European Journal of Pharmaceutics and Biopharmaceutics 76 (2010) 215–221

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Enhancement of dissolution rate and oral absorption of a poorly water-soluble drug, K-832, by adsorption onto porous silica using supercritical carbon dioxide

Hiroshi Miura [a,*], Makoto Kanebako [a], Hiroyuki Shirai [a], Hiroshi Nakao [a], Toshio Inagi [a], Katsuhide Terada [b]

[a] Fuji Research Laboratories, Pharmaceutical Division, Kowa Company, Ltd., Shizuoka, Japan
[b] Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan

ARTICLE INFO

Article history:
Received 11 April 2010
Accepted in revised form 25 June 2010
Available online 14 August 2010

Keywords:
Porous silica
Supercritical carbon dioxide
Amorphous
Dissolution
Absorption

ABSTRACT

The aim of this study was to enhance the dissolution rate and oral absorption of a poorly water-soluble drug, 2-benzyl-5-(4-chlorophenyl)-6-[4-(methylthio)phenyl]-2*H*-pyridazin-3-one (K-832) by adsorbing it onto the porous silica Sylysia 350 using supercritical CO_2 ($scCO_2$) as a solvent. K-832-silica formulations were prepared using $scCO_2$ or dichloromethane (DCM) as the solvent (K-832-silica $scCO_2$ and K-832-silica DCM). Scanning electron microscopy, polarizing microscopy, differential scanning calorimetry, and powder X-ray diffraction observations revealed that in both formulations, K-832 existed mainly in an amorphous state. In a dissolution test, 70.2% and 13.3% of K-832 were released from K-832-silica $scCO_2$ and K-832-silica DCM, respectively, within 5 min, whereas only 2.3% of K-832 was released from a physical mixture within 120 min. Results of an *in vivo* absorption test showed that the area under the plasma concentration–time curve and peak concentration of K-832-silica $scCO_2$ were 8.3- and 13.3-fold greater than those of K-832 crystal, whereas the corresponding values of K-832-silica DCM were 5.0- and 8.3-fold greater than those of K-832 crystal. These results suggest that the method of using $scCO_2$ as the solvent is effective in enhancing the dissolution rate and oral absorption of poorly water-soluble drugs because it does not require a toxic solvent and surfactant.

1. Introduction

It has been previously reported that 2-benzyl-5-(4-chlorophenyl)-6-[4-(methylthio)phenyl]-2*H*-pyridazin-3-one (K-832, Fig. 1) exhibits the high inhibitory activity for the production of interleukin-1β, a cytokine and is effective as a preventive and therapeutic drug for immune, inflammatory, and ischemic diseases [1,2]. Therefore, we started the formulation development of K-832. However, K-832 exhibits extremely poor solubility in water and in *in vivo* study, the plasma concentration of K-832 was not sufficient; thus, considerable effort has been devoted to improving its dissolubility and absorbability.

Attempts to increase the dissolution rate of poorly water-soluble drugs have been made in several studies, by approaches such as decreasing the particle size (for example, by milling [3,4]), precipitation of micro- [5–7] or nanoparticles [8–10], and producing micronized particles using supercritical CO_2 ($scCO_2$) [11,12]. $scCO_2$ is an important commercial and industrial solvent because of its high dissolution capability, low toxicity, and low environmental impact. Because CO_2 attains the critical point under mild conditions (7.38 MPa and 31.0 °C [13]), $scCO_2$ seems to be most suitable for preparing pharmaceutical products. However, the fine particles obtained by the aforementioned methods form aggregates because of van der Waals attraction. In another study, a different method was adopted for increasing the dissolution rate; this method involved the adsorption of a drug onto a high-surface-area carrier [14]. By this method, the dissolution rate of a relatively insoluble drug (e.g. Indomethacin or Aspirin) can be improved; this was achieved by equilibration of the drug in an organic solvent (e.g. acetone or dichloromethane) on a high-surface-area carrier. $scCO_2$ can also be used as a solvent in this method. For example, Smirnova et al. and Sanganwar et al. have reported the feasibility of using a system with a high-surface-area carrier and $scCO_2$ (as the solvent) as a drug delivery system (DDS) [15–20]. The aforementioned method is useful because no residual solvent remains in the formulation and the dissolution rate of the drug is enhanced. However, thus far, a detailed comparison between a formulation prepared by the adsorption of the drug from $scCO_2$ onto the high-surface-area carrier and that prepared by the adsorption of the drug from a conventional organic solvent onto the same carrier has not been carried out; the only thing known for certain is that the former formulation has the advantage of no residual solvent. Furthermore, drug absorption by a formulation in which the drug is adsorbed from $scCO_2$ onto the high-surface-area carrier has not yet been reported.

* Corresponding author. Address: Fuji Research Laboratories, Pharmaceutical Division, Kowa Company, Ltd., 332-1 Ohnoshinden, Fuji, Shizuoka 417-8650, Japan. Tel.: +81 545 33 1716; fax: +81 545 33 1805.
E-mail address: h-miura@kowa.co.jp (H. Miura).

0939-6411/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2010.06.016

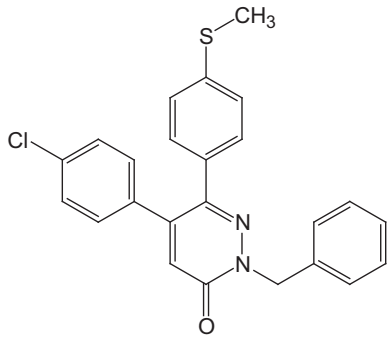

Fig. 1. Chemical structure of K-832.

With this background, in this study, we investigated the K-832-silica formulation prepared by the adsorption of K-832 from $scCO_2$ onto the porous silica Sylysia 350; Sylysia 350 has a large internal surface area with a large number of pores and is a three-dimensional silicate structure; it is used as an inactive ingredient in pharmaceutical products. We also confirmed the applicability of this formulation as a DDS by an *in vivo* evaluation.

2. Materials and methods

2.1. Materials

K-832 used in this study was manufactured by Kowa Company, Ltd. Sylysia 350 (specific surface area: 300 m^2/g; pore size: 21 nm; pore volume: 1.6 mL/g; particle size: 3.9 μm) was supplied by Fuji Silysia Chemical, Ltd. Liquid CO_2 was purchased from Fujisanso Industry Co., Ltd. Dichloromethane (DCM, special grade reagent) was purchased from Wako Pure Chemical Industries, Ltd.

2.2. Preparation of K-832-silica $scCO_2$ by $scCO_2$ method

The experimental apparatus used in the study is shown in Fig. 2. It mainly consists of a CO_2 tank, CO_2 pump, pressure-resistant vessel with jacket, a paddle stirrer, pressure gauges, valves, and a safety valve. The volume of the pressure-resistant vessel is 500 mL. The experimental procedure was as follows. Eight hundred milligrams of K-832 and 4.0 g of Sylysia 350 (K-832:Sylysia 350 = 1:5 (by weight)) were placed in the pressure-resistant vessel.

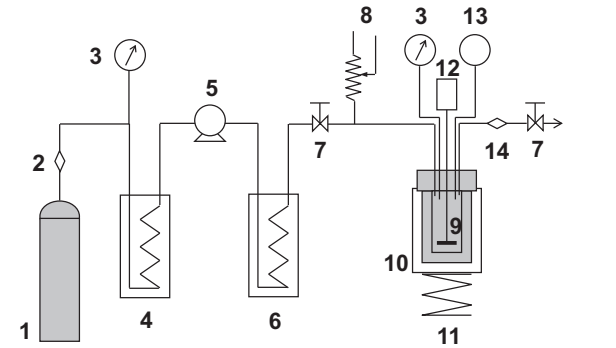

Fig. 2. Schematic diagram of experimental apparatus.

The vessel was closed and heated to 60 °C, and then, 460 g of CO_2 was flowed into the vessel. After it reached a pre-selected pressure (18 MPa), the temperature and pressure were maintained for 5 h under stirring at 500 rpm. Thereafter, CO_2 was gradually discharged from the vessel through the valve, thus yielding K-832-silica $scCO_2$ (Table 1).

2.3. Preparation of K-832-silica DCM by solvent method

Two hundred twenty milligrams of K-832 was dissolved in 150 mL of DCM; then, 1.1 g of Sylysia 350 was dispersed in this solution (K-832:Sylysia 350 = 1:5 (by weight)), and the suspension was sonicated for 5 min. Subsequently, DCM was removed from the suspension. Thereafter, the residue was dried for 6 h at 30–35 °C under vacuum, thus yielding K-832-silica DCM (Table 1).

2.4. Morphology

The morphology of the K-832-silica $scCO_2$ and K-832-silica DCM samples was investigated by scanning electron microscopy (SEM, Hitachi S-3000 N, Japan) and polarizing microscopy (Olympus BX51, Japan).

2.5. Evaluation of crystallinity

The crystallinity of K-832 was evaluated by powder X-ray diffraction (PXRD, Rigaku RINT2000, Japan) with Cu Kα radiation at 40 kV, 20 mA and differential scanning calorimetry (DSC, Shimadzu DSC-60, Japan). In the PXRD measurement, a sample was scanned from 5° to 35° (2θ) at a scanning speed of 5°/min. The DSC measurement was performed at 10 °C/min under N_2 gas flow (20 mL/min).

A test for evaluating the stability of the K-832-silica $scCO_2$ and K-832-silica DCM during long storage was also conducted. The samples stored at room temperature for 9 months were measured by DSC.

2.6. Measurement of specific surface area and pore volume

The specific surface area and pore volume of the samples were measured by the N_2 adsorption method using TriStar 3000 (Micromeritics Instrument Corporation, USA) after degassing the samples at 120 °C for 15–20 h.

2.7. Dissolution test

A dissolution test was carried out by the second dissolution test method (paddle method) at 50 rpm; this method is a general test method specified by Japanese Pharmacopoeia. The amount of tested sample was 5 mg of K-832. The dissolution medium was 900 mL of water containing 0.1% Triton X-100 [21] at 37 ± 0.5 °C. The amount of K-832 dissolved in the test solution was determined by high-performance liquid chromatography (HPLC, Shimadzu LC-10A system) using a reversed-phase column (Inertsil ODS-2, GL Sciences Inc., Japan), and then, the percent dissolution (%) of K-832 was calculated. The flow rate of the mobile phase (acetonitrile/water (7:3, v/v)) was 1.1 mL/min. The eluent was detected by a UV detector at 280 nm.

2.8. Oral absorption study in dogs

HRA beagle dogs (male, body weight 9.4–11.4 kg, Japan Laboratory Animals, Inc.) were housed in the animal facility of Fuji Research Laboratories of Kowa Company, Ltd., with free access to water all day and free access to food until about 17 h before administration. All animals care and procedures were conducted accord-

ing to the Regulation of Animal Experiments in Kowa Company, Ltd. The study was approved by the Ethics Committee on Animal Research of Kowa Company, Ltd. The K-832-silica formulation suspended in 30 mL of water was administered orally along with 20 mL of water to male beagle dogs via a Nelaton catheter (dose: 10 mg/kg of K-832). Blood (1.0 mL) was taken from the antebrachial vein using heparinized syringes; this blood was taken 0.5, 1, 1.5, 2, 3, 5, 8, and 24 h after the administration and then centrifuged immediately. The plasma obtained was stored at −20 °C until an assay. Then, 400 μL of the plasma samples was mixed with 1 mL of an internal standard solution, 750 μL of 1 mol/L glycine buffer solution (pH 10), and 5 mL of methyl *tert*-butyl ether. The supernatant fluid after centrifugation was dried at 40 °C under N_2 gas flow. The residue was dissolved in 250 μL of the mobile phase (methanol/0.05 mol/L formic acid buffer solution (pH3)/acetonitrile (12:5:2, v/v)), and 80 μL of the resultant solution was subjected to HPLC analysis of K-832 under conditions described later.

Concentrations of K-832 in the plasma samples were determined by HPLC, and then, the area under the plasma concentration–time curve (AUC) and peak concentration (C_{max}) of K-832 were calculated. The HPLC analysis was carried out on the LC-10A system using an Inertsil ODS-P column (GL Sciences Inc., Japan). The flow rate of the mobile phase was 0.8 mL/min. The eluent was detected by the UV detector at 280 nm.

3. Results

3.1. Morphology

The SEM and polarizing microscopy images of K-832, Sylysia 350, a physical mixture, K-832-silica $scCO_2$, and K-832-silica DCM are shown in Figs. 3 and 4. The average diameters of K-832 crystal and Sylysia 350 particles are ca. 30 μm and 3.9 μm, respectively. Therefore, larger particles of K-832 crystal when compared to those of Sylysia 350 were observed in the physical mixture (Figs. 3c, 4c). The original particles of K-832 were not observed in K-832-silica $scCO_2$ (Fig. 3d) and K-832-silica DCM (Fig. 3e). In addition, the shapes of K-832-silica $scCO_2$ (Fig. 3d) and K-832-silica DCM (Fig. 3e) were similar to that of Sylysia 350 (Fig. 3b). However, polarizing microscopy revealed that K-832-silica $scCO_2$ (Fig. 4d) and K-832-silica DCM (Fig. 4e) contained a small amount of residual K-832 particles.

3.2. Evaluation of crystallinity

DSC curves of K-832 crystal, Sylysia 350, the physical mixture, K-832-silica $scCO_2$, and K-832-silica DCM are shown in Fig. 5. An endothermic peak in the case of the K-832 crystal was observed at 158 °C (Fig. 5a, −102.14 J/g, K-832 melting point (155–159 °C)). This endothermic peak was not observed for K-832-silica DCM (Fig. 5e), but a small peak was observed for K-832-silica $scCO_2$ (Fig. 5d, −1.32 J/g, 11.1% of K-832 crystal). PXRD patterns of K-832 crystal, Sylysia 350, the physical mixture, K-832-silica $scCO_2$, and K-832-silica DCM are shown in Fig. 6. Pure K-832 crystal showed many distinctive diffraction peaks, which indicates high crystallinity (Fig. 6a). The distinctive peaks of the physical mixture were retained, with a marked decrease in the intensity from that of pure K-832 crystal, whereas K-832-silica $scCO_2$ and K-832-silica DCM did not retain any diffraction peaks and showed a halo pattern.

In the stability test for K-832-silica $scCO_2$ and K-832-silica DCM, the endothermic peaks of the samples stored at room temperature for 9 months did not increase; thus, both products were physically stable for at least 9 months.

3.3. Specific surface area and pore volume

The specific surface areas and pore volumes of K-832 crystal, Sylysia 350, K-832-silica $scCO_2$, and K-832-silica DCM are shown in Table 2. The specific surface areas of K-832-silica $scCO_2$ and K-832-silica DCM were higher than that of pure K-832 (0.85 m^2/g). A comparison of K-832-silica $scCO_2$ with K-832-silica DCM showed

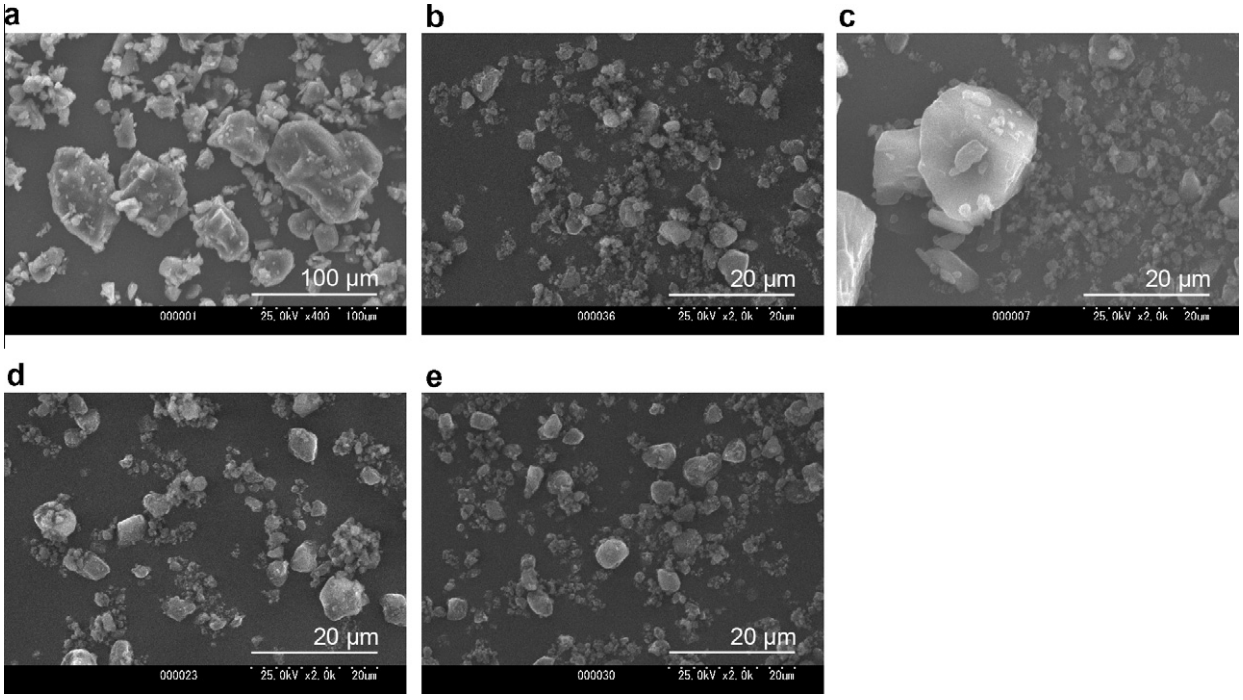

Fig. 3. SEM images of K-832, silica, physical mixture (PM), K-832-silica $scCO_2$, and K-832-silica DCM. (a) K-832 intact; (b) Sylysia 350; (c) K-832-silica PM; (d) K-832-silica $scCO_2$; (e) K-832-silica DCM.

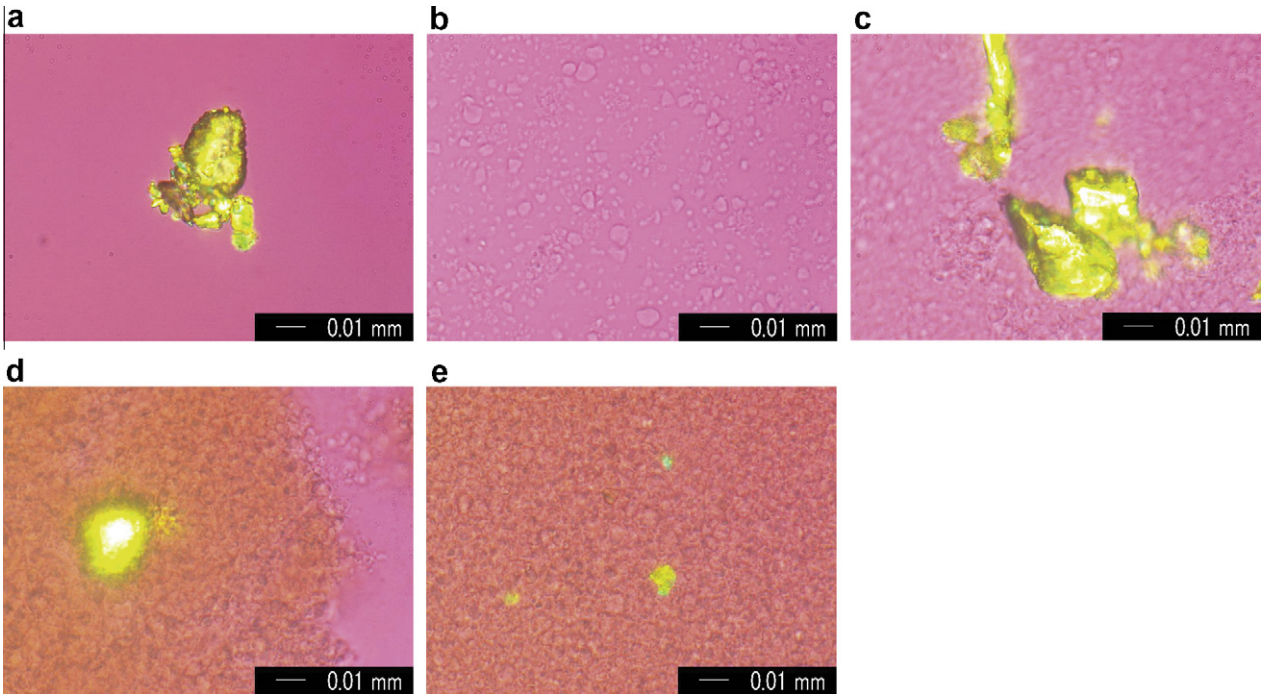

Fig. 4. Polarizing microscopy images of K-832, silica, PM, K-832-silica $scCO_2$, and K-832-silica DCM. (a) K-832 intact; (b) Sylysia 350; (c) K-832-silica PM; (d) K-832-silica $scCO_2$; (e) K-832-silica DCM.

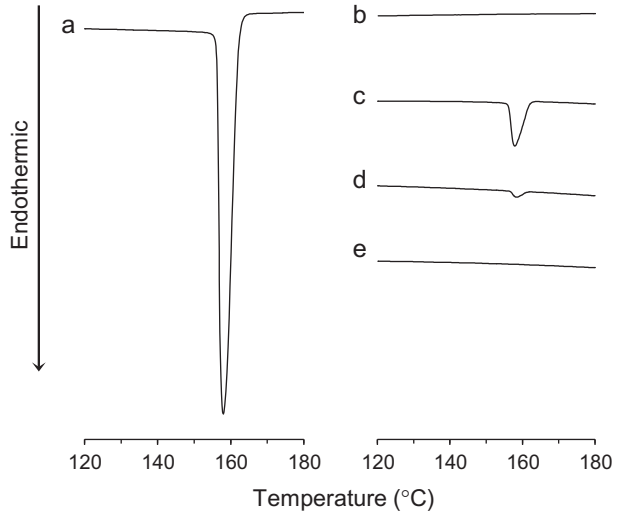

Fig. 5. DSC curves of K-832, silica, PM, K-832-silica $scCO_2$, and K-832-silica DCM. (a) K-832 intact; (b) Sylysia 350; (c) K-832-silica PM; (d) K-832-silica $scCO_2$; (e) K-832-silica DCM.

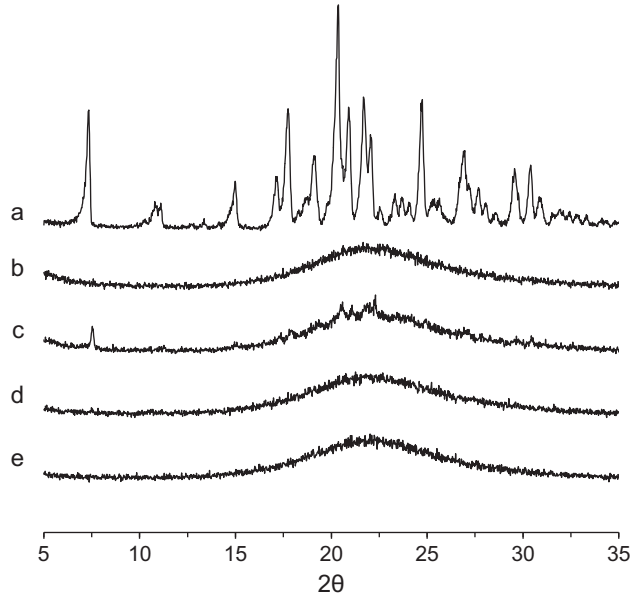

Fig. 6. Powder X-ray diffraction patterns of K-832, silica, PM, K-832-silica $scCO_2$, and K-832-silica DCM. (a) K-832 intact; (b) Sylysia 350; (c) K-832-silica PM; (d) K-832-silica $scCO_2$; (e) K-832-silica DCM.

that the specific surface area and pore volume of the latter were larger than those of the former.

3.4. Drug dissolution profiles

The dissolution profiles of K-832 are shown in Fig. 7. It can be seen that within 5 min, 70.2% and 13.3% of K-832 were released from K-832-silica $scCO_2$ and K-832-silica DCM, respectively, whereas only 2.3% of K-832 was released from the physical mixture within 120 min. In particular, the dissolution of K-832-silica $scCO_2$ occurred much faster than that of K-832-silica DCM.

3.5. Oral absorption study in dogs

Fig. 8 shows the plasma concentrations of K-832 in beagle dogs after oral administration and Table 3 lists the pharmacokinetic parameters. Plasma concentrations of K-832 after the dosing of K-832-silica $scCO_2$ and K-832-silica DCM were markedly greater than those after the dosing of the corresponding crystalline drug. The AUC values of K-832-silica $scCO_2$, K-832-silica DCM, and crystalline drug were 2.40 ± 0.58, 1.44 ± 0.25, and 0.29 ± 0.07 μg h/mL, respectively. The C_{max} values of K-832-silica $scCO_2$, K-832-silica

Table 1
Composition and formulation procedure for K-832-silica formulations and corresponding physical mixture.

Formulation	Component and ratio		Formulation procedure
	K-832	Sylysia 350	
K-832-silica $scCO_2$	1.0	5.0	Adsorbing K-832 onto Sylysia 350 from $scCO_2$ solution
K-832-silica DCM	1.0	5.0	Adsorbing K-832 onto Sylysia 350 from DCM solution
K-832-silica PM	1.0	5.0	Physical mixture of K-832 and Sylysia 350

Table 2
Specific surface areas and pore volumes of K-832, silica, K-832-silica $scCO_2$, and K-832-silica DCM.

Sample	Specific surface area (m^2/g)	Pore volume (mL/g)
K-832 intact	0.85	0.000
Sylysia 350	291.16	1.796
K-832-silica $scCO_2$	218.45	1.266
K-832-silica DCM	227.80	1.345

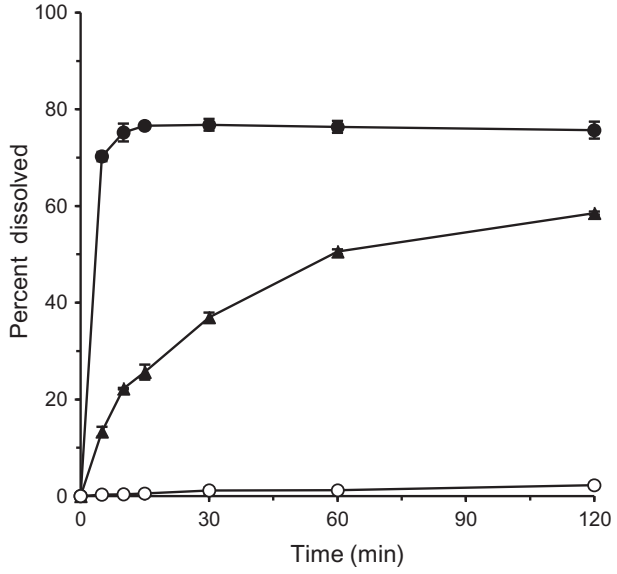

Fig. 7. Dissolution profiles of K-832 in 900 mL of 0.1% Triton X-100 solution at 37 °C. ●, K-832-silica $scCO_2$; ▲, K-832-silica DCM; ○, K-832-silica PM. mean ± SD, n = 3.

DCM, and crystalline drug after oral administration were 0.40 ± 0.04, 0.25 ± 0.03, and 0.03 ± 0.01 μg/mL, respectively. That is, the AUC and C_{max} values of K-832-silica $scCO_2$ were 8.3- and 13.3-fold greater than those of K-832 crystal (i.e. crystalline drug), whereas the AUC and C_{max} values of K-832-silica DCM were 5.0- and 8.3-fold greater than those of K-832 crystal.

4. Discussion

The aim of this study was to increase (i.e. enhance) the dissolution rate and oral absorption of K-832 and compare the performances of K-832-silica $scCO_2$ and K-832-silica DCM as a DDS for the poorly water-soluble drug K-832.

K-832 used in this study has extremely low solubility (ca. 10 ng/mL in water at 25 °C). Therefore, the technique of rapid expansion

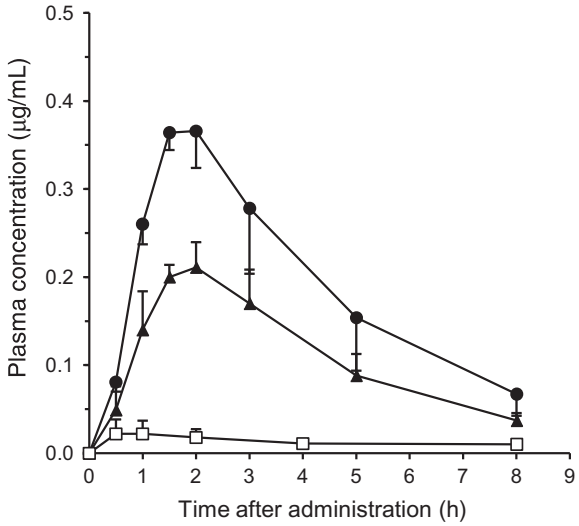

Fig. 8. Plasma concentrations of K-832 in beagle dogs after oral administration. ●, K-832-silica $scCO_2$; ▲, K-832-silica DCM; □, K-832 crystal. Dose, 10 mg/kg, mean ± SE, n = 5.

Table 3
Pharmacokinetic parameters of K-832 after single oral administration to dogs for a dose of 10 mg/kg of K-832-silica $scCO_2$, K-832-silica DCM, and K-832 crystal.

Sample	AUC		T_{max} (h)	C_{max}	
	(μg h/mL)	Ratio		(μg/mL)	Ratio
K-832 crystal	0.29 ± 0.07	as 1	4.3 ± 3.7	0.03 ± 0.01	as 1
K-832-silica $scCO_2$	2.40 ± 0.58	8.3	1.8 ± 0.3	0.40 ± 0.04	13.3
K-832-silica DCM	1.44 ± 0.25	5.0	1.8 ± 0.3	0.25 ± 0.03	8.3

Each value represents the mean ± SE (n = 5).

of supercritical solutions (RESS) [13,22], which is one of the techniques for particle formation using supercritical fluid, was applied for the preparation of K-832 fine particles with a large surface area and excellent solubility. However, this technique encounters the following problems: (1) particle collection from the gaseous stream is difficult because the compressed CO_2 solution is sprayed through a small orifice to the tank; (2) K-832 particles obtained by this technique are microparticles and not nanoparticles, by which solubility enhancement cannot be expected; and (3) only a small number of microparticles are obtained.

Sylysia 350, the porous silica used in this study, has a hydrophilic surface and large surface area. Therefore, we expected that the method of adsorbing K-832 onto silica having a large surface area and good wettability would increase the surface area of K-832 and enhance its dissolution. In addition, because the average particle diameter of Sylysia 350 is 3.9 μm, we expected good handling characteristics of particles and ease of collection of silica particles that adsorbed the drug.

An organic solvent such as DCM is used in the conventional method for producing a drug-adsorbed high-surface-area carrier; this solvent dissolves a poorly water-soluble drug and adsorbs it onto a high-surface-area carrier in the production process [14]. In addition, in the aforementioned RESS technique, a co-solvent (entrainer) can also be used to enhance the solubility of the drug in the supercritical phase [22]. However, the use of a general solvent has the drawback that a residual solvent remains in the formulation. Therefore, in this study, we examined the possibility of use of $scCO_2$ as a solvent and found that no residual solvent remained in the obtained formulation.

First, we examined the morphology and crystallinity of the formulation and the N_2 adsorption method, in order to investigate the

state of existence of a drug with a porous silica. As mentioned earlier, Sylysia 350 has a large surface area; in particular, the internal surface area of its particles is much larger than their outer surface area. Therefore, it is thought that most drugs are present on the internal surface of the Sylysia 350 particles and thus the shape of the product is derived from that of the Sylysia 350 particles (i.e. non-spherical, Fig. 3b). The morphology examination revealed that K-832 was mainly present on the surface of the Sylysia 350 particles or inside the particle pores and a part of K-832 existed in the crystal form. The shapes of the products (Fig. 3d and e) were also non-spherical. The DSC measurement revealed that the formulation prepared by adsorption from $scCO_2$ contained a small amount of the crystalline form of K-832 (11.1% of K-832). It was inferred from these data that some of K-832 was deposited as fine particles from $scCO_2$ solution or it did not be dissolved in $scCO_2$. However, it is thought that generally, when a drug adsorbs onto a high-surface-area carrier, the drug is in the amorphous state [23,24]. The DSC and PXRD measurements showed that in K-832-silica DCM, K-832 existed in the amorphous state, whereas in K-832-silica $scCO_2$, it mainly existed in the amorphous state and partially in the crystalline state. Therefore, we expected that the K-832-silica DCM drug dissolved faster than the K-832-silica $scCO_2$ drug because of the existence of only the amorphous state of K-832 in the former.

Results of the dissolution test showed that the dissolution rate of K-832 from the formulations was remarkably better than that of the corresponding physical mixture (Fig. 7). This behaviour can be attributed to an increase in the surface area of K-832 by its adsorption onto the porous silica (Table 2), the existence of K-832 in the amorphous state (confirmed by DSC and PXRD), and wettability of the hydrophilic porous silica. Sylysia 350 particles have a hydrophilic surface because of the silanol groups; therefore, this silica has good wettability for aqueous media. Further, Smirnova et al. reported that one of the reasons for the release enhancement was the immediate collapse of the carrier (silica aerogel) network in aqueous media [17]. However, Sylysia 350 particles, which acted as the carrier in this study, do not collapse in aqueous media, because a primary particle of Sylysia 350 chemically binds with other primary particles. Therefore, we thought that K-832 immediately eluted through the pore network of Sylysia 350 without the collapse of the structure. However, despite the coexistence of the crystalline form of K-832 (11.1%) in K-832-silica $scCO_2$, dissolution from K-832-silica $scCO_2$ was faster than that from K-832-silica DCM (Fig. 7). We speculated that the dispersion state of K-832 in the porous structure of Sylysia 350 in K-832-silica $scCO_2$ might be different from that in K-832-silica DCM because of the high diffusibility of $scCO_2$ solution dissolving K-832. The fact that the specific surface area and pore volume of K-832-silica $scCO_2$ were smaller than those of K-832-silica DCM might indicate that the drug-porous silica system with $scCO_2$ is highly homogeneous.

The absorption studies revealed that the plasma concentrations of K-832 after the dosing of K-832-silica $scCO_2$ and K-832-silica DCM were markedly greater than those after the dosing of the corresponding drug crystals. We thought that the dissolution and absorption characteristics of K-832 were enhanced because of an increase in the surface area of K-832 by its adsorption onto porous silica, the existence of K-832 in the amorphous state, the wettability of the hydrophilic silica, and the high dispersion of the drug. Consequently, a solid dispersion with a silica structure (mesopores) appeared to have been prepared. Therefore, porous silicas such as Sylysia 350 used as a carrier of a poorly water-soluble drug were effective in enhancing the dissolution rate and oral absorption of the drug. In addition, we found that the dispersion states of the drug molecule in K-832-silica $scCO_2$ and K-832-silica DCM were different, and this difference affected the *in vivo* absorption of the drug.

In our study, we adopted the $scCO_2$ method because it requires a lower preparation temperature, which is generally more favourable for ensuring drug stability in the preparation process. For example, Kinoshita et al. reported that when a poorly water-soluble drug was treated with porous calcium silicate (Florite RE) by means of a twin screw extruder, the drug melted and was adsorbed onto Florite RE in an amorphous state. The melt-adsorbed product had good solubility and the absorption of the poorly water-soluble drug was high as well. However, a higher temperature is required for the preparation of the melt-adsorbed product using the twin screw extruder, in consideration of the melting point of the drug during preparation (Kinoshita's temperature condition: 250 °C) [25,26]. Therefore, this method is unsuitable for heat-unstable drugs. In the case of our $scCO_2$ method, the temperature condition of the vessel containing the drug was mild (60 °C), and this temperature is much lower than the melting point of the drug (155–159 °C). Furthermore, K-832-silica $scCO_2$ was also obtained under a milder condition (35 °C) by using $scCO_2$, which could easily change the preparation condition. Therefore, the $scCO_2$ method adopted in this study is suitable for a drug vulnerable to heat. In addition, the long-term stability test revealed that K-832-silica $scCO_2$ was physically stable. Furthermore, $scCO_2$ is a non-toxic and non-flammable alternative to hazardous organic solvents used in conventional preparation methods such as the solvent method. In our study, CO_2 could be easily removed from the products through the valves. Our results suggested that the use of $scCO_2$ might yield high solubility of the drug and its good absorption without any residual solvent, which is not the case with the use of DCM as a solvent. Moreover, the $scCO_2$ method does not require the use of a surfactant, which is otherwise used for preparations of a solid dispersion system.

5. Conclusions

The investigation of a new solid dispersion system containing a porous silica Sylysia 350 as a carrier and $scCO_2$ as a solvent revealed that using such a system, it is possible to achieve high solubility and good absorption *in vivo* of a poorly water-soluble drug, without the use of any organic solvent and surfactant and under mild temperature conditions. Therefore, this method of using $scCO_2$ seems to be one of the superior methods for achieving excellent absorption of drugs in DDSs.

Acknowledgements

The authors thank Mr. Yoshiharu Ishikawa (Tokyo New Drug Research Laboratories, Kowa Company, Ltd.) for his assistance in the oral absorption study. The authors also acknowledge Fuji Silysia Chemical, Ltd. (Aichi, Japan) for providing the porous silica used in the study.

References

[1] M. Ohkuchi, Y. Kyotani, H. Shigyo, T. Koshi, T. Kitamura, T. Ohgiya, T. Matsuda, Y. Yamazaki, N. Kumai, K. Kotaki, H. Yoshizaki, Y. Habata, Pyridazine compounds and compositions containing the same, US patent 6, 348,468, 2002.
[2] Y. Tabunoki, T. Edano, K. Murakami, H. Kobayashi, T. Koshi, M. Ohkuchi, S. Ohshima, T. Mima, T. Ishii, Y. Hattori, Y. Saeki, Anti-arthritic effects of a novel anti-cytokine low molecular weight compound, K-832, Arthritis Rheum. 48 (Suppl. S555) (2003).
[3] N. Kondo, T. Iwao, H. Masuda, K. Yamanouchi, Y. Ishihara, N. Yamada, T. Haga, Y. Ogawa, K. Yokoyama, Improved oral absorption of a poorly water-soluble drug, HO-221, by wet-bead milling producing particles in submicron region, Chem. Pharm. Bull. 41 (1993) 737–740.
[4] V.N. Mochalin, A. Sagar, S. Gour, Y. Gogotsi, Manufacturing nanosized fenofibrate by salt assisted milling, Pharm. Res. 26 (2009) 1365–1370.

[5] N. Rasenack, B.W. Müller, Dissolution rate enhancement by in situ micronization of poorly water-soluble drugs, Pharm. Res. 19 (2002) 1894–1900.
[6] N. Rasenack, H. Hartenhauer, B.W. Müller, Microcrystals for dissolution rate enhancement of poorly water-soluble drugs, Int. J. Pharm. 254 (2003) 137–145.
[7] X.S. Li, J.X. Wang, Z.G. Shen, P.Y. Zhang, J.F. Chen, J. Yun, Preparation of uniform prednisolone microcrystals by a controlled microprecipitation method, Int. J. Pharm. 342 (2007) 26–32.
[8] J.Y. Zhang, Z.G. Shen, J. Zhong, T.T. Hu, J.F. Chen, Z.Q. Ma, J. Yun, Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants, Int. J. Pharm. 323 (2006) 153–160.
[9] T.L. Rogers, I.B. Gillespie, J.E. Hitt, K.L. Fransen, C.A. Crowl, C.J. Tucker, G.B. Kupperblatt, J.N. Becker, D.L. Wilson, C. Todd, C.F. Broomall, J.C. Evans, E.J. Elder, Development and characterization of a scalable controlled precipitation process to enhance the dissolution of poorly water-soluble drugs, Pharm. Res. 21 (2004) 2048–2057.
[10] T. Lee, C.W. Zhang, Dissolution enhancement by bio-inspired mesocrystals: the study of racemic (R,S)-(+/−)-sodium ibuprofen dihydrate, Pharm. Res. 25 (2008) 1563–1571.
[11] E. Reverchon, G. Della Porta, A. Spada, A. Antonacci, Griseofulvin micronization and dissolution rate improvement by supercritical assisted atomization, J. Pharm. Pharmacol. 56 (2004) 1379–1387.
[12] J. Kerč, S. Srčič, Ž. Knez, P. Senčar-Božič, Micronization of drugs using supercritical carbon dioxide, Int. J. Pharm. 182 (1999) 33–39.
[13] J.W. Tom, P.G. Debenedetti, Particle formation with supercritical fluids – a review, J. Aerosol. Sci. 22 (1991) 555–584.
[14] D.C. Monkhouse, J.L. Lach, Use of adsorbents in enhancement of drug dissolution I, J. Pharm. Sci. 61 (1972) 1430–1435.
[15] I. Smirnova, J. Mamic, W. Arlt, Adsorption of drugs on silica aerogels, Langmuir 19 (2003) 8521–8525.
[16] I. Smirnova, S. Suttiruengwong, W. Arlt, Feasibility study of hydrophilic and hydrophobic silica aerogels as drug delivery systems, J. Non-Cryst. Solids 350 (2004) 54–60.
[17] I. Smirnova, S. Suttiruengwong, M. Seiler, W. Arlt, Dissolution rate enhancement by adsorption of poorly soluble drugs on hydrophilic silica aerogels, Pharm. Dev. Technol. 9 (2004) 443–452.
[18] I. Smirnova, M. Türk, R. Wischumerski, M.A. Wahl, Comparison of different methods for enhancing the dissolution rate of poorly soluble drugs: case of griseofulvin, Eng. Life Sci. 5 (2005) 277–280.
[19] I. Smirnova, S. Suttiruengwong, W. Arlt, Aerogels: tailor-made carriers for immediate and prolonged drug release, Kona 23 (2005) 86–97.
[20] G.P. Sanganwar, R.B. Gupta, Dissolution-rate enhancement of fenofibrate by adsorption onto silica using supercritical carbon dioxide, Int. J. Pharm. 360 (2008) 213–218.
[21] E. Galia, J. Horton, J.B. Dressman, Albendazole generics – a comparative in vitro study, Pharm. Res. 16 (1999) 1871–1875.
[22] E.M. Phillips, V.J. Stella, Rapid expansion from supercritical solutions: application to pharmaceutical processes, Int. J. Pharm. 94 (1993) 1–10.
[23] Y. Nakai, K. Yamamoto, K. Terada, J. Ichikawa, Interaction of medicinals and porous powder. I. Anomalous thermal behavior of porous glass mixtures, Chem. Pharm. Bull. 32 (1984) 4566–4571.
[24] Y. Nakai, K. Yamamoto, K. Terada, T. Oguchi, M. Yamamoto, Study of the interaction between light anhydrous silicic acid and drugs, Yakugaku Zasshi 107 (1987) 294–300.
[25] M. Kinoshita, K. Baba, A. Nagayasu, K. Yamabe, T. Shimooka, Y. Takeichi, M. Azuma, H. Houchi, K. Minakuchi, Improvement of solubility and oral bioavailability of a poorly water-soluble drug, TAS-301, by its melt-adsorption on a porous calcium silicate, J. Pharm. Sci. 91 (2002) 362–370.
[26] M. Kinoshita, K. Baba, M. Azuma, H. Houchi, K. Minakuchi, Improvement of solubility and oral bioavailability of a poorly water-soluble drug by its melt-adsorption on silicate compounds, Pharm. Tech. Japan 21 (2005) 113–120.