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In vitro evaluation of the effect of cyclodextrin complexation on pulmonary deposition of a peptide, cyclosporin A

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Abstract

The effect of hydroxypropyl-α-cyclodextrin (HP-α-CD) complexation on in vitro pulmonary deposition of a cyclic peptide cyclosporin A (CsA) was studied. In addition, the effect of storage (32 days, 40 °C, 75% RH) on CsA/HP-α-CD complexes was studied. The complexation of CsA with CDs was evaluated by a phase-solubility method. Solid CsA/HP-α-CD complexes were prepared by freeze drying. Three inhalation formulations were prepared: CsA/lactose reference formulation (LF) (drug:carrier 1:364, w/w), CsA/HP-α-CD complex formulation (CDF) (drug:CD 1:269, w/w) and CsA/HP-α-CD complex/lactose formulation (CDLF) (complex:carrier 100:114, w/w). The inhalation studies were performed in vitro using Andersen Sampler (Ph. Eur.) and Taifun® multi-dose dry powder inhalers (DPIs). Before the storage, the respirable fraction value (RF%) of CsA was 19.8 ± 0.7%, 33.0 ± 7.0% and 34.6 ± 1.1% (mean ± S.D., $n = 4 \times 20$) with LF, CDF and CDLF, respectively. When exposed to moisture (storage in a permeable polystyrene tube), the RF% values of CsA from formulations containing CsA/HP-α-CD complexes were lower than before the storage. However, when stored in the Taifun® DPI, the RF% value of CsA from any of the formulations did not decrease. In conclusion, an acceptable RF% value of a peptide CsA from freeze-dried, simply micronized CsA/HP-α-CD complex powder was achieved before and after storage in the DPI.

Keywords: Peptide; Cyclodextrin; Inhalation powder; Deposition

1. Introduction

The lungs offer a potential site of administration for the delivery of peptides or proteins (Adjei and Gupta, 1994; Smith, 1997; Agu et al., 2001). However, e.g. low aqueous solubility or stability, rapid clearance in the lungs or inadequate absorption may complicate the pulmonary delivery of peptide or protein drugs (Yamamoto et al., 1996; Yang et al., 1998; Fukaya et al., 2003; Hussain et al., 2003; Lombry et al., 2004; Mahesh Kumar and Misra, 2004).

Cyclodextrins (CDs) are a group of cyclic oligosaccharides which have been shown to improve the physicochemical and biopharmaceutical properties of peptides and proteins (Frömming and Szejtli, 1994; Irie and Uekama, 1999). The inner cavity of a CD molecule is non-polar whereas its outer surface is hydrophilic (Frömming and Szejtli, 1994). Thus, the hydrophobic side chains of peptides or proteins (e.g. aromatic tryptophan and tyrosine residues) may be inserted into the CD cavity, and this may increase the aqueous solubility of peptides or proteins (Irie and Uekama, 1999). For example, β-CD has been shown to improve the aqueous solubility, and hence, to increase the pulmonary bioavailability of a poorly water soluble peptide, FK224 (Nakate et al., 2003). Furthermore, it has been shown that the use of maltosyl-α-CD improved the efficiency of a cyclic peptide cyclosporin A in the inhalation therapy of asthma with an experimental animal model (Fukaya et al., 2003). CDs are also known to stabilize some peptides or proteins against chemical, enzymatic or physical degradation (Brewster et al., 1991; Haeberlin et al., 1996; Fredholt et al., 1999; Sigurjónsdóttir et al., 1999; Tavornvipas et al., 2004) and some CDs have been found to act as absorption enhancers in the lungs (Hussain et al., 2003; Mahesh Kumar and Misra, 2004). These properties represent advantages when developing peptide or protein drug formulations for pulmonary delivery. However, the number of studies

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dealing with the effect of CD complexation on the inhalation properties of drugs is still very limited (Leite Pinto and Cabral Marques, 1999; Srichana et al., 2001; Kinnarinen et al., 2003). To our knowledge, no studies about the effect of CD complexation on the pulmonary deposition of peptides or proteins have yet been published.

In the present study, the effect of CD complexation on in vitro pulmonary deposition of a peptide cyclosporin A (CsA) was studied. Some CDs have been shown to be absorbed from the lungs to the systemic circulation (Cabral Marques et al., 1991), and thus, CDs used in pulmonary applications have to be parenterally safe. In this study, hydroxypropyl-α-cyclodextrin (HP-α-CD) was used due to the good parenteral safety of hydroxyalkylated CD-derivatives (Irie and Uekama, 1997). CsA, which is a cyclic undecapeptide with a low aqueous solubility (0.019 mM at 25 °C) (Miyake et al., 1999), was used as a model peptide since there is increased interest of pulmonary delivery of CsA for use in the treatment of rejection of lung transplants and also in asthma therapy (Sihra et al., 1997; Corcoran et al., 2004). Taifun® (Pitcairn et al., 2000) was used as a multi-dose, reservoir based dry powder inhaler (DPI).

2. Materials and methods

2.1. Materials

Cyclosporin A was purchased from Fluka (Buchs SG, Switzerland). Crystalline α-lactose monohydrate (Pharmatose® 110 M) was purchased from DMV (The Netherlands). α-CD, HP-α-CD, HP-β-CD and γ-CD were purchased from Wacker Chemie GmbH (Burghausen, Germany) and (SBE_{7m})-β-CD from CyDex L.C. (Kansas, USA). Methanol (HPLC grade) was purchased from Labscan Ltd. (Dublin, Ireland) and acetonitrile (HPLC grade) from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Taifun® multi-dose reservoir based DPIs (dose volume 3.46 mm^3) and polystyrene tubes (Tamro, Vantaa, Finland) were kindly donated by Lab Pharma Ltd. (Turku, Finland).

2.2. Analysis of cyclosporin A

The content of CsA in the samples was determined by HPLC. The Agilent 1100 HPLC system (Agilent Technologies Inc., Waldbronn, Karlsruhe, Germany) consisted of a binary pump G1312A, a vacuum degasser G1379A or 1322A, an automated injector system autosampler G1313A, an UV-detector G1315B DAD, an HPLC column oven G1316A and an analyst software Agilent ChemStation for LC 3D Systems Rev. A.08.03. Reversed-phase HPLC was conducted with a LiChroCART® Purospher RP-18e column (125 mm × 4 mm, 5 μm) (Merck KGaA, Germany). The following conditions were used: the mobile phase consisted of acetonitrile, methanol and water, either 60:30:10 (v/v/v) (for the samples from the phase-solubility studies) or 80:40:30 (v/v/v) (for the samples from the in vitro inhalation studies). The detector wavelength was 214 nm, flow rate was 1.0 ml/min, column temperature was either 50 or 70 °C and an injection volume was either 20 or 50 μl. The HPLC method was validated with respect to linearity, repeatability and the limit of quantitation. Prior to HPLC analyses, the samples containing solid particles were filtered (0.45 μm, Millex®-HV, low protein binding Durapore PVDF, Millipore Corporation, Ireland).

The inhalation powders were analyzed by HPLC-ESI-MS/MS-method after the storage (32 days, 40 °C, 75% RH) in order to ensure the chemical stability of CsA during the storage. The HPLC-ESI-MS/MS apparatus consisted of an LCQ quadrupole ion trap mass spectrometer equipped with an ESI ion source (Finnigan MAT, San Jose, CA, USA), a Rheos 4000 HPLC pump (Flux Instruments, Danderyd, Sweden), an autosampler (Merck Hitachi) and a column oven (MetaTherm HPLC Column Temperature Control; Meta Chem Technologies Inc., Torrance, CA, USA). The HPLC-ESI-MS/MS-analyses were performed using a LiChroCART® Purospher RP-18e column (125 mm × 4 mm, 5 μm) (Merck KGaA, Germany). The following conditions were used in HPLC-ESI-MS/MS-analyses: solution A contained 10 mM ammonium acetate in water and solution B contained 10 mM ammonium acetate in 95% ACN. The gradient used was 70–99% of B in 6 min, followed by an isocratic period with 99% B (2 min) and a decrease of B% back to 70%. Flow rate was 500 μl/min and the column temperature was set to 70 °C. The mass spectrometer was used in the positive ion electrospray mode. The spray needle was set to 4.0 kV in the positive ion mode. The spray was stabilised by a nitrogen sheath flow, the value was set to 100 (arbitrary units). The inlet capillary temperature was 225 °C and tube lens potential was 10 V. Injection volume was 5 μl.

2.3. Phase-solubility of cyclosporin A

The phase-solubility studies were performed using the Higuchi and Connors method (Higuchi and Connors, 1965). An excess amount of CsA was added to aqueous CD-solutions (α-CD, HP-β-CD, γ-CD and (SBE_{7m})-β-CD at concentrations of 0%, 2.5%, 5%, 7.5% and 10% (w/v) and HP-α-CD at concentrations of 0%, 2.5%, 5%, 7.5%, 10%, 15% and 20% (w/v). The suspensions were shaken (300 rpm; IKA® KS 260 basic shaker) for 3 days at room temperature (RT) in order to attain an equilibrium. After shaking, the samples were filtered (0.45 μm, Millex®-HV, low protein binding Durapore PVDF, Millipore Corporation, Ireland) in order to remove undissolved CsA. The filtrates were analyzed for CsA by HPLC. The phase-solubility diagrams were plotted as aqueous solubility of CsA [mol/l] as a function of CD concentration [mol/l]. Eq. (1) was used to calculate the stability constants ($K_{1:1}$) between CsA and HP-β-CD, γ-CD, (SBE_{7m})-β-CD and HP-α-CD:

$$K_{1:1} = \frac{\text{slope}}{[S_0](1 - \text{slope})} \quad (1)$$

where $[S_0]$ is the intrinsic solubility [mol/l] of CsA and the slope is that of the phase-solubility diagram (Higuchi and Connors, 1965).

In the case of α-CD, the Eq. (2) was used in order to calculate the stability constants $K_{1:1}$ and $K_{1:2}$:

$$\frac{[S_t] - [S_0]}{[L_t]} = K_{1:1}[S_0] + K_{1:1}K_{1:2}[S_0][L_t] \quad (2)$$

where $[S_t]$ is the total concentration of CsA at CD concentration $[L_t]$. $[S_0]$ is the intrinsic solubility of CsA (Higuchi and Connors, 1965).

2.4. Preparation of solid CsA/HP-α-CD complexes

Solid CsA/HP-α-CD complexes were prepared by agitating an excess amount of CsA in 20.0% (w/v) HP-α-CD aqueous solution (3 days, at room temperature). After shaking, the suspension was filtered (0.45 μm, Millex®-HV, low protein binding Durapore PVDF, Millipore Corporation, Ireland) and the filtrate was freeze-dried (FTS® Systems Inc., N.Y., USA).

2.5. Preparation and characterization of inhalation powders

Three inhalation powder formulations were prepared: (1) reference lactose formulation LF, which consisted of micronized CsA and α-lactose monohydrate 110 M (drug:carrier ratio 1:364, w/w), (2) cyclodextrin complex formulation CDF, which consisted of micronized CsA/HP-α-CD complex (drug:CD ratio 1:269, w/w) and (3) cyclodextrin complex/lactose formulation CDLF, which consisted of micronized CsA/HP-α-CD complex and α-lactose monohydrate 110 M (complex:carrier ratio 100:114, w/w). The amount of lactose in the reference formulation LF was selected so that the mass proportion of CsA in one dose of LF was comparable with that in one dose of CDF. Due to practical reasons, the ratios of drug to lactose in LF (1:364, w/w) and drug to CD in CDF (1:269, w/w) were somewhat different but nevertheless, the ratios were considered equal.

Before preparing the formulations, CsA and freeze-dried CsA/HP-α-CD complex powder were micronized by manually sieving them (15 μm sieve) in order to generate particles with the correct particle size for pulmonary delivery. The particle size distribution of micronized CsA was determined by laser diffraction (wet measurement in water) (Malvern Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK). The particle size (d50) of micronized CsA was 8.8 ± 0.1 μm (mean ± S.D., $n = 17$) (measured by laser diffraction). d10 and d90 values for micronized CsA were 1.7 ± 0.0 and 26.5 ± 0.8 μm, respectively. The particle size distribution of micronized CsA/HP-α-CD complex powder was not determined due to the lack of a proper dispersant. When preparing LF and CDLF formulations, the materials were weighed and mixed in a mortar by a spatula.

Prior to filling the inhalers, each formulation was balanced at RT and 33% RH for 5 days in an open container. Required humidity was achieved by keeping saturated $MgCl_2 \cdot 6H_2O$ solution at the bottom of the desiccator. An accurately weighed amount (550 mg) of each formulation was loaded into the Taifun® DPIs. After loading, the Taifun® DPIs were balanced for 1 day at RT and 33% RH. Four inhalers per each formulation were filled for tests for uniformity of dose and two of them were used also in in vitro inhalation studies.

For stability tests, one Taifun® DPI and one permeable polystyrene tube (25 ml, sealed with a screw cap) were filled with an accurately weighed amount (550 mg) of a formulation and stored at 40 °C and 75% RH for 32 days. Required humidity was achieved by keeping saturated NaCl solution at the bottom of the desiccator. Taifun® inhalers protect powder against humidity (Lankinen, 2000), whereas the polystyrene tubes are very permeable to moisture (Bellamy et al., 1980). After the storage, the powders in the polystyrene tubes were moved into the Taifun® DPIs (1 inhaler/formulation) and the inhalers were stored for 1 day at RT and 33% RH.

The CsA contents of the inhalation powders were quantified by HPLC. The homogeneity of the formulations was checked by analyzing the CsA contents of 10 accurately weighed samples (4–7, 10–16 or 9–11 mg of LF, CDF or CDLF, respectively) taken from different sides of each powder. The samples were dissolved in solvent (10.0–25.0 ml) which consisted of MeOH and H_2O (1:1, v/v) and CsA concentrations were analyzed by HPLC. The CsA content in the LF formulation varied from 2.5 to 2.9 μg/mg powder (RSD 4.0%, $n = 10$). The CsA content in CDF and CDLF formulations was 3.7 μg/mg powder (RSD 0.9%, $n = 10$) and 1.7–1.8 μg/mg powder (RSD 4.1%, $n = 10$), respectively.

The bulk densities (ρ_b) of the formulations were determined by pouring an accurately weighed amount of the powder (1000 mg of LF, 400 mg of CDF or 600–800 mg of CDLF) into a graduated glass cylinder (10 ml; 45° angle). The surface of the powder was leveled gently with a spatula and the volume of the powder was measured using a scale of the graduated glass cylinder.

The formulations were examined before and after the storage by scanning electron microscopy (SEM) (accelerating voltage 15 kV, spot size 3.0 or 4.0, working distance 10 or 13 mm) (XL30 ESEM TMP microscope, FEI Company/Oy Philips Ab). The samples were sputter coated with gold (voltage 2.5 kV, current 20 mA, coating time 2.5 min) (Advanced Sputter Coater, Series II-E5100, Polaron Equipment Ltd., UK).

2.6. Uniformity of emitted CsA dose

Tests for uniformity of emitted dose were performed at RT and 20–28% RH (not controlled) before and after the storage (32 days, 40 °C, 75% RH). Dose collecting tubes (according to the European Pharmacopoeia, 4th ed., 2002) were connected to a differential pressure meter (DVR2 Vacuubrand GMBH + CO KG, Wertheim, Germany), a three-way valve and a vacuum pump. The flow control valve was adjusted until the pressure drop across the inhaler was 4 kPa. The flow rate was measured (28.0–30.3 l/min) (TopTrak™ model no. 826-CE-NX-OV1 PV1-V1, Sierra Instruments Inc., Monterey, CA, USA) and the test flow duration (7.9–8.6 s) was defined so that 4 l of air were drawn through the inhaler per released dose. Critical flow occurrence was ensured by the procedure described in European Pharmacopoeia, 4th ed. (2002). Doses 1–25 from each inhaler (four inhalers before storage, one inhaler after the storage in

an inhaler and one inhaler after the storage in a polystyrene tube) were released into the dose collection tubes. The samples were dissolved in 3.0 or 5.0 ml of solvent which consisted of methanol and water (1:1, v/v). The CsA content of delivered doses was analyzed using HPLC. When analyzing the results, the first five doses from each inhaler were ignored, as described earlier (Harjunen et al., 2002, 2003; Kinnarinen et al., 2003). Eq. (3) was used to calculate the theoretical CsA doses:

$$\text{theoretical CsA dose} = \rho_b V_{dose} c_{CsA} \quad (3)$$

where ρ_b is the bulk density of the formulation, V_{dose} the volume of the dose and c_{CsA} is the CsA content of the formulation.

2.7. The effect of CD complexation on in vitro pulmonary deposition of CsA

An Andersen Sampler (European Pharmacopoeia, 4th ed., 2002) was used in order to investigate whether freeze-dried and micronized CsA/HP-α-CD complex powder was able to deposit in the lungs in vitro. The Andersen Sampler consisted of a mouthpiece adapter, an induction port, a pre-separator, eight aluminium stages (each of them consisted of a frame and a non-coated collection plate; no viscous liquid on the stages was used in this study) and a backup filter. The Andersen Sampler was connected to a three-way valve and a vacuum pump (flow rate 28.3 l/min, flow-time 8.0 s). When adjusting a flow rate, a flow meter (TopTrak™ model no. 826-CE-NX-OV1 PV1-V1) (Sierra Instruments Inc., Monterey, CA, USA) was used. Before every experiment, the airtightness of the Andersen Sampler was ensured and the critical flow occurrence was ensured by the procedure described in European Pharmacopoeia, 4th ed. (2002). The in vitro inhalation studies were performed at RT, 20–28% RH (not controlled) before and after the storage (32 days, 40 °C, 75% RH).

After the test for uniformity of emitted dose, the in vitro inhalation studies were carried out in duplicate (2 × 20 doses) for each inhaler (two inhalers before storage, one inhaler after the storage in an inhaler and one inhaler after the storage in a polystyrene tube). Twenty doses per experiment (doses 26–45 for the first determination and doses 46–65 for the second determination) were released into the Andersen Sampler. After each dose, there was a 1 min interval prior to the release of the next dose. The stages and a backup filter, a mouthpiece adapter and an induction port and a pre-separator were rinsed with 2.0–10.0 ml of solvent consisting methanol and water (1:1, v/v) in order to dissolve deposited CsA or CsA/HP-α-CD complex. The CsA content of the samples was analyzed using HPLC.

The total recovered mass of CsA (RM) and the total fine particle mass of CsA (FPM) (particles, whose aerodynamic diameter is <5.8 μm) emitted from the 20 doses were calculated. RM was the amount of CsA emitted from 20 doses. FPM was the amount of CsA which was deposited onto the stages 2–7 and onto the backup filter. Eq. (4) was used when calculating the respirable fraction RF% values:

$$\text{RF\%} = \frac{\text{FPM}}{\text{RM}} \times 100 \quad (4)$$

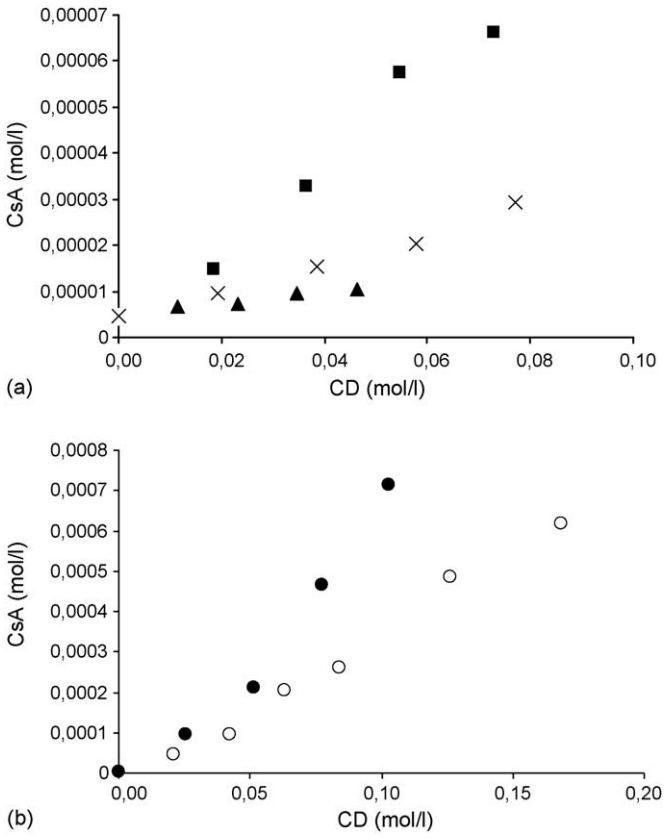

Fig. 1. The phase-solubility diagrams of cyclosporin A (CsA) in the presence of: (a) HP-β-CD (■), γ-CD (×) and (SBE7m)-β-CD (▲), and (b) α-CD (●) and HP-α-CD (○) at room temperature. Note the differences of the scales on the axes.

In order to determine approximate mass median aerodynamic diameters (MMADs), the cumulative particle size diagram was created. The total mass of CsA deposited onto the stages 0–7 and onto the backup filter was calculated for each stage. Cumulative mass% was represented as a function of the effective cutoff aerodynamic diameter of each stage. An approximate MMAD was read from the curve at the 50% point.

3. Results and discussion

3.1. Cyclodextrin complexation of CsA

Fig. 1 shows the phase-solubility diagram of CsA with various CDs. The CsA phase-solubility behavior was classified as A_L-type (Higuchi and Connors, 1965) for HP-α-CD, HP-β-CD, γ-CD and (SBE_{7m})-β-CD with the stability constants ($K_{1:1}$) being 866, 199, 66 and 22 M^{-1}, respectively. With α-CD, CsA exhibited an A_P-type (Higuchi and Connors, 1965) phase-solubility profile and the stability constants were 288 M^{-1} ($K_{1:1}$) and 43 M^{-1} ($K_{1:2}$). The results correspond well with earlier studies where CsA has been shown to complex efficiently with natural α-CD (Sasamoto et al., 1991) and derivatives (Miyake et al., 1999; Okada et al., 1999; Fukaya et al., 2003). In the present study, HP-α-CD was chosen for in vitro inhalation studies because it is thought to be parenterally safe and thus suitable

Table 1
Theoretical and emitted CsA doses, respirable fraction values (RF%) of CsA and mass median aerodynamic diameters (MMAD) of CsA from the formulations LF (micronized CsA/α-lactose monohydrate (drug:carrier ratio 1:364, w/w)), CDF (micronized CsA/HP-α-CD complex (drug:CD ratio 1:269, w/w)) and CDLF (micronized CsA/HP-α-CD complex/α-lactose monohydrate (complex:carrier ratio 100:114, w/w, drug:CD ratio of complex 1:269, w/w)) before and after storage) (32 days, 40 °C, 75% RH) in a Taifun® dry powder inhaler and in a permeable polystyrene tube

	Theoretical CsA dose (μg)	Emitted CsA dose (μg) (mean ± S.D.)	RF (%) value	Approximate MMAD (μm)
Before storage				
LF	6.2	6.6 ± 0.6 (n = 80)	19.8 ± 0.7[a]	3.1
CDF	2.2	1.3 ± 0.5 (n = 80)	33.0 ± 7.0[a]	2.7
CDLF	2.2	1.4 ± 0.2 (n = 80)	34.6 ± 1.1[a]	2.7
After storage (in an inhaler)				
LF	6.2	6.5 ± 0.4 (n = 20)	23.6; 20.0[b]	2.8
CDF	2.2	1.7 ± 0.5 (n = 20)	28.5; 34.4[b]	3.1
CDLF	2.2	1.1 ± 0.2 (n = 20)	34.3; 37.6[b]	2.6
After storage (in a polystyrene tube)				
LF	6.2	5.6 ± 0.4 (n = 20)	20.6; 19.4[b]	3.0
CDF	2.2	2.1 ± 1.1 (n = 20)	9.8; 8.2[b]	n.d.
CDLF	2.2	0.8 ± 0.2 (n = 20)	17.2; 22.0[b]	3.9

n.d. = not determined.
[a] mean ± S.D., n = 4, 20 doses/experiment.
[b] n = 2, values from two experiments, 20 doses/experiment.

for pulmonary delivery (Cabral Marques et al., 1991; Irie and Uekama, 1997). These aqueous solubility studies indicate that CDs are able to increase the solubility of CsA in the lungs and thus maybe reduce the required dose of CsA. Indeed, Fukaya et al. have previously shown that the effective dose of CsA for inhalation may be decreased by using maltosyl-α-CD (Fukaya et al., 2003).

Freeze-dried CsA/HP-α-CD complex contained CsA and HP-α-CD in a ratio of 1:269 (w/w) (1:272 mol/mol), evidence of the presence of empty CD molecules in the powder. A low drug:CD ratio in the complex may limit the use of CDs in inhalation powders, as discussed earlier (Kinnarinen et al., 2003). However, it must be noted that the complexation efficiency of the present samples might be improved by utilizing different methods for preparing the complexes (Frömming and Szejtli, 1994).

The bulk densities (g/ml) of the powders were 0.65 ± 0.03 (n = 3), 0.16/0.18 (values from two parallel experiments) and 0.37/0.35 (values from two parallel experiments) for LF, CDF and CDLF, respectively.

3.2. The effect of CD complexation on in vitro pulmonary delivery of CsA

Mass median aerodynamic diameters (MMADs) of CsA were suitable for pulmonary delivery for every formulation before the storage (Table 1). RF% values of CsA before the storage were 19.8%, 33.0% and 34.6% for LF, CDF and CDLF, respectively (Table 1). Typically with DPIs, approximately 12–40% of the emitted dose is deposited in the lungs (Labiris and Dolovich, 2003). Thus, RF% values of CsA from the present formulations can be considered to be acceptable.

All the formulations were analyzed by HPLC-ESI-MS/MS after the storage (32 days, 40 °C, 75% RH), and no chemical degradation of CsA was observed. However, amorphous materials are known to be hygroscopic (Zeng et al., 2001) and thus, physical stability of amorphous CsA/HP-α-CD complex powders had to be evaluated. Storage in the polystyrene tube affected the performance of formulations containing CsA/HP-α-CD complexes (Table 1). After the storage in the permeable polystyrene tube, the RF% values of CsA from CDF (9.8%, 8.2%, n = 2 × 20) and from CDLF (17.2%, 22.0%, n = 2 × 20) were lower than those measured before the storage (33.0% and 34.6%, respectively). SEM micrographs (Fig. 2) reveal that CDF formed large agglomerates when stored in a polystyrene tube. Due to the formation of large agglomerates, the MMAD of CDF formulation could not be determined.

After storage in a polystyrene tube, the RF% value of CsA decreased to a lesser extent in the case of CDLF than occurred with CDF (Table 1). In addition, SEM micrographs show that the agglomeration during the storage in the polystyrene tube was not as marked in CDLF as in CDF (Fig. 2). These results indicate that the presence of crystalline lactose protected the CsA/HP-α-CD complex powder against agglomeration. However, the RF% value and MMAD of CsA from CDLF were impaired if the powder was stored in a polystyrene tube. Storage in a permeable polystyrene tube did not substantially affect the in vitro inhalation properties of LF; neither the RF% value nor the MMAD of CsA changed during the storage (Table 1).

Contrary to the present study, Kinnarinen et al. have previously shown that the storage in a permeable polystyrene tube (1 month, 40 °C, 75% RH) did not affect the deposition of a budesonide/γ-cyclodextrin complex from a DPI (Kinnarinen et al., 2003). This may be attributable to the different physical properties of the cyclodextrins used in these two studies. γ-cyclodextrin used by Kinnarinen et al. is crystalline whereas HP-α-CD used in the present study is amorphous. In addition, it must be noted that the amounts of crystalline lactose in the formulations in the study of Kinnarinen et al. were higher than used in the present study.

The storage of the formulations (32 days, 40 °C, 75% RH) in the Taifun® DPI did not alter the RF% values of CsA to any major

extent (Table 1). This can be explained by the fact that Taifun® DPI protected the powders from moisture. In the Taifun® inhaler, powder is stored in a tightly sealed medicament chamber which is very impermeable to moisture (Lankinen, 2000). In addition, in the medicament chamber there is a desiccant in a moisture permeable container. Acceptable RF% values of CsA both from CDF (28.5%; 34.4%) and CDLF (34.3%; 37.6%) were achieved after the storage in DPI.

3.3. Uniformity of emitted CsA dose

The average emitted CsA dose ± S.D. before the storage was 6.6 ± 0.6, 1.3 ± 0.5 and 1.4 ± 0.2 μg for LF, CDF and CDLF, respectively (Table 1), which corresponds to 106%, 59% or 64% of the theoretical CsA doses. The theoretical CsA doses from CDF and CDLF were lower than that from LF because of the lower bulk densities of CDF and CDLF.

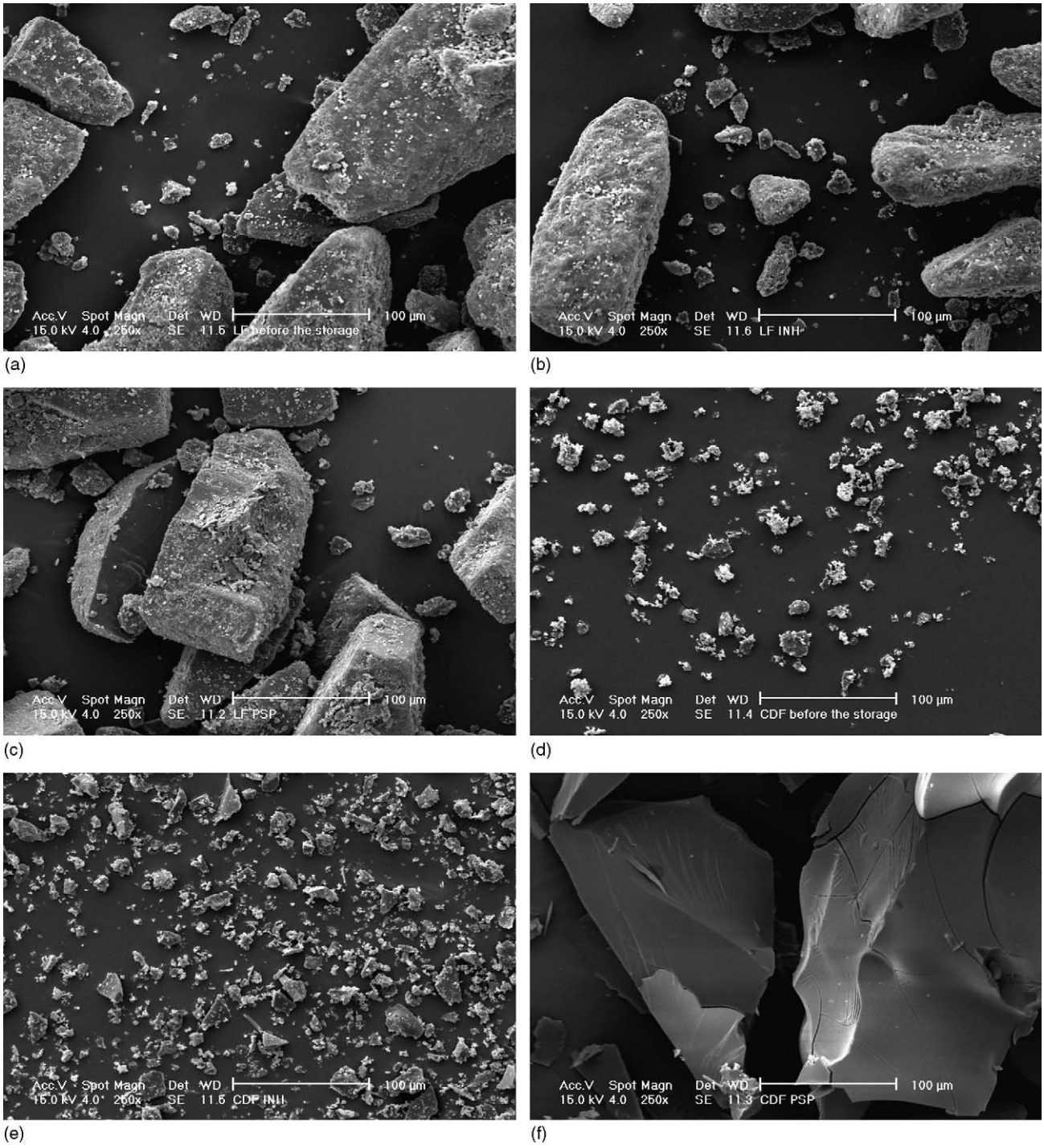

Fig. 2. Scanning electron microscopic (SEM) images of inhalation powders before and after storage (32 days, 40 °C, 75% RH): (a) LF before storage, (b) LF after storage in a Taifun® DPI, (c) LF after storage in a polystyrene tube, (d) CDF before storage, (e) CDF after storage in a Taifun® DPI, (f) CDF after storage in a polystyrene tube, (g) CDLF before storage, (h) CDLF after storage in a Taifun® DPI, and (i) CDLF after storage in a polystyrene tube. Scale bars are 100 μm.

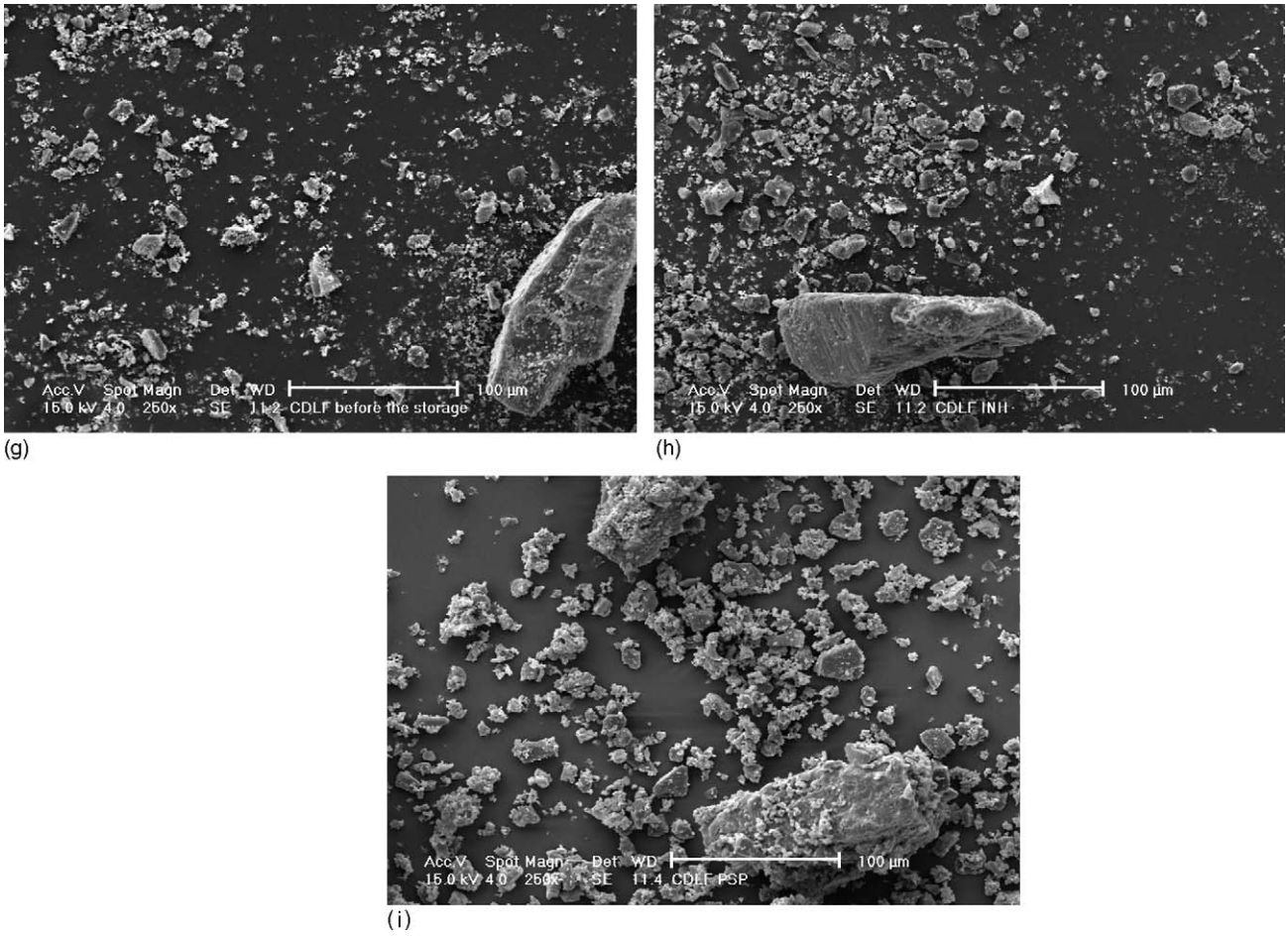

Fig. 2. (*Continued*).

Table 1 shows that CsA/HP-α-CD complex with and without lactose was not totally expelled from the inhaler. This result indicates that the flowability of CDF and CDLF needs to be improved. Micronized powders are usually cohesive since the surface electric forces outweigh the gravitational forces (Staniforth, 1987). In addition, non-uniform particle shape of freeze-dried and micronized powders may result in poor flow properties (Zeng et al., 2001). A coarse carrier (e.g. lactose) typically improves the flowability of an inhalation powder and enhances the dosing accuracy (Labiris and Dolovich, 2003). In the present study, the dosing repeatability of CsA was enhanced when crystalline α-lactose monohydrate was added in CsA/HP-α-CD complex powder (RSD of CsA dose from CDF and CDLF was 34% and 14% before the storage, respectively) (Table 1). However, the emitted CsA dose was not improved by addition of α-lactose monohydrate (CDF versus CDLF, Table 1). It is also possible that the emitted dose of CsA from cyclodextrin formulations can be increased simply by increasing the amount of lactose in the formulation. In the present study, the lactose amount was not increased due to practical reasons (the quantitation limit of the HPLC method).

After storage in a permeable polystyrene tube, CDF formed large agglomerates (Fig. 2) and the uniformity of emitted CsA dose was impaired (Table 1). Correspondingly to the results observed before the storage, CDLF resulted in better dosing repeatability (RSD of CsA dose 24%) than CDF (RSD of CsA dose 54%) after the storage in the polystyrene tube (Table 1). It must be noted that the emitted CsA dose from CDLF was only 36% of the theoretical dose after the storage in the polystyrene tube (Table 1). The storage in the polystyrene tube also tended to decrease the emitted CsA dose from LF.

The storage in Taifun® DPI did not affect the emitted dose of CsA from LF (106% and 105% of the theoretical CsA dose before and after the storage, respectively). Interestingly, the emitted dose of CsA from CDF tended to increase after storage in Taifun® DPI (59% and 77% of the theoretical CsA dose before and after the storage, respectively). In contrast, the emitted dose of CsA from CDLF tended to decrease after storage in Taifun® DPI (64% and 50% of the theoretical CsA dose before and after the storage, respectively). The effect of storage on the emitted dose of a drug from Taifun® has been shown to be strongly affected by formulation (Harjunen et al., 2003).

4. Conclusions

In the present in vitro study, an acceptable RF% value of a peptide CsA from freeze-dried, simply micronized CsA/HP-α-CD complex powder was achieved. The CsA/HP-α-CD complex powder was physically unstable when exposed to moisture (storage in a permeable polystyrene tube 32 days, 40 °C, 75% RH).

As a result, the RF% value of CsA from the cyclodextrin formulations decreased after the storage in the polystyrene tube. However, the storage (32 days, 40 °C, 75% RH) in a Taifun® dry powder inhaler, which protects formulations against the effect of moisture, did not decrease the RF% value of CsA from CsA/HP-α-CD complex powder. The addition of crystalline α-lactose monohydrate improved the physical stability of CsA/HP-α-CD complex powder and dosing repeatability of CsA.

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