Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 55 (2003) 147–154
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with β-cyclodextrin

Toshiomi Nakate[a,*], Hiromitsu Yoshida[a], Atsuo Ohike[a], Yuji Tokunaga[a], Rinta Ibuki[a], Yoshiaki Kawashima[b]

[a]*Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan*
[b]*Gifu Pharmaceutical University, Gifu, Japan*

Received 18 June 2002; accepted in revised form 31 October 2002

Abstract

FK224 is a cyclopeptide drug with a low aqueous solubility. Following oral administration to rats, poor absorption was observed due to proteolysis in the gastrointestinal tract. The objective of this study was to investigate the effect of the pulmonary route on the systemic absorption of FK224 in comparison with other administration routes, and to determine the bioavailability (BA) of FK224 following pulmonary administration in rats using various dosage forms. From absorption studies on the Polyethylene Glycol 400 solution given by various routes (intranasal, subcutaneous, intratracheal and intravenous as reference), it was shown that pulmonary administration was a potentially attractive route for FK224. In the pulmonary absorption studies, after administration of the aqueous suspension, the BA was reduced to 2.7% compared with 16.8% for the solution. However, β-cyclodextrin (β-CyD) was found to be an effective additive as far as improving the solubility of FK224 was concerned. The BA of the aqueous suspension containing β-CyD was increased to 19.2%. Pressurized metered dose inhalers were prepared by formulating β-CyD with various molar ratios of 1:0, 1:1 and 1:7 (FK224/β-CyD), and the resulting BAs were 4.3%, 29.0% and 91.2%, respectively. It was observed that both the C_{max} and AUC of FK224 were increased as the amount of β-CyD increased. The plasma profiles showed sustained absorption. In conclusion, we have seen that the lung is a suitable route for absorption of FK224, and β-CyD is an extremely effective additive as far as improving the pulmonary absorption of FK224 is concerned. β-CyD or derivatives with various degrees of aqueous solubility are potential drug carriers for controlling pulmonary absorption.

Keywords: Lung; β-Cyclodextrin; Absorption enhancement; Metered dose inhaler; Peptide

1. Introduction

Most drugs on the market, designed for application to the respiratory organs, have been developed for the treatment of respiratory diseases such as asthma or other localized lung diseases. However, in the last 10 years, the pulmonary route has been recognized as a potential way of delivering drugs to the systemic circulation [1]. Many peptides and proteins must be administered intravenously due to their inadequate stability caused by proteolysis, hepatic metabolism, and low membrane permeability while in the gastrointestinal (GI) tract.

FK224 (L-Ser-L-Thr-L-Leu-D-Phe-L-allo-Thr-L-Asp-NH_2, MW:1041) is a novel cyclopeptide which has been investigated for its potential as a substance P agonist and neurokinin antagonist. It has a very low aqueous solubility, and following oral administration to rats, poor absorption was observed due to proteolysis in the GI tract. In the present study, the bioavailabilities (BAs) of FK224 following administration via a number of different routes (intraperitoneal, subcutaneous, intratracheal and intravenous as reference) were compared in rats to evaluate the potential of pulmonary administration. In pulmonary absorption studies, effective additives have been investigated, with the aim of improving systemic absorption. We chose β-cyclodextrin (β-CyD) as an appropriate enhancing agent because of its high solubilizing capability as far as FK224 is concerned. Furthermore, as a practical dosage form, a pressurized metered dose inhaler (p-MDI) has been developed for a formulation incorporating β-CyD, and the effectiveness of this additive on pulmonary absorption has been confirmed in rats. Based on the pharmacokinetic

* Corresponding author. Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka, 532-8514, Japan. Tel.: +81-6-6390-2439; fax: +81-6-6304-1399.
E-mail address: toshiomi_nakate@po.fujisawa.co.jp (T. Nakate).

0939-6411/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0939-6411(02)00158-3

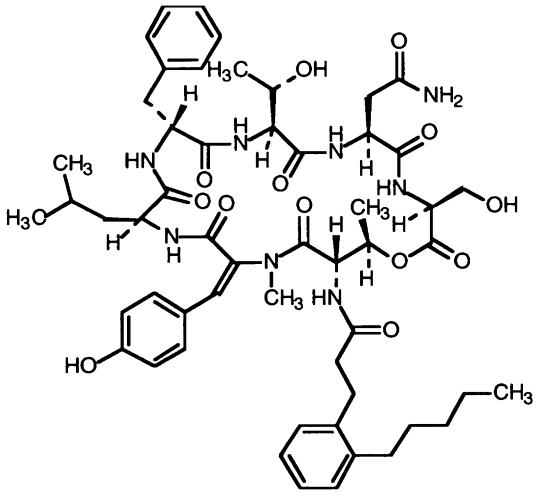

Fig. 1. Chemical structure of FK224.

parameters obtained, a possible mechanism of enhancement of FK224 absorption by β-CyD is discussed in this report. There have also been attempts to improve the systemic absorption of peptides using the pulmonary route [2–5]. However, there seems to be no established explanation for this, in particular in the case of CyDs.

2. Materials and methods

2.1. Materials

FK224 was provided by Fujisawa Pharmaceutical Co. Ltd. (Osaka Japan). The chemical structure of FK224 is shown in Fig. 1. Polyethylene Glycol (PEG) 400 was obtained from Wako Ltd. (Tokyo Japan) and was used as the sample solution medium for the administration study. Methanol, diethyl ether and acetonitrile, all of analytical grade, were purchased from Hayashi Pure Chemical Industries Ltd. (Tokyo, Japan). A pH 6.0 buffer (Puffer-Titrisol®) was purchased from Merck, and α-CyD, β-CyD and γ-CyD of guaranteed grade were purchased from Tokyo Kasei Ltd. (Tokyo, Japan). All chemicals were used as received.

2.2. Preparation of dosage forms

2.2.1. FK224 solutions

FK224 was dissolved in PEG 400 (12.5 and 100 mg/ml as FK224).

2.2.2. FK224 suspension

Micronized FK224 was dispersed in an aqueous solution containing 0.5% (w/v) methylcellulose (MC) (12.5 mg/ml as FK224).

2.2.3. FK224/β-CyD suspension

FK224 and β-CyD (1:1 molar ratio) were dissolved in a 50% (v/v) ethanolic solution and then evaporated to dryness. The dried mixture was pulverized using a jet mill and the micronized mixture was dispersed in an aqueous solution containing 0.5% (w/v) MC immediately before administration (12.5 mg/ml as FK224).

2.2.4. FK224 p-MDI

FK224 and β-CyD (1:0, 1:1 and 1:7 molar ratio) were dissolved in a 50% (v/v) ethanolic solution and then evaporated to dryness. The dried mixture was pulverized using a jet mill and suspended in chlorofluorocarbon (CFC) propellant (CFC11/CFC12/CFC114 = 17.5/65/17.5) including 1.0% (w/v) soybean lecithin as the dispersing agent and to lubricate the valve mechanism. The concentrations of FK224 with 0.125, 0.25 and 1.25 mg/100 μl were prepared, and administration involved a single activation.

2.3. Animal experiments

Young male Sprague–Dawley rats weighing 250–300 g were used and anesthetized in an ether-saturated chamber prior to administration. After administration, 0.2 ml blood was collected from the inferior vena cava at designated time intervals. Each blood sample was centrifuged immediately after collection at 3000 rev./min for 10 min in order to obtain plasma.

2.3.1. Rat absorption studies involving various administration routes

FK224 (5 mg/kg) was given intravenously, intranasally or intratracheally, and 100 mg/kg FK224 was given orally or subcutaneously with FK224 solutions to each rat. The area under the plasma FK224 concentration vs. time curve (AUC; 0–6 h) was calculated by the trapezoidal method after administration by each route. The absolute BA was determined by comparing the AUC following intravenous administration using Eq. (1).

$$\mathrm{BA}\% = (\mathrm{AUC}_{\mathrm{route}}/\mathrm{AUC}_{\mathrm{i.v.}}) \times (\mathrm{dose}_{\mathrm{i.v.}}/\mathrm{dose}_{\mathrm{route}}) \times 100 \quad (1)$$

2.3.2. Pulmonary absorption studies involving solution, suspension, and p-MDI

The trachea was exposed and an incision was made between the fifth and sixth tracheal rings caudal to the thyroid cartilage. A polyethylene tube was attached to a microsyringe, and inserted from the tracheal incision into the lung. Then, 5 mg/kg FK224 was given as FK224 solution, suspension and FK224/β-CyD suspension. In the case of p-MDI, a stainless-steel needle was attached to the valve of the p-MDI, and inserted into the tracheal incision. FK224 (0.5–5.0 mg/kg) was given at the bifurcation of the trachea via the p-MDI needle.

The BA was calculated by comparing the area under the curve over 24 h ($\mathrm{AUC}_{0-24\ \mathrm{h}}$) vs. that for an i.v. dose of 5 mg/kg using Eq. (1).

2.4. Assay of FK224 in plasma

Plasma samples (0.1 ml) were mixed with 0.5 ml phosphate buffer (pH 6), 0.1 ml methanol and 4 ml diethyl ether in 10 ml glass-stoppered centrifuge tubes. Subsequently, the mixed solution was shaken for 5 min and centrifuged at 2700 rev./min for 5 min. A 3 ml volume of the organic phase containing FK224 was transferred to a 5 ml pear-shaped flask, and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 150 μl methanol and 20 μl of this solution was subjected to HPLC under the following conditions: a μBondasphere column 5 μm C18-100 Å (Waters, 15 cm × 3.9 mm I.D.), acetonitrile solution (50:50, v/v) as a mobile phase at 0.8 ml/min and detection at a wavelength of 280 nm.

2.5. FK224 solubility profiles

The solubility of FK224 was determined at various pH values (pH 2, 4, 6, 8). Sodium editic acid, glycerin, ethanol, carboxymethylcellulose and Polysorbate 80, which have all been widely used for commercial products applied to the lung, were evaluated for their effect on solubility. Moreover, hydroxymethylcellulose and three kinds of CyDs, as shown in Table 2, were also evaluated as common solubilizing agents. Then 1.0% (w/v) of each agent was added in water, and mixed with excess FK224 in a jacketed glass vessel by stirring at 25 °C, until solubility equilibria were reached (12 h). The concentrations of the CyDs were changed from 0.5% to 5% to examine the solubility profiles of each CyD. After equilibration, the suspension of FK224 was centrifuged at 3000 rev./min for 10 min, and the clear supernatant solution was filtered through a filter of pore size 0.22 μm. The concentration of FK224 in the filtered solution was determined by HPLC.

2.6. FK224 dissolution profiles of mixtures with β-CyD

Using the pulverized mixtures used for p-MDI preparation, the dissolution profiles were investigated to compare the dissolution rates. The dissolution tests were performed using saline solution according to the paddle method described in JP XIII (100 mg as FK224, 100 rev./min paddle rotating speed, 900 ml dissolution medium, 37 ± 0.5 °C temperature of the dissolution medium). The samples collected from the vessel at specified times were immediately filtered after sampling. FK224 in the sample solution was assayed by HPLC, and the quantity dissolved in a specified time was expressed as a percentage of the labeled amount.

2.7. Aerodynamic particle size distribution of FK224

The aerodynamic particle size distributions of FK224, using the three formulations of p-MDI, were determined by the multi-stage liquid impinger (MSLI) method at an air flow rate of 60 l/min. When FK224 was discharged from the p-MDI to the MSLI, the adapter provided by Valois (Type: NK1, 0.3) was used. An appropriate volume of methanol was used as the dissolving solvent for FK224.

3. Results

3.1. FK224 absorption studies following various routes of administration

Fig. 2 summarizes the absolute BAs following administration of FK224 solution (PEG 400) to rats when the compound was given orally (p.o.) or subcutaneously (s.c.) at a dose of 100 mg/kg, or intranasally (i.n.) or intratracheally (i.t.) at a dose of 5 mg/kg. FK224 was not absorbed from the GI tract. After administration via the s.c. and i.n. routes, the BAs were only 4.0% and 2.2%, respectively. Poor absorption was observed by both routes. On the other hand, it was found that more than 15% FK224 was absorbed from the lung when given as a solution, indicating that the pulmonary route could deliver FK224 into the systemic circulation.

3.2. Pulmonary absorption studies

Fig. 3 summarizes the BAs of FK224 in rats when the compound was given i.t. at a dose of 5 mg/kg in solution and as a suspension. In the case of the aqueous suspension without β-CyD, the BA was only 2.7%. However, in the case of the suspension of FK224 containing β-CyD, the BA was 19.2% which is almost equivalent to the absorption ratio with PEG 400 solution.

Fig. 4 shows the concentration–time profiles of FK224 in plasma after administration of 5 mg/kg FK224 containing various ratios of β-CyD by p-MDI. Fig. 5 shows the concentration–time profiles of FK224 in plasma after

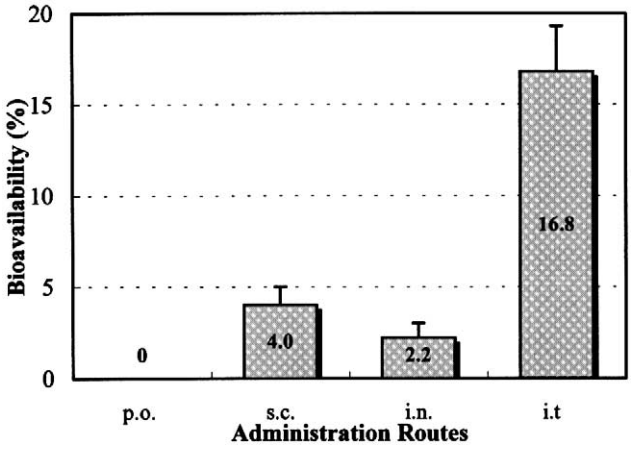

Fig. 2. Comparison of absolute BAs of FK224 for various administration routes to rats after oral (p.o.) and subcutaneous (s.c.) doses of 100 mg/kg with PEG solution, and intranasal (i.n.) and intratracheal (i.t.) doses of 5 mg/kg with PEG solution. Mean ± SE of three rats.

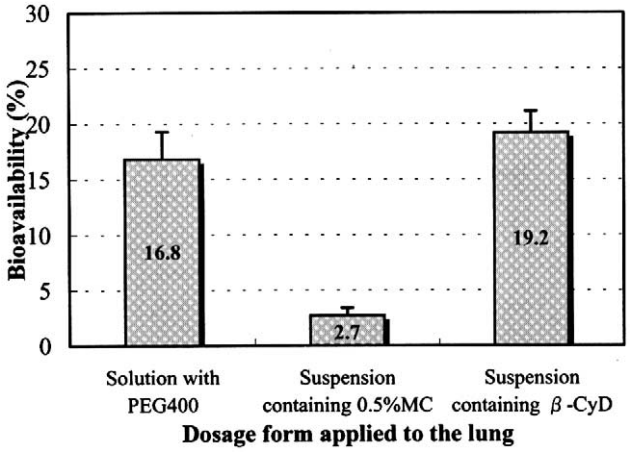

Fig. 3. Comparison of absolute BAs of FK224 following intratracheal administration to rats with various dosage forms at 5 mg/kg. Mean ± SE of three rats.

administering various doses of FK224 with a 1:7 ratio of β-CyD by p-MDI and the pharmacokinetics parameters are summarized in Table 1. The BAs of FK224 increased in parallel with the amount of β-CyD. In the case of the formulation without β-CyD (FK224 alone), the C_{max} was only 0.05 ± 0.03 μg/ml. However, the corresponding values for the formulation with β-CyD in ratios of (FK224/β-CyD) 1:1 and 1:7 were 0.17 ± 0.09 and 0.43 ± 0.22 μg/ml, respectively. The AUC of each formulation also increased in relation to the ratio of β-CyD. In the case of the 1:1 formulation, the AUC was 2.15 ± 0.25 μg·h/ml, i.e. approximately seven (7) times that of the drug alone. Furthermore, the 1:7 formulation showed extremely good absorption (6.76 ± 0.92 μg·h/ml), and the BA was 91.2%. In addition, the T_{max} was 0.25 h and this was the same for the 1:1 and 1:7 formulations. In the presence of β-CyD, the plasma level profiles reflected a period of sustained absorption.

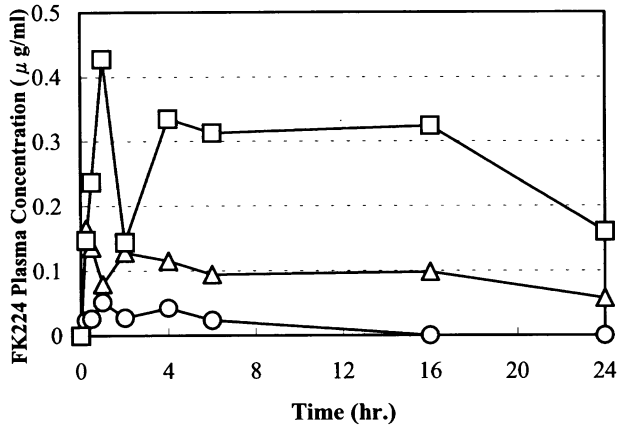

Fig. 4. Mean plasma concentrations of FK224 in rats after intratracheal administration of 5 mg/kg with p-MDI. (○) FK224 alone; (△) FK224/β-CyD, 1:1; (□) FK224/β-CyD, 1:7 ($n = 3$).

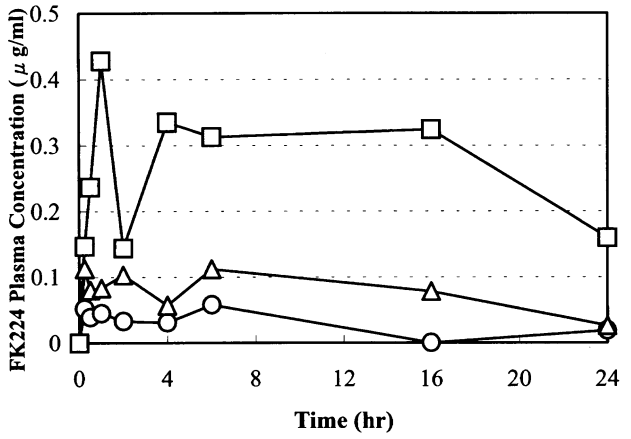

Fig. 5. Mean plasma concentrations of FK224 in rats after intratracheal administration with p-MDI (FK224/β-CyD, 1:7). (○) 0.5 mg/kg; (△) 1 mg/kg; (□) 5 mg/kg ($n = 3$).

3.3. FK224 solubility

The solubility of FK224 in solutions of various pH values and the effectiveness of a number of solubilizing agents were investigated using solutions containing 1.0% (w/v) of each solubilizing agent. As shown in Table 2, the solubility of FK224 was unaffected by the solution pH. Most additives did not improve FK224 solubility, except for polysorbate 80 and CyDs. As far as CyDs were concerned, β-CyD significantly improved FK224 solubility. There was over a 50-fold difference compared with FK224 alone. The FK224 solubility profiles following the addition of CyDs are shown in Fig. 6. The solubility of FK224 increased in parallel with the concentration of the CyDs. β-CyD had the highest slope of the CyDs studied, up to a concentration of 3.0×10^{-2} M, and over this concentration of β-CyD the solubility remained steady because of the limited solubility of β-CyD in water (2.5×10^{-2} M at 25 °C).

Based on the linearity of the solubility profile between β-CyD and FK224, it was concluded that the 1:1 β-CyD complex with FK224 was formed in the solution up to a β-CyD concentration of 3.0×10^{-2} M. From the slope of the approximate line described in Fig. 6, it was calculated that approximately seven (7) molecules of β-CyD are required to dissolve one molecule of FK224. The stability constant, $K_{1:1}$, was calculated to be approximately 8000 M^{-1} according to Eq. (2), by using the phase solubility method of Higuchi and Connors [6].

$$K_{1:1} = \text{slope}/(\text{intercept} \times (1 - \text{slope})) \quad (2)$$

3.4. FK224 dissolution profiles from mixtures with β-CyD

The dissolution rates of FK224 from the mixtures with β-CyD, prepared by the co-precipitation method, were investigated. The dissolution profiles are shown in Fig. 7.

Table 1
Summary of the pharmacokinetics parameters (mean ± SE; $n = 3$) of FK224 after intratracheal administration of various doses with p-MDI to rats

Formulations of FK224/β-CyD	Dose (mg/kg)	T_{max} (h)	C_{max} (μg/ml)	$AUC_{0-24\ h}$ (μg h/ml)	BA (%)[a]
1:0	5	1 ± 0.3	0.05 ± 0.03	0.32 ± 0.13	4.3
1:1	5	0.25 ± 0.1	0.17 ± 0.09	2.15 ± 0.25	29.0
	0.5	0.25 ± 0.1	0.06 ± 0.03	0.60 ± 0.21	81.0
1:7	1	0.25 ± 0.1	0.11 ± 0.08	1.87 ± 1.26	126.0
	5	0.25 ± 0.2	0.43 ± 0.22	6.76 ± 0.92	91.2

[a] BA, ratio against the $AUC_{0-\infty}$ of intravenous administration in rats.

The dissolution rates increased in parallel with the increase in the ratio of β-CyD. The time to reach 50% dissolution of FK224 ($T_{50\%}$) for 1:1 and 1:7 FK224/β-CyD ratios was 25 and 3 min, respectively. In the case of FK224 alone, the dissolution rate after 60 min remained 40%.

3.5. Aerodynamic particle size distribution of FK224

The aerodynamic particle size distribution of FK224 was determined after discharge from the p-MDI. The effect of the different ratios of β-CyD was studied by means of a MSLI at an air flow rate of 60 l/min.

All formulations showed similar profiles for the aerodynamic particle size distribution of FK224 as shown in Fig. 8. About 12% of the FK224 adhered to the actuator in all of the formulations, so that the emitted dose was about 88%. The fine particle fractions, which are those with a cut-off diameter under 5.9 μm, accounted for 36% of all formulations.

4. Discussion

FK224 exhibits poor oral absorption in rats due to proteolysis in the GI tract. As far as the nasal or subcutaneous route is concerned, the BA was 2.2% or 4.0%, respectively, a slight improvement compared with oral absorption. However, 16.8% of the administered drug was absorbed from the lung when given as a solution of PEG 400 to rats (Fig. 2). This shows that the pulmonary route is an effective method of administration of FK224, compared with other routes. When FK224 was given i.t. as an aqueous suspension, the BA was only 2.7% as shown in Fig. 3. The difference in BA between the solution and suspension strongly suggests that the dissolved quantity of FK224 on the surface of the alveolar epithelial membrane influences absorption into the systemic circulation. In other words, a pharmaceutical approach involving the improved solubilization of FK224 would likely increase the permeation of the drug via the lung. Although the solubility of FK224 was unaffected by the solution pH, polysorbate 80 and CyDs were found to be effective as shown in Table 2.

CyDs have been investigated as additives to improve certain physical or chemical properties of drugs, such as their solubilization and stabilization, as well as to control drug release [7–10]. There have been many reports of enhanced drug BA following oral or nasal administration involving the use of CyDs [11–14]. However, there are few studies involving the pulmonary application of CyDs [15–20].

From the results of FK224 absorption studies in rats using a suspension including β-CyD, it was found that β-CyD was an effective additive for enhancing the absorption of FK224, as shown in Fig. 3. It appears that the addition of

Table 2
Solubilities of FK224 in aqueous solution containing 1.0% (w/v) of each solubilizer at 25 °C

Solubilizing agents		Solubility (μg/ml)
None	Water	30
	pH 2	35
	pH 4	24
	pH 6	21
	pH 8	23
Sodium editic acid		40
Glycerin		40
Ethanol		40
Carboxymethylcellulose		10
Polysorbate 80		460
Hydroxymethylcellulose		60
α-CyD		270
β-CyD		1100
γ-CyD		280

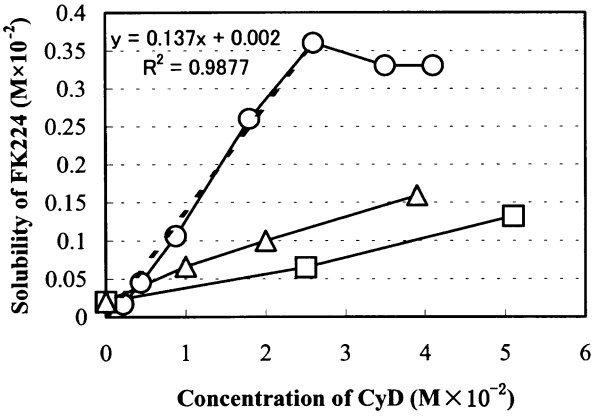

Fig. 6. Solubility profiles of FK224 in water containing various cyclodextrins at 25 °C. (○) α-CyD; (△) β-CyD; (□) γ-CyD.

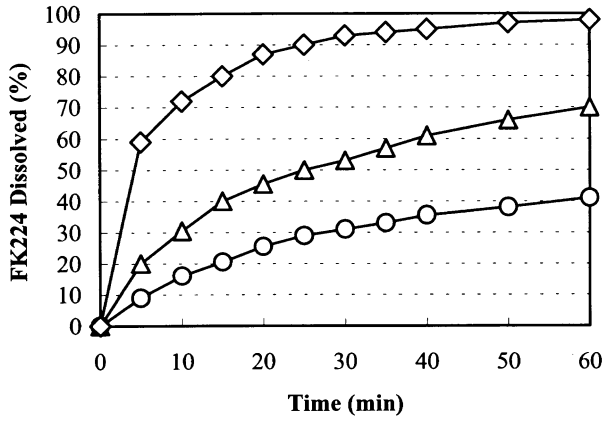

Fig. 7. Dissolution profiles of FK224 (100 mg) from mixtures with various ratios of β-CyD in saline (900 ml) at 37 °C measured by the paddle method at 100 rev./min. (○) FK224 alone; (△) FK224/β-CyD, 1:1; (◇) FK224/β-CyD, 1:7.

β-CyD leads to an increase of the quantity of FK224 dissolving in the alveolae.

As far as polysorbate 80 is concerned, a number of studies have described formulations with absorption enhancers [21,22], or protease inhibitors [23], which showed marked enhancement of absorption. However, there was also damage to the alveolar epithelium because the surfactant may cause the destruction of epithelial cells [22]. Furthermore, the addition of surfactant may also have an effect on the alveolar ventilation system because the alveolar epithelium cells are normally covered with enough natural surfactant to ensure efficient inflation and deflation of the alveolae. From what has been discussed above, it seems reasonable to conclude that polysorbate 80 is not suitable as an FK224 enhancer.

In the pulmonary absorption studies in rats using p-MDI, the BA increased linearly in parallel with the β-CyD ratio. When no β-CyD was present, the BA was only 4.3%, but a 1:1 formulation (FK224/β-CyD) increased this to 29%, and

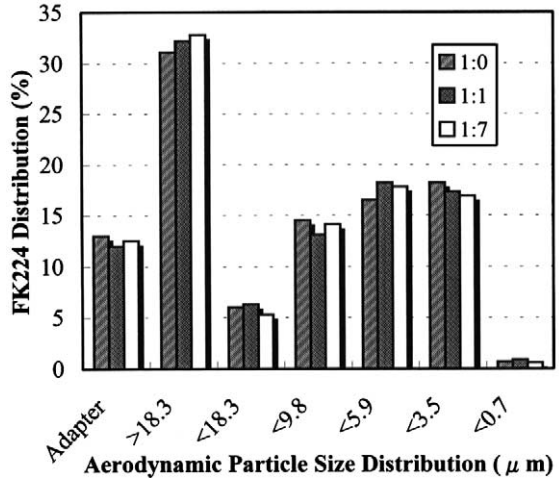

Fig. 8. Aerodynamic particle size distribution of FK224 after discharging from p-MDI containing various ratios of β-CyD determined by MSLI at an air flow rate of 60 l/min.

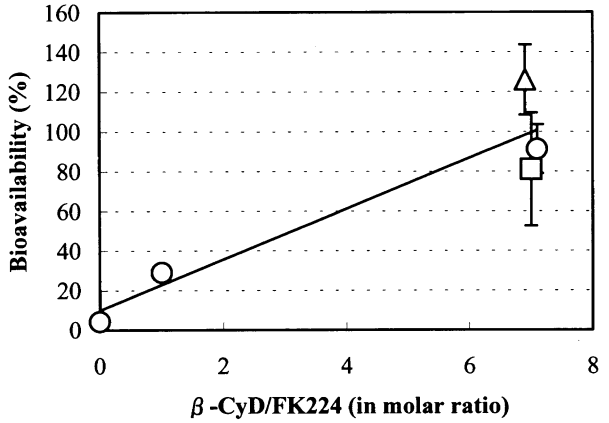

Fig. 9. Correlation between the pulmonary absorption of FK224 and the molar ratio of β-CyD in formulations applied to rats. (○) 0.5 mg/kg; (△) 1 mg/kg; (□) 5 mg/kg. Mean ± SE of three rats.

a 1:7 formulation showed that most of the FK224 applied to the lung was absorbed, irrespective of the dose, as shown in Fig. 9. In the case of p-MDI, the influence of the particle size distribution of FK224 after discharge from p-MDIs needs to be considered. As shown in Fig. 8, similar particle distribution profiles were obtained for all the p-MDIs. This suggests that the difference in absorption seen with various formulations might not be related to the particle size distribution of FK224, but to the quantity of β-CyD.

Pulmonary absorption is generally rapid and, in the present absorption studies using p-MDI, the T_{max} values were only 0.25 h after administration, except for FK224 alone. However, the plasma level profiles are very interesting because they show a sustained absorption in the presence of β-CyD. It will be worthwhile discussing one possible mechanism for such a phenomenon, because there seems to be no current explanation for the enhancing effect of β-CyD on pulmonary absorption.

The first point that needs to be considered is the state of FK224 and β-CyD on the alveolae after administration to rats, because it has been found that the solubility of FK224 has a great effect on absorption. Kobayashi et al. [24] have estimated the volume of the fluid phase in rat lung as 3–12 μl. If the volume is 10 μl for each rat, 283 μg β-CyD could be dissolved based on its solubility (28.3 mg/ml at 37 °C) in the fluid, with 40 μg FK224 being dissolved from the solubility profile. On the other hand, the quantity of FK224 applied to rats in this absorption study was 5 mg/kg, equivalent to 1.25 mg/rat (1.36 mg/rat as β-CyD), if the body weight is 250 g, and only 3–4% of the dose was dissolved even in the presence of β-CyD. In the absence of β-CyD, less than 1 μg (less than 0.1% of the dose) FK224 could be dissolved in the fluid phase. Therefore, it appears that both FK224 and β-CyD exist as two phases, solid and partially dissolved, following administration of a dose of 5 mg/kg to the lungs of rats.

Secondly, the formation of a complex between FK224 and β-CyD was determined using the mixture which was

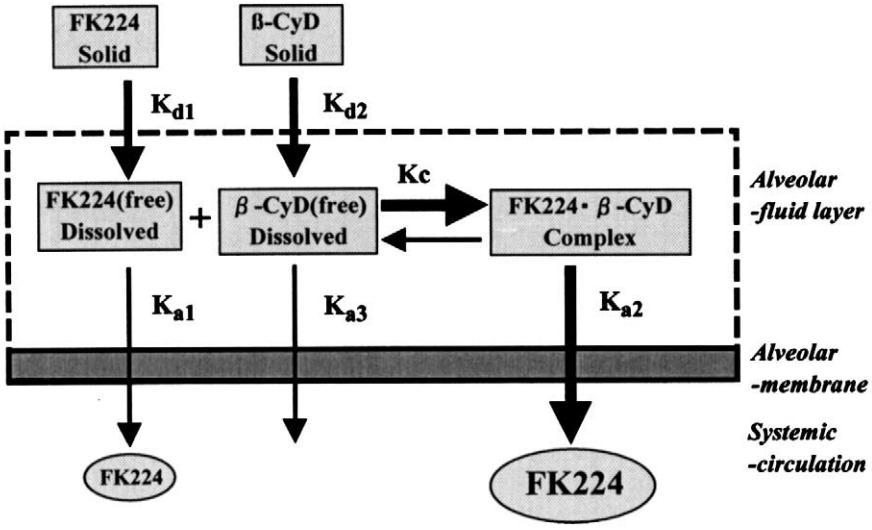

Fig. 10. Overall process of FK224 absorption from the mixture containing β-CyD in the lung.

used for the p-MDI preparation following analysis by powder X-ray, IR and DSC. However, the X-ray, IR spectra and DSC thermograms of the mixture had the same properties as the physical mixture of FK224 and β-CyD. Thus, the solid mixture did not form a complex.

Based on the results obtained and the above considerations, the assumed mechanism is described in Fig. 10, where K_{d1} is the FK224 dissolution rate constant, K_{d2} is the β-CyD dissolution rate constant, K_c is the stability constant of the complex of FK224 with β-CyD, K_{a1} is the absorption rate constant for FK224 (free), K_{a2} is the absorption rate constant for the FK224 complex with β-CyD and K_{a3} is the absorption rate constant for β-CyD.

When FK224 and β-CyD are applied to the lung in rats by p-MDI, they exist in a solid state, independently of the alveolar epithelial membrane, as discussed above. Initially, β-CyD would be partially dissolved in the fluid layer because of the higher solubility of FK224, and then FK224 would dissolve depending on the concentration of β-CyD as a consequence of complex formation according to the stability constant (K_c; 8000 M^{-1}). Therefore, most of the dissolved FK224 exists as the complexed form with β-CyD in the fluid layer. In the GI tract, it is well known that only the free form of a drug, which is in equilibrium with the complexed form of the drug in solution, is capable of entering the systemic circulation, and CyDs cannot cross the GI membrane [9]. Masson et al. [25] have demonstrated the mechanism of CyD effect on drug permeability from aqueous solutions through biological membranes (hairless mouse skin and semi-permeable cellophane) using the following Eq. (3) based on the diffusion model.

$$J = \frac{(P_m/K_d)[D/CD]}{M_{1/2} + [CD]} \quad (3)$$

where J is a drug flux across the membrane, P_m/K_d is a constant which is the membrane permeability divided by the stability constant of the drug/CyD complex in the donor phase, $M_{1/2}$ is a constant which is equal to the CyD concentration in the donor phase when the flux is half the maximum flux, $[CD]$ is the CyD concentration in the donor phase, and $[D/CD]$ is the concentration of the complex in the donor phase. In the pulmonary absorption of FK224, it is assumed that the dissolved quantity of β-CyD is constant in the fluid layer and following the dissolved quantity of FK224 (free and complexed form) there would be no difference between 1:1 and 1:7 formulations. If the same thing as the above happens in the lung, the C_{max} should be the same in both formulations. However, the C_{max} and AUC increased in parallel with the increase in β-CyD. Therefore, the absorption process should differ from that in the GI tract.

Marques et al. [26] have reported that β-CyD is well absorbed from the lung (BA: 66%, following i.t. administration to rabbit lung). Matsukawa et al. [27] have reported that proteins, ranging in molecular weight from 12,300 to 150,000 Da, are capable of transport to cultured rat alveolar epithelial cells. Now, the molecular weights of FK224 (1041) and β-CyD (1135) are much lower than the above proteins so that if the transportability and the increase in absorption depending on the ratio of β-CyD are considered, this suggests that FK224 is absorbed from the lung not only as the free form but also the complexed form. By absorption of the complex, free FK224 and free β-CyD would be required to maintain the equilibrium between the free and complex forms according to the absorption constant (K_{a2}). Then, there needs to be a way of compensating for the lack of free FK224 and free β-CyD based on each dissolution rate. In the dissolution studies using the mixture with β-CyD, as shown in Fig. 7, the apparent dissolution rates (K_{d1}) increased in parallel with the increase in the ratios of β-CyD because of the increase in the dissolution rate of β-CyD corresponding to the entire surface of the excess β-CyD. Consequently, a dependence on the C_{max} and AUC corresponding to the quantity of β-CyD would be observed,

namely in the case of the 1:1 formulation; although the absorption of FK224 improved, there was a limit due to exhaustion of the β-CyD reserve. On the other hand, in the 1:7 formulation, at which ratio β-CyD can dissolve all the FK224 to form the complex, most of the FK224 was found to be absorbed in rats. As for the sustained absorption, it was considered that the phenomenon occurred because the excess drug stayed on the alveolae and was absorbed according to the above consideration. This assumption leads us to conclude that it should be possible to control pulmonary absorption of drugs by using the derivatives of β-CyD, with different degrees of solubility as far as their complexes are absorbed.

5. Conclusion

This study has shown that by using FK224, a novel cyclopeptide, pulmonary administration is a potentially good route for drugs that exhibit poor permeability from the GI tract, and β-CyD is an extremely effective additive for improving the absorption of FK224 because of the increased drug solubility. In the case of FK224 pulmonary absorption using β-CyD, it appears that most of the FK224 is absorbed as the complexed form. Derivatives of β-CyD could have a potential role, not only as absorption enhancers, but also as drug carriers by controlling pulmonary absorption by allowing the appropriate aqueous solubility to be selected.

References

[1] P.L. Smith, Peptide delivery via the pulmonary route: a valid approach for local and systemic delivery, J. Control. Release 46 (1997) 99–106.
[2] K. Okumura, S. Iwakawa, T. Yoshida, T. Seki, F. Komada, Intratracheal delivery of insulin. Absorption from solution and aerosol by rat lung, Int. J. Pharm. 88 (1992) 63–73.
[3] P.L. Smith, J. Marcello, D.C. Chiossone, D. Orner, I.J. Hidalgo, Absorption of an RGD peptide (SK&F 106760) following intratracheal administration in rats, Int. J. Pharm. 106 (1994) 95–101.
[4] J.S. Patton, P. Trinchero, R.M. Platz, Bioavailability of pulmonary delivered peptides and proteins: α-interferon, calcitonins and parathyroid hormones, J. Control. Release 28 (1994) 79–85.
[5] P.L. Nicklin, D. Bayley, J. Giddings, S.J. Craig, L.L. Cummins, J.G. Hastewell, J.A. Phillips, Pulmonary bioavailability of a phosphorothioate oligonucleotide (CGP 64128A): comparison with other delivery routes, Pharm. Res. 15 (1998) 583–591.
[6] T. Higuchi, K.A. Connors, Phase-solubility techniques, Adv. Anal. Chem. Instrum. 4 (1965) 117–212.
[7] R.A. Rajewski, V.J. Stella, Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery, J. Pharm. Sci. 85 (1996) 1142–1169.
[8] T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization, J. Pharm. Sci. 85 (1996) 1017–1025.
[9] F. Hirayama, K. Uekama, Cyclodextrin-based controlled drug release system, Adv. Drug Del. Rev. 36 (1999) 125–141.
[10] T. Irie, K. Uekama, Cyclodextrins in peptide and protein delivery, Adv. Drug Del. Rev. 36 (1999) 101–123.
[11] N. Nambu, M. Shimoda, Y. Takahashi, H. Ueda, T. Nagai, Bioavailability of powdered inclusion compounds of nonsteroidal antiinflammatory drugs with β-cyclodextrin in rabbits and dogs, Chem. Pharm. Bull. 26 (1978) 2952–2956.
[12] M.D. Dhanaraju, K.S. Kumaran, T. Baskaran, M.S.R. Moorthy, Enhancement of bioavailability of griseofulvin by its complexation with β-cyclodextrin, Drug. Dev. Indust. Pharm. 24 (1998) 583–587.
[13] F.W.H.M. Merkus, J.C. Verhoef, S.G. Romeijn, N.G.M. Schipper, Absorption enhancing effect of cyclodextrins on intranasally administered insulin in rats, Pharm. Res. 8 (1991) 588–592.
[14] F.W.H.M. Merkus, J.C. Verhoef, E. Marttin, S.G. Romeijn, P.H.M. van der Kuy, W.A.J.J. Hermens, N.G.M. Schipper, Cyclodextrins in nasal drug delivery, Adv. Drug Del. Rev. 36 (1999) 41–57.
[15] H.M.C. Marques, J. Hadgraft, I.W. Kellaway, G. Taylor, Studies of cyclodextrin inclusion complexes. 3. The pulmonary absorption of β-, DM-β- and HP-β-cyclodextrins in rabbits, Int. J. Pharm. 77 (1991) 297–302.
[16] D.A. Wall, J. Marcello, D. Pierdomenico, A. Farid, Administration as hydroxypropyl-beta-cyclodextrin complexes does not slow rates of pulmonary drug absorption in rat, STP Pharm. Sci. 4 (1994) 63–68.
[17] Z.Z. Shao, Y.P. Li, A.K. Mitra, Cyclodextrins as mucosal absorption promoters of insulin. 3. Pulmonary route of delivery, Eur. J. Pharm. Biopharm. 40 (1994) 283–288.
[18] Z.Z. Shao, A.K. Mitra, Pulmonary absorption of recombinant human growth hormone in rats, Eur. J. Pharm. Biopharm. 42 (1996) 199–203.
[19] D.J. Freeman, R.W. Niven, The influence of sodium glycocholate and other additives on the in vivo transfection of plasmid DNA in the lungs, Pharm. Res. 13 (1996) 202–209.
[20] J.M.C.L. Pinto, H.M.C. Marques, Beclomethasone/cyclodextrin inclusion complex for dry powder inhalation, STP Pharm. Sci. 9 (1999) 253–256.
[21] S. Kobayashi, S. Kondo, K. Juni, Study on pulmonary delivery of salmon calcitonin in rats: effects of protease inhibitors and absorption enhancers, Pharm. Res. 11 (1994) 1239–1243.
[22] A. Yamamoto, S. Okumura, Y. Fukuda, M. Fukui, K. Takahashi, S. Muranishi, Improvement of the pulmonary absorption of ($Asu^{1,7}$)-eel calcitonin by various absorption enhancers and their pulmonary toxicity in rats, J. Pharm. Sci. 86 (1997) 1144–1147.
[23] A. Yamamoto, T. Fujita, S. Muranishi, Pulmonary absorption enhancement of peptides by absorption enhancers and protease inhibitors, J. Control. Release 41 (1996) 57–67.
[24] S. Kobayashi, S. Kondo, K. Juni, Pulmonary delivery of salmon calcitonin dry powders containing absorption enhancers in rats, Pharm. Res. 13 (1996) 80–83.
[25] M. Masson, T. Loftsson, G. Masson, E. Stefansson, Cyclodextrins as permeation enhancers: some theoretical evaluations and in vitro testing, J. Control. Release 59 (1999) 107–118.
[26] H.M.C. Marques, J. Hadgraft, I.W. Kellaway, G. Taylor, Studies of cyclodextrin inclusion complexes. 4. The pulmonary absorption of sulbutamol from a complex with 2-hydroxypropyl-β-cyclodextrin in rabbits, Int. J. Pharm. 77 (1991) 303–307.
[27] Y. Matsukawa, H. Yamahara, F. Yamashita, V.H.L. Lee, E.D. Crandall, K.J. Kim, Rates of protein transport across rat alveolar epithelial cell monolayers, J. Drug Target. 7 (2000) 335–342.