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The *in vitro* Pulmonary Deposition of a Budesonide/γ-Cyclodextrin Inclusion Complex

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Abstract

The interest in dry powder inhalers (DPIs) has recently increased because the problems associated with the propellants used in pressurized metered-dose inhalers (PMDIs) will be avoided. Cyclodextrins (CDs) may be used as excipients in inhalation powders; e.g., in order to increase the solubility, stability and absorption of an inhaled drug. In the present study, the effect of complexation of budesonide with γ-CD on its pulmonary deposition was studied *in vitro*. In the presence of γ-CD, the aqueous solubility of budesonide followed B_S-type phase-solubility behaviour. A precipitation complexation method was used to prepare the solid budenoside/γ-CD complexes. The pulmonary *in vitro* deposition of budenoside was evaluated after inhalation of plain budesonide and budenoside/γ-CD complexes (lactose carrier used in both formulations) by using the "Andersen" cascade impactor. The novel Taifun® was used as the DPI. The respirable fractions of the emitted budesonide dose (initially and after the storage in 40 °C, RH 75%) were comparable for both plain budesonide and budesonide/γ-CD complexes. The present study indicates that a drug/CD-complex can be used in inhalation powders without lowering the pulmonary deposition of the drug.

Introduction

Pulmonarily administered drugs are usually intended for topical action, i.e. to treat asthma, chronic obstructive pulmonary disease (COPD) or other lung diseases [1]. However, it also is possible to administer drugs via lungs for systemic action.

In the lungs, there is a large surface area, high blood flow and low enzymatic activity, which produce promising circumstances for absorption [1]. When administered via lungs, the first pass metabolism of the drug will also be avoided. That is why the pulmonary delivery of drugs is considered as an interesting alternative for systemic drug delivery.

The poor aqueous solubility and dissolution of a drug may be an absorption rate limiting step in pulmonary drug delivery [2]. Insoluble particles are removed by the mucociliary clearance in the upper airways and by macrophages in the alveoli [1]. CDs might be of a great value in pulmonary delivery by increasing the solubility and dissolution rate of the complexed drug. This might lead to decreased clearance, increased absorption and faster onset of the action of the drug. Furthermore, there is a possibility to convert a liquid drug in a solid form by CD complexation, which enables the formulation of an inhalation powder. The DPIs have some common advantages compared to the pMDIs, e.g., the need of co-ordination between breathing and releasing the dose will be avoided when using a DPI instead of a pMDI.

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In the dry powder formulations, a carrier (e.g., lactose) is typically needed to improve the flow properties of the micronized drug. The drug must detach from the surface of the carrier in order to reach the lower parts of the lungs. If the flow properties of the drug are acceptable, the use of the carrier is not essential.

The number of studies dealing with pulmonary applications of CDs is limited. Drugs of small molecular weight, such as steroids [3, 4] and β_2-receptor agonists [5–7] have been complexed with CDs or CD derivatives. The *in vitro* pulmonary deposition of the drug/CD complexes [3, 5] and *in vivo* pulmonary absorption [6–8] or toxicity [5, 9] of the CDs or the drug/CD complexes have been studied.

In the present study, the complexation of budesonide with γ-CD was determined and a precipitation complexation method was developed to prepare solid budesonide/γ-CD complexes. The effect of γ-CD complexation on the pulmonary deposition of budesonide was studied *in vitro*.

Materials and methods

Materials

Micronized budesonide and the Taifun® DPIs [10] were supplied from Focus Inhalation, Ltd (Finland). γ-CD (Gamma® W8 or Cavamax® W8) was obtained from Wacker-Chemie GmbH (Germany). α-lactose monohydrate

(Pharmatose® 110 M) was obtained from DMV (Netherlands). Methanol was from Labscan, Ltd. (Ireland), acetonitrile from Rathburn Chemicals (Scotland) and ethanol from Primalco (Finland).

HPLC method for budesonide

The samples obtained from the *in vitro* inhalation studies were analyzed using a method in which the mobile phase consisted of methanol, acetonitrile and 0.017 M sodium dihydrogen phosphate buffer (pH 3.2) (30:30:40 V/V) (flow rate 1.0 ml/min, wavelength 249 nm) [11]. The samples from the phase-solubility studies were analyzed using a method in which the mobile phase consisted of methanol and water (72:28 V/V) (flow rate 1.0 ml/min, wavelength 250 nm). The HPLC system (Merck Hitachi) consisting of UV-detector (L-7400), interface module (D-7000), pump (L-7100), autosampler (L-7250) and Purospher® reversed phase column (RP-18e, 5 μm, 125 $\times$ 4 mm) was used in both methods.

Phase-solubility studies

Phase-solubility studies were performed by stirring excess budesonide with γ-CD aqueous solution (0, 0.5, 1, 2, 4, 6, 8 and 10% (m/V)) for 3 days. Budesonide concentration of the filtered (Ø 0.45 μm) solutions was analyzed by using HPLC.

Preparation of the budesonide/γ-CD complex

The preparation method of solid budesonide/γ-CD complexes based on the phase solubility behaviour of budesonide (Figure 1). To prepare budesonide/γ-CD complex, excess budesonide (200 mg) was agitated with 250 ml of 1% γ-CD solution for 3 days. The solution was filtered and 46 ml of 20% γ-CD solution (per 245 ml 1% γ-CD solution) was added to produce a final 4% γ-CD concentration. After 3 days agitation, the precipitate formed was collected, lyophilized and micronized.

Preparation of the inhalation powders

The effect of γ-CD complexation on *in vitro* pulmonary deposition of budesonide was determined with three formulations. The cyclodextrin formulation (CDF) was prepared by mixing micronized budesonide/γ-CD complex powder and α-lactose monohydrate in a complex:carrier ratio of 1:15. Two control formulations (CF) were prepared by mixing micronized budesonide and α-lactose monohydrate in a drug:carrier ratio of 1:15 (CF1) and 1:159 (CF2). The mixing was performed using Turbula® apparatus.

Tests for inhalation formulations

Taifun® DPIs were filled with 550 mg of each formulation. The formulations were tested initially (two inhalers) and after one month stability test (1 month in 40 °C, 75% RH) (two inhalers) as described below. The DPIs were placed into a climate chamber (25 °C, 60% RH).

Uniformity of budesonide dose

The tests for the inhalation formulations were performed in the climate chamber (25 °C, 65% RH). The doses 1–25 were released in separate sample collection tubes connected to a ballast tube, 3-way valve and a vacuum pump (air flow 30 l/min; flow time 8 s). The tubes were carefully washed with 10 ml of sample solvent (methanol and 0.017 M sodium dihydrogen phosphate buffer (pH 3.2) in a volume ratio of 50:50). The budesonide concentration of the samples was analyzed using HPLC. The first 5 samples were ignored when calculating the results (i.e., the doses 6–25 are included in the results).

In vitro Pulmonary Deposition

The cascade impactor test was performed twice for each inhaler in the climate chamber (25 °C, 65% RH). The doses 26–45 and 46–65 from each inhaler were released into the Andersen cascade impactor, consisting of a throat, a preseparator, 8 frames with non-coated plates and a filter, connected to a 3-way valve and a vacuum pump (air flow 28.3 l/min; flow time 8 s). The budesonide deposited onto each frame and plate, throat, preseparator and filter was dissolved in 10–50 ml of sample solvent (methanol:0.017 M sodium dihydrogen phosphate buffer (pH 3.2) in a volume ratio of 50:50), and the concentration was analyzed using HPLC.

The total recovered mass (RM) and the total fine particle mass (FPM) ($\leq$ 5.8 μm particles) of emitted budesonide were calculated. The respirable fraction (RF%) is expressed as a percentage ratio of FPM to RM. The mass median aerodynamic diameter (MMAD) is the median value of a curve representing the cumulative particle size distribution.

Results and discussion

Phase-solubility studies

Budesonide formed a B_S-type [12] phase-solubility diagram with γ-CD (Figure 1). The apparent solubility of budesonide increased linearly until 1% γ-CD concentration, but after this point the apparent solubility of budesonide decreased and the precipitation of the budesonide/γ-CD complex occurred. The results are in good agreement with earlier studies [13] which show that steroids form B-type phase-solubility diagrams with γ-CD.

The stability constants ($K_{1:1}$) were calculated from the linear part of the phase-solubility diagram by using Equation (1) [12],

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

where S_0 is the intrinsic solubility of budesonide. The value of $K_{1:1}$ for the budesonide/γ-CD complex was 4578 M^{-1}.

Preparation of budesonide/γ-CD complex

A solid white powder of budesonide/γ-CD complexes was obtained by using the precipitation complexation method.

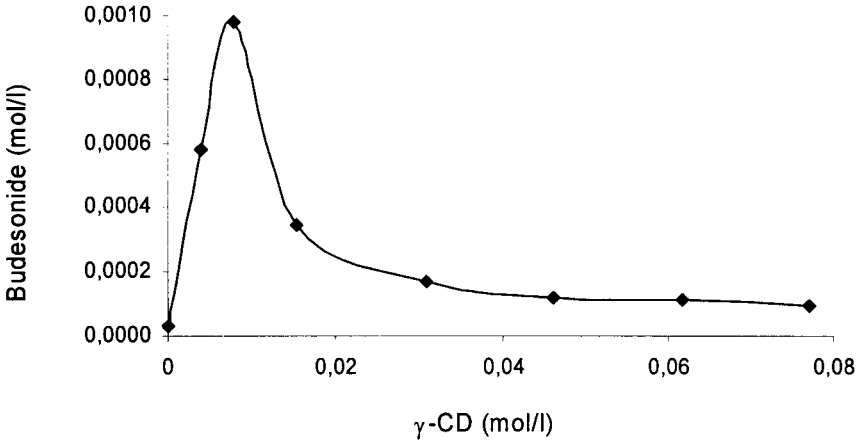

Figure 1. The phase-solubility diagram of budesonide with γ-CD.

Table 1. Emitted dose, respirable fraction (RF%) of the emitted dose and mass median aerodynamic diameter (MMAD) of budesonide initially and after storage (one month in 40 °C, RH 75%) (mean)

Formulation[a]		Theoretical dose (μg)	Emitted dose (μg) ($n = 40$)	RF% ($n = 4$)	MMAD (μm)
CF 1	Initially	100	69	45	2.9
	After storage	100	79	51	3.1
CF 2	Initially	10	7.0	35	3.7
	After storage	10	7.5	31	4.0
CDF	Initially	15	9.3	35	4.7
	After storage	15	15.2	31	4.9

[a] CF 1 = budesonide:lactose 1:15.
CF 2 = budesonide:lactose 1:159.
CDF = budesonide/γ-CD complex:lactose 1:15.

The yield of the budesonide/γ-CD complex was 528–558 mg ($n = 4$). The budesonide content of the complex powder was 0.11–0.16 mg budesonide/mg powder ($n = 4$).

Tests for inhalation formulations

Uniformity of budesonide dose

The average emitted dose of budesonide varied from 69 to 79% (CFs) and from 62 to 101% (CDF) of the theoretical dose (Table 1). It must be noted that the emitted budesonide dose was higher after storage than initially. This is probably due to some changes in interparticulate interactions during the storage which result in the easier detachment of budesonide or budesonide/γ-CD complex from the carrier particles.

In vitro *pulmonary deposition*

The respirable fraction (RF%) of budesonide was comparable for the CDF (15 μg budesonide/dose) and the CF 2 (10 μg budesonide/dose) (Table 1). The RF% was somewhat higher for the CF 1 (100 μg budesonide/dose). This result indicates that the use of CDs does not impair the properties of a budesonide inhalation powder. This result is in agreement with previous results of other studies, where the use of CDs even improved the *in vitro* pulmonary deposition of salbutamol (γ-CD) [5] and beclomethasone (HP-β-CD) [3].

If the use of a carrier is essential, the amount of inhalation powder in one inhaled dose may become a limiting factor in the development of cyclodextrin containing inhalation formulations. In order to avoid the mass and volume becoming too large, the dose of the drug should be low enough or the complexation efficiency should be very high to enable the use of drug/CD complexes in an inhalation powder. Naturally, in some cases, when a carrier is not essential, this matter can be ignored.

Conclusions

The present study shows that the precipitation complexation method produces a solid powder of budesonide/γ-CD complexes and the complexation of budesonide with γ-cyclodextrin does not decrease the pulmonary deposition of the drug *in vitro*. The results indicate that CDs can be used in inhalation powders in order to improve the biopharmaceutical properties of the drug without lowering its pulmonary deposition.

Acknowledgments

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