. (Eds.), Methods and Principles in Medical Chemistry, vol. 12. Wiley–VCH, Weinheim, pp. 53–87.

Dunbar, C., Scheuch, G., Sommerer, K., DeLong, M., 2002. In vitro and in vivo dose delivery characteristics of large porous particles for inhalation. Int. J. Pharm. 245, 179–189.

Edwards, D.A., Hanes, J., Caponetti, G., Hrkach, J., Ben-Jebria, A., Eskew, M.L., Mintzes, J., Deaver, D., Lotan, N., Langer, R., 1997. Large porous particles for pulmonary drug delivery. Science 276, 1868–1871.

Irie, T., Uekama, K., 1997. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J. Pharm. Sci. 86, 147–161.

Jones, J., 1984. Clearance of inhaled particles from the alveoli. In: Clarke, S.W. (Ed.), Aerosol and the Lung: Clinical and Experimental Aspects. Butterworth, London.

Kim, H.K., Chung, H.J., Park, T.G., 2006. Biodegradable polymeric microspheres with "open/closed" pores for sustained release of human growth hormone. J. Controlled Release 112, 167–174.

Kohler, D., 1990. Aerosols for systemic treatment. Lung 168, 677–684.

Koushik, K., Dhanda, D.S., Cheruvu, N.P.S., Kompella, U.B., 2004. Pulmonary delivery of deslorelin: large-porous PLGA particles and HPβCD complexes. Pharm. Res. 21, 1119–1126.

Lee, E.S., Kwon, M.J., Lee, H., Kim, J.J., 2006a. Long acting poly(lactide-co-glycolide) microsphere containing sucrose acetate isobutyrate (SAIB). Eur. J. Pharm. Sci. 29, 435–444.

Lee, J., Jallo, G.I., Penno, M.B., Gabrielson, K.L., Young, G.D., Johnson, R.M., Gillis, E.M., Rampersaud, C., Carson, B.S., Guarnieri, M., 2006b. Intracranial drug-delivery scaffolds: biocompatibility evaluation of sucrose acetate isobutyrate gels. Toxicol. Appl. Pharmacol. 215, 64–70.

Musante, C.J., Schroeter, J.D., Rosati, J.A., Crowder, T.M., Hickey, A.J., Martonen, T.B., 2002. Factors affecting the deposition of inhaled porous drug particles. J. Pharm. Sci. 91, 1580–1590.

Okumu, F.W., Dao, L.N., Fielder, P.J., Dybdal, N., Brooks, D., Sane, S., Cleland, J.L., 2002. Sustained delivery of human growth hormone from a novel gel system: SABER^{TM}. Biomaterials 23, 4353–4358.

Pandey, R., Sharma, A., Zahoor, A., Sharma, S., Khuller, G.K., Prasad, B., 2003. Poly(DL-lactide-*co*-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. J. Antimicrob. Chemother. 52, 981–986.

Sandor, M., Enscore, D., Weston, P., Mathiowitz, E., 2001. Effect of protein molecular weight on release from micron-sized PLGA microspheres. J. Controlled Release 76, 297–311.

Shinha, V.R., Trehan, A., 2005. Formulation, characterization, and evaluation of ketorolac tromethamine-loaded biodegradable microspheres. Drug. Deliv. 12, 133–139.

Steckel, H., Brandes, H.G., 2004. A novel spray-drying technique to produce low densitiy particles for pulmonary delivery. Int. J. Pharm. 287, 187–195.

Tomoda, K., Kojima, S., Kajimoto, M., Watanabe, D., Nakajima, T., Makino, K., 2005. Effects of pulmonary surfactant system on rifampicin release from rifampicin-loaded PLGA microspheres. Colloids Surf. B 45, 1–6.

Wang, F.J., Wang, C.H., 2002. Sustained release of etanidazole from spray dried microspheres prepared by non-halogenated solvents. J. Controlled Release 90, 263–280.