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Research paper

Comparison of the lung absorption of FK224 inhaled from a pressurized metered dose inhaler and a dry powder inhaler by healthy volunteers

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Abstract

FK224 is a cyclopeptide drug with poor oral absorption due to proteolysis in the gastrointestinal tract. The objectives of this study were to investigate the absorption of FK224 from the lung in healthy volunteers, and compare the pharmacokinetic profiles of FK224 after inhalation from a pressurized metered dose inhaler (pMDI) and dry powder inhaler (DPI). The pMDI (Suspension type, 1 mg as FK224/puff) and DPI (4 mg and 10 mg as FK224/capsule, using Spinhaler as the device) were developed by formulating the same micronized particles of FK224 which were premixed with β-cyclodextrin (β-CyD) to improve the solubility of FK224. In the case of pMDI, 1, 4 or 8 mg was inhaled by the corresponding number of puffs with the pMDI. In addition, the in vitro drug delivery characteristics of the inhalers were evaluated using a multistage liquid impinger. In both inhalers, it was observed that FK224 could be absorbed into the systemic circulation from the lungs of the healthy volunteers, and the AUC and C_{max} were proportionally increased depending on the emitted dose after inhalation. However, the pharmacokinetic (PK) parameters for DPI were significantly higher than that of pMDI, in spite of usage of the same fine particles for the formulations in both inhalers. Based on the distribution from the in vitro examination, the fine particle dose, which is defined as the dose region delivered as particles < 3.8 μm, was calculated from the emitted dose inhaled by the healthy volunteers. It was found that the PK parameters for both inhalers were proportionally increased depending on the predicted fine particle dose regardless of the type of inhaler. This suggests that the absorption from the lung is influenced by the fine particle dose. We concluded that DPI is a suitable inhaler for FK224, and the alveolus, which is generally known as the site of action of the fine particles, is a possible absorptive site for FK224.

Keywords: Metered dose inhaler; Dry powder inhaler; Lung deposition; Lung absorption; Peptide; β-Cyclodextrin

1. Introduction

A pressurized metered dose inhaler (pMDI) has been commonly used for the treatment of respiratory diseases such as asthma and other localized lung diseases [1]. The pMDI is portable, convenient and easy to use. However, there have been some reports suggesting a difficulty of co-ordination with the respiratory cycle [2–5]. A dry powder inhaler (DPI) has also been developed to improve the coordination, and it is generally understood that DPI is suitable on environmental grounds because it does not contain chlorofluorocarbons (CFC) which contribute to stratospheric ozone depletion [6].

In the last decade, there has been interest in employing the pulmonary route as an alternative pathway for the systemic application of drugs [7,8]. Peptides and proteins have been especially studied because most of them must presently be administered intravenously due to the break-down caused by proteolysis and hepatic metabolism, and low membrane permeability in the environment of the gastrointestinal tract [9]. Another interesting aspect of pulmonary absorption is the option of investigating the deposition of the drug in the lung as well as at the absorptive site using scintigraphic imaging techniques [10–13].

FK224 (L-Ser-L-Thr-L-Leu-D-Phe-L-allo-Thr-L-Asp-NH^2, M_w 1041), is a novel cyclopeptide which has been investigated for its potential as a substance P agonist and

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neurokinin antagonist. In absorption studies using rats, it had been found that the pulmonary administration route was effective for FK224, although no plasma levels were measured following oral administration. Furthermore, it has been found that β-CyD is a very effective additive as far as the absorption of FK224 is concerned because of the improvement in its solubility [14].

In the present study, pMDI which is a suspension type device with CFC propellants and DPI using a Spinhaler were prepared using a micronized mixture of FK224 with β-CyD, and the systemic absorption was evaluated in healthy volunteers. The difference in absorption between pMDI and DPI was compared to evaluate the superiority of the dosage forms for FK224. Furthermore, based on the difference in the pharmacokinetic parameters obtained in the healthy volunteers, a possible absorptive site in the lung is discussed in this report.

2. Materials and methods

2.1. Preparation of the mixed powder of FK224 and β-CyD

FK224 was provided by Fujisawa Pharmaceutical Co. Ltd. (Osaka Japan). The compound was dissolved in a 50% (v/v) ethanolic solution with β-CyD, (FK224/β-CyD, 1:1 molar ratio), which was then evaporated to obtain the mixed powder of FK224 and β-CyD. The dried mixture was then pulverized using a jet mill into fine particles for the preparation of pMDI and DPI. The particle size distribution of the pulverized FK224 was measured with a laser diffraction size analyzer (LDSA-2400A, Tohnichi, Japan). The pulverized mixture of FK224 and β-CyD was dispersed in the FK224 saturated water solution to dissolve the β-CyD prior to the measurements. The median particle size was 2.2 ± 0.4 μm.

2.2. Preparation of pMDI

The fine particles were suspended in chlorofluorocarbon propellant (CFC11/CFC12/CFC114 = 17.5:65:17.5) containing 1.0% (w/v) of soybean lecithin (ICI Chemicals) as the dispersing agent and for lubrication of the operating valve. The suspension was transferred to an empty aluminum canister (20 ml in capacity, Bespak, UK) under cool conditions using liquid nitrogen. A 100 μl metering valve (Valois, France) was immediately crimped on the canister. The concentration of FK224 was 1 mg/100 μl as one actuation. Type NK1 (Valois) was used as an adapter for inhalation.

2.3. Preparation of DPI

The fine particles were blended with a conventional α-lactose monohydrate (Pharmatose Mesh 200, DMV, Holland) at two different ratios of fine particles/lactose (1:9 and 1:3 as a weight ratio). Each 40 mg aliquot of the blended powder was transferred to a No. 2 size capsule (Shionogi Qualicaps, Japan). The nominal dose in each formulation was 4 mg and 10 mg, respectively. Spinhaler (Fisons, UK) was used as the device for inhalation.

2.4. In vitro distribution studies

The aerodynamic particle size distributions of FK224 in the formulation of pMDI and DPIs were determined by the Multi Stage Liquid Impinger (MSLI, Fisons) method at an air flow rate of 60 l/min. In the case of pMDI, ten continuous actuations were discharged per experiment, and a single capsule was used per experiment for DPIs. An appropriate volume of methanol was placed on each stage of the MSLI. After discharging to the MSLI, the solution on each stage was collected separately, and FK224 was assayed by UV spectrophotometry (Model U-2000, Hitachi, Japan) at 326 nm. The distributed ratio of FK224 at each stage was referenced against the recovery from the MSLI (excluding throat deposition) using Eq. (1):

$$\text{Distributed ratio at each stage (\%)} = \text{Stage } N/(\text{Stage 1} + \text{Stage2} + \text{Stage3} + \text{Stage4} + \text{Filter}) \times 100 \quad (1)$$

The depositions were determined as undischarged ratio% (device retention for pMDI, capsule retention + device retention for DPI), throat deposition and MSLI depositions (Stage 1–Filter), and were calculated against the recovered dose (i.e. total dose = undischarged dose + throat deposition + MSLI depositions). It was confirmed that the recovered dose in each experiment was between 95 and 105% of the nominal dose. All values are expressed as the means of triplicate runs ($n = 3$).

2.5. Pharmacokinetic (PK) studies in healthy volunteers

Twenty-seven healthy male volunteers aged 20–48 years (mean age 30.1 years) with a body weight ranging from 56.5 to 75.0 kg (mean weight 64.0 kg), having normal lung function for age, were recruited. The studies were conducted according to a randomized open design. All subjects were required to practice the correct inhaler technique using Spinhaler or pMDI with placebo in advance of the study. In the case of DPI, the inspiratory flow rate was controlled at about 60 l/min by training before inhalation.

Three subjects inhaled 1 mg FK224 by one actuation of pMDI, and six subjects inhaled 4 or 8 mg FK224 by four or eight actuations of pMDI, respectively. Each of the six subjects received each dose of 4 and 10 mg in the case of DPI as shown in Table 1. Blood samples were collected from an arm vein at designated time periods (15, 30 min, 1, 2, 4, 6, 8, 12 and 24 h) after inhalation. Plasma levels of FK224 were determined by EIA. The area under

Table 1
Summary of the pharmacokinetics parameters (mean ± SE; $n = 3$ or 6) of FK224 after inhalation in normal healthy volunteers

Inhaler	Nominal dose (mg/body)	No. of volunteers	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-24\ h}$ (ng h/ml)
pMDI	1	3	2.7 ± 1.3	0.07 ± 0.02	0.13 ± 0.05
	4	6	3.0 ± 0.8	0.36 ± 0.07	3.16 ± 0.80
	8	6	2.7 ± 0.6	0.55 ± 0.09	5.88 ± 1.57
DPI	4	6	2.2 ± 1.2	1.36 ± 0.17	14.44 ± 2.69
	10	6	0.7 ± 0.1	3.66 ± 0.56	30.51 ± 2.86

the concentration/time curve (AUC) from 0 to 24 h was calculated using the trapezoidal method.

In order to determine the deposition of FK224, mouth rinsing with gargling was required immediately after inhalation. The residue in the capsules and material adhering to the devices were also determined by HPLC. The delivered dose to the trachea/lung was calculated using Eq. (2):

$$\text{Delivered dose (mg)} = \text{Nominal dose} - \text{Retained quantity (Adapter for pMDI, Capsule and Device for DPI)} - \text{Deposited quantity in mouth} \quad (2)$$

2.6. Statistical tests

The in vitro distribution data and the pharmacokinetics parameters were evaluated for statistically significance differences by Student's unpaired *t*-test. A P value of less than 0.05 was considered significant.

3. Results

3.1. In vitro deposition of FK224

Fig. 1 shows the distribution of FK224 after discharging from pMDI or DPI at an air flow rate of 60 l/min with MSLI. In the case of pMDI, 19.6% of the formulated FK224 was retained in the adapter, 29.2% was trapped in the throat and 51.2% reached the stages of MSLI. For the 4 and 10 mg DPI formulations using Spinhaler, the amounts retained in the capsule were 17.3 and 15.5%, and the amounts adhering to the device were 12.5 and 17.1%, respectively. The amounts trapped in the throat of MSLI were 10.3 and 11.4%, respectively. Consequently, 59.9 and 56.0% reached the stages of MSLI, respectively.

The emitted doses obtained for pMDI and DPIs at two formulations were 80.4, 70.2 and 67.4%, respectively. It was found that pMDI was significantly greater in the emitted dose than those of DPIs. However, in the pMDI, the amount trapped in the throat was 29.2%, and it was much greater than that of DPI formulations. Consequently, it was found that about 50–60% of FK224 was distributed in the stages of MSLI for pMDI and DPI formulations.

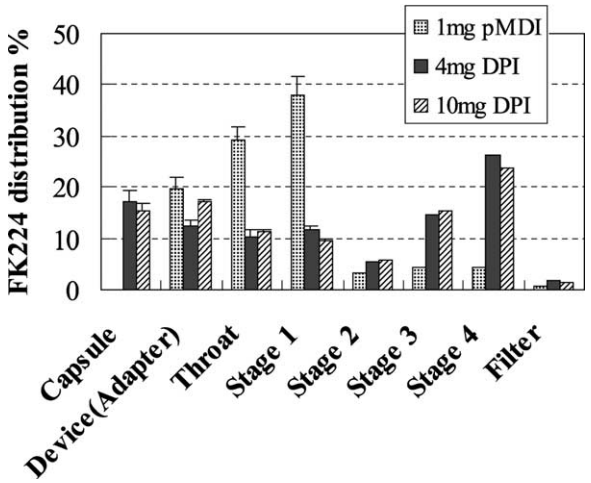

Fig. 1. Comparison of distributed FK224 ratio discharged from 1 mg pMDI, 4 mg DPI or 10 mg DPI using MSLI at 60 l/min in flow rate. Mean ± SD ($n = 3$).

3.2. In vitro particle size distribution of FK224

Fig. 2 shows the aerodynamic particle size distribution of FK224 calculated from the quantity distributed in each stage of MSLI using Eq. (1). In the case of DPI, similar aerodynamic particle size characteristics were observed between 4 mg DPI and 10 mg DPI. In addition, 80.4 and 83.0% were distributed at a particle size of < 10.7 μm, and 47.2 and 45.2% were distributed at a particle size of < 3.8 μm for 4 mg DPI and 10 mg DPI, respectively. On the other hand, pMDI showed that a greater amount of particles was distributed to the upper stages in MSLI, compared with DPIs, 25.7 and 10.5% for 10.7 and 3.8 μm, respectively.

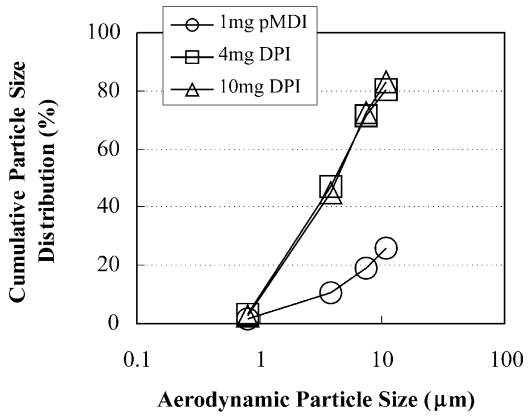

Fig. 2. Particle size distribution of FK224 discharged from pMDI and DPIs by MSLI at air flow rate of 60 l/min.

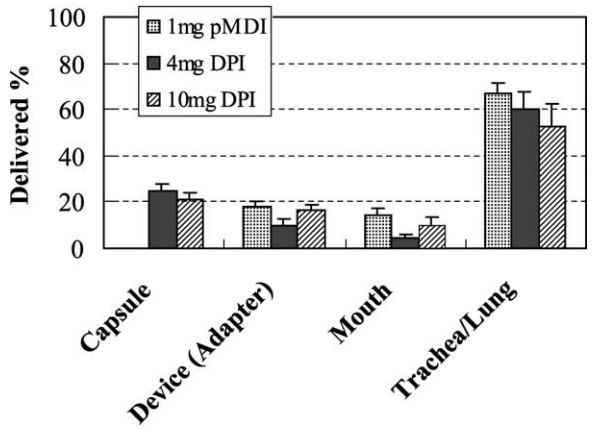

Fig. 3. Comparison of distributed FK224 ratio inhaled from 1 mg pMDI, 4 mg DPI or 10 mg DPI in healthy volunteers. Mean ± SD ($n = 6$).

3.3. In vivo deposition of FK224 in healthy volunteers

Fig. 3 shows the distribution of FK224 after inhalation using pMDI or DPI in the healthy volunteers. In the case of pMDI, 18.3% of the formulated FK224 was retained in the adapter, and 14.5% of the nominal dose was collected from the mouth. Consequently, 67.2% was calculated as the amount inhaled into the trachea/lung. In the 4 and 10 mg DPI formulations using Spinhaler, the amounts retained in the capsule were 25.1 and 20.9%, and the amounts adhering to the device were 9.8 and 16.7%, respectively. The amounts collected from the mouth were 4.7 and 9.5%, respectively. It was estimated that 60.4 and 52.9% reached the trachea/lung in the case of 4 mg DPI and 10 mg DPI, respectively.

3.4. PK studies in healthy volunteers

The PK parameters are summarized in Table 1. Fig. 4 shows the plots of C_{max} against the delivered dose to the trachea/lung after inhalation using pMDI and DPIs. The C_{max} of DPI is significantly higher than that of pMDI (4 mg pMDI vs. 4 mg DPI). In both inhalers the C_{max} increased in relation to the delivered dose to the trachea/lung.

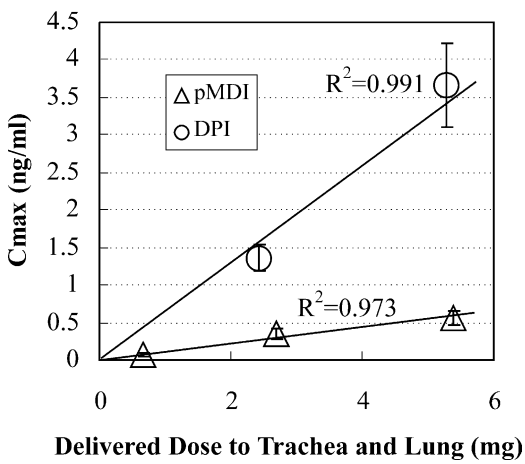

Fig. 4. Relationship between FK224 delivered from pMDI and DPI to trachea/lung and C_{max} after inhalation of FK224 to healthy volunteers ($n = 6$). Mean ± SE.

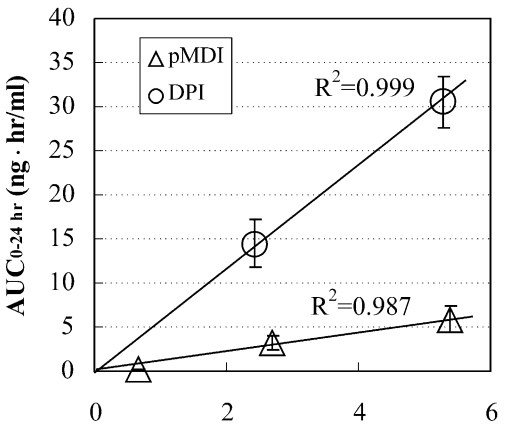

Fig. 5. Relationship between FK224 delivered from pMDI and DPI to trachea/lung and AUC after inhalation of FK224 to healthy volunteers ($n = 6$). Mean ± SE.

Fig. 5 shows the plots of AUC against the delivered dose to the trachea/lung after inhalation using pMDI and DPIs. The AUC of DPI is also significantly higher than that of pMDI (4 mg pMDI vs. 4 mg DPI). In both inhalers, the AUC increased in parallel with the dose delivered to the trachea/lung.

4. Discussion

In the PK studies with normal healthy volunteers, it was observed that FK224 as a cyclopeptide could be absorbed into the systemic circulation via the lung using either pMDI or DPI. Furthermore, the C_{max} and AUC were found to increase proportionally depending on the increase in the dose delivered to the trachea/lung in both inhalers as shown in Figs. 4 and 5. On the other hand, when the absorption of FK224 was compared between the inhalers, it was found that DPI was significantly higher than that of pMDI in spite of the fact that the same FK224 fine particles were formulated in both inhalers. Although it is clear that DPI is an appropriate inhaler for FK224 via systemic absorption, what we have to consider is the cause of the difference in absorption. As for the apparent difference between the inhalers, the FK224 fine particles were dispersed into the propellants or blended with conventional lactose for pMDI or DPI, respectively. However, the propellants are volatilized on their way to the lung, and only a small amount of lactose is generally deposited in the lungs as Karhu et al. [15] have reported. Therefore, it was considered that the FK224 fine particles could only be distributed to the lung by both inhalers. With regard to

the absorptive site, Pitcairn et al. [13] have reported that there was an increase in the C_{max} and AUC corresponding to the increased delivered dose of LAS 31025 in the body as the sum of lung and oropharyngeal deposition. However, in the case of the LAS 31025, the absorption occurred via both the respiratory and gastrointestinal tract. Thus, it was not possible to discuss the absorption from the lung on its own. In contrast, since the FK224 is not absorbed from the gastrointestinal tract, the systemic absorption is due solely to its lung absorption.

From the above considerations, it is reasonable to suppose that the difference in absorption between pMDI and DPI may be associated with the difference in the deposited site of FK224 in the lungs. Furthermore, the correlation of the PK parameters with the delivered dose to the trachea/lung suggests that the absorption of FK224 via the lung proportionally depends on the quantity of drug delivered to the absorptive site in the lungs. Colthorpe et al. [16] have investigated the pulmonary deposition and absorption of human growth hormone (hGH), administered by aerosol and instillate by gamma scintigraphic imaging using ^{99m}Tc-DTPA in five male New Zealand White rabbits. Their studies indicated that the peripheral/central deposition ratio for aerosol was greater than for the instillate from the gamma scintigraphy, and the bioavailability of aerosolized hGH was also greater than that of instilled hGH.

In order to investigate drug deposition within the lung, there have been many attempts using gamma scintigraphy [10,17]. It is generally understood that the scintigraphic method is useful for investigating the deposition visually in the lung. On the other hand, it is important to demonstrate the similarity of aerodynamic properties between radiolabeled and original drug particles before the radiolabeled drug is administered to subjects. Furthermore, it must be noted that there is a report by Bondesson et al. [18], demonstrating a potential risk of producing a radiolabeled dry powder aerosol with aerodynamic properties different from those of the unlabeled one. As a different approach, it may be useful to consider the drug deposition based on the in vitro particle size distribution and PK parameters of FK224 examined in this study.

As shown in Fig. 2, in the case of DPI, it was found that over 80% or about 45% of FK224 reaching the stages of MSLI was distributed as particles < 10.7 μm or 3.8 μm (aerodynamic particle size), respectively. On the other hand, about 25% or 10% of pMDI was distributed as < 10.7 μm or 3.8 μm, respectively. There is a significant difference between the aerodynamic particle size distributions.

We will now develop the hypothesis a little further as the in vitro aerodynamic particle size distribution should reflect to the deposition in the lungs. The delivered fine particle dose (FPD), which is defined as a quantity of drug distributed as particles < 3.8 μm in the lungs, is then calculated based on the in vitro particle size distribution as shown in Table 2. The PK parameters were plotted against the calculated FPD and, for both C_{max} and AUC, the points were located on the same proportional line as shown in Figs. 6 and 7. These results lead us to the conclusion that the dose related to the absorption is the delivered dose < 3.8 μm, and the alveolus, which is generally regarded as the site of delivery of particles of around 3 μm, is the most likely absorptive site in the lungs for FK224.

It was found that DPI was an appropriate inhaler for FK224 because of the better systemic absorption. However, even in this type of DPI, there have been many reports of the influence of the physical properties of powders and inhaler devices [19–21]. When an improvement in absorption is needed, an in vitro investigation is useful for predicting absorption.

Lastly, it may be helpful to consider the reason why the aerodynamic particle size distribution of pMDI is greater than that of DPI. In pMDI, the FK224 fine particles are presented as a solid in a liquid suspension. When the drug is inhaled, a small mist of the liquid suspension containing

Table 2
In vitro and in vivo FK224 particle deposition characteristics

	In vitro (MSLI)			In vivo (healthy volunteers)		
	1 mg ($n = 3$)	4 mg DPI ($n = 3$)	10 mg ($n = 3$)	1 mg ($n = 3$)	4 mgDPI ($n = 6$)	10 mg ($n = 6$)
Adapter (%)	19.6 ± 2.3	–	–	18.3 ± 2.0	–	–
Capsule (%)	–	17.3 ± 2.1	15.5 ± 1.4	–	25.1 ± 2.7	20.9 ± 3.5
Device (%)	–	12.5 ± 0.9	17.1 ± 0.6	–	9.8 ± 2.8	16.7 ± 2.3
Throat (%)	29.2 ± 2.6	10.3 ± 1.3	11.4 ± 0.5	–	–	–
Mouth (%)	–	–	–	14.5 ± 2.5	4.7 ± 1.5	9.5 ± 4.1
Stages in MSLI (%)	51.2 ± 4.9	59.9 ± 3.9	56.0 ± 2.4	–	–	–
Trachea/Lung (%)	–	–	–	67.2 ± 4.5	60.4 ± 7.0	52.9 ± 9.8
FPF (%)[a]	10.5 ± 1.6	47.2 ± 3.1	45.2 ± 3.5	–	–	–
Predicted FPF (%)[b]				7.1 ± 0.5	28.5 ± 3.3	23.9 ± 4.4
Predicted FPD (Mg)[c]				0.07	1.14	2.39

[a] Percentage of delivered quantity as particles < 3.8 μm against deposited quantity in MSLI.
[b] Percentage of delivered quantity as particles < 3.8 μm (% of Trachea/Lung × FPF).
[c] Predicted fine particle dose at < 3.8 μm (Predicted FPF × Nominal dose).

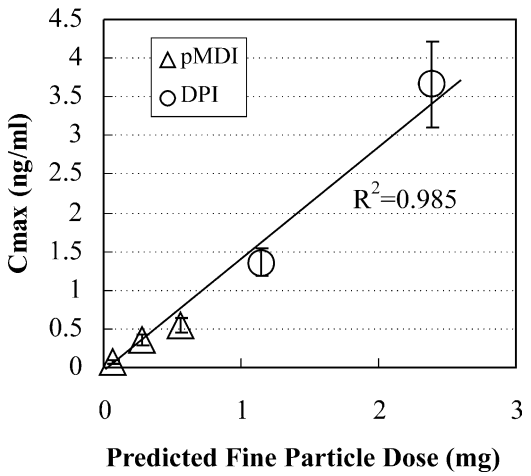

Fig. 6. Relationship between FK224 fine particle dose in the aerodynamic particle size range of less than 3.8 μm and C_{max} after inhalation of FK224 using pMDI and DPI to healthy volunteers ($n = 6$). Mean ± SE.

the FK224 fine particles is discharged. However, the liquid phase is volatilized on the way to the lungs. The aggregates settle as the result of the volatilization of the propellants. On the other hand, FK224 fine particles adhere to the surface of the carrier lactose in DPI and, after inhalation, the FK224 fine particles are dispersed from the surface of the lactose by the airstream exerting a drag force [22].

In conclusion, the pulmonary route of administration was confirmed to be an appropriate way of transporting FK224 into the systemic circulation. It was also found that DPI was a suitable inhaler for FK224 because of the better distribution properties and systemic absorption. The fine particle dose defined as the dose distributed as < 3.8 μm is related to the absorption, and it was assumed that the alveolus, which is generally regarded as the site reached by the fine particles, is a likely site of absorption for FK224. We conclude that the fine particle dose from the in vitro study helps to promote absorption when the DPI formulation is modified.

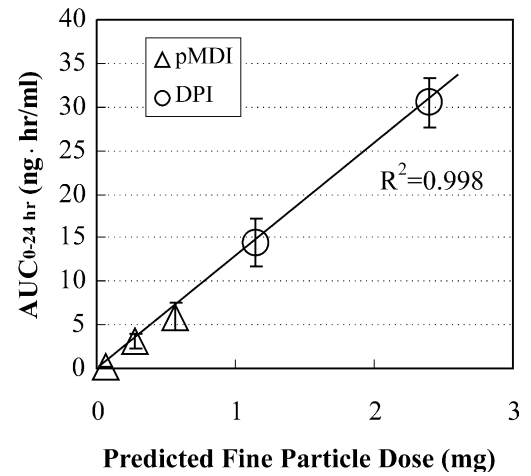

Fig. 7. Relationship between FK224 fine particle dose in the aerodynamic particle size range of less than 3.8 μm and AUC after inhalation of FK224 using pMDI and DPI to healthy volunteers ($n = 6$). Mean ± SE.

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