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Establishment of new preparation method for solid dispersion formulation of tacrolimus

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Abstract

The aim of this study was to establish a new preparation method for solid dispersion formulation (SDF) of tacrolimus, a poorly water-soluble drug, without dichloromethane, because no use of dichloromethane is recommended by ICH harmonized tripartite guideline. To select the appropriate carrier, three different SDFs with polyethylene glycol 6000 (PEG 6000), polyvinylpyrrolidone (PVP) and hydroxypropylmethylcellulose (HPMC) were prepared by the conventional solvent method, in which tacrolimus and the carrier were completely dissolved in the mixture of dichloromethane and ethanol. Powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC) patterns indicated that tacrolimus exists in an amorphous state in all three SDFs. The supersaturated dissolution profiles of tacrolimus were observed in all SDFs, and the highest level of supersaturation for tacrolimus was obtained and maintained for 24 h from SDF with HPMC. On the other hand, the supersaturated level from SDF with PEG 6000 or PVP decreased rapidly. The in vivo oral absorption study in dogs showed that bioavailability of tacrolimus from SDF with HPMC was remarkably improved compared with the crystalline powder. It was clarified that HPMC is the most appropriate carrier for SDF of tacrolimus. Then, SDF of tacrolimus was prepared by the new method, which allows us to make SDF of tacrolimus by swelling HPMC with ethanol, in which tacrolimus was completely dissolved. This new method does not need dichloromethane. The physicochemical properties of SDF with HPMC prepared by the new method were the same as those of SDF prepared by the conventional solvent method. Furthermore, SDF with HPMC prepared by the new method was still stable after stored at 40 °C for 3 months. The pharmacokinetic parameters after oral administration in monkeys showed no significant difference ($P > 0.01$) between SDFs with HPMC prepared by the two methods. In conclusion, we have established the new preparation method for SDF of tacrolimus with HPMC and the new method makes it possible to prepare SDF of tacroliumus without dichloromethane.

Keywords: Tacrolimus; Solid dispersion formulation; Hydroxypropylmethylcellulose; Powder X-ray diffraction; Differential scanning calorimetry; Supersaturation; Oral administration

1. Introduction

Tacrolimus (Fig. 1), which is a 23-member macrolide lactone with potent immunosuppressive activity, was isolated from *Streptomyces tsukubaensis* in 1984 (Kino et al., 1987a,b). Tacrolimus is a poorly water-soluble compound, which has the low solubility in water, about 1–2 μg/ml (Hane et al., 1992) and showed relatively low bioavailability (Honbo et al., 1987). In order to enhance the oral absorption of tacrolimus, Honbo et al. (1987), reported that oily ethanol formulation and solid dispersion formulation

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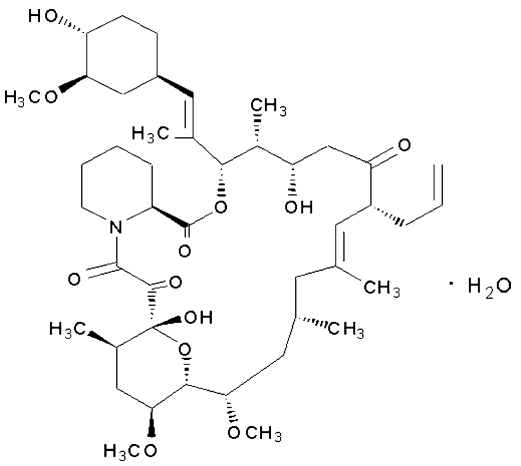

Fig. 1. Chemical structure of tacrolimus.

(SDF) are most potent among many different formulations of tacrolimus examined.

SDF, which was developed by Chiou and Riegelman (1971), is the formulation that possibly enhances the dissolution rate, solubility and oral absorption of a poorly water-soluble drug. Swarbrick (1990), Shargel (1993) and Craig (2001) discussed the increase in drug dissolution rate from SDF and they concluded that the dissolution rate was increased by the following factors: (1) the reduction of the drug particle size to molecular level, (2) the solubilizing effect on the drug by the water-soluble carrier, and (3) the enhancement of the wettability and dispersibility of the drug by the carrier material. According to the definition of SDF, both a given drug and carrier should be completely dissolved with organic solvents (solvent method) or fused by the heating (melting method) in order to prepare SDF. With regard to carriers for SDF, many carriers such as polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, gelucires, eudragits and chitosans have been reported to improve the solubility and bioavailability of poorly water-soluble drugs (Okimoto et al., 1997; Portero et al., 1998; Jung et al., 1999; Kohri et al., 1999; Trapani et al., 1999; Damian et al., 2002; Tantishaiyakul et al., 1999; Yamada et al., 2000; Cilurzo et al., 2002; Kushida et al., 2002; Nakamichi et al., 2002), but among them HPMC is considered as one of the most suitable carriers for SDF (Kohri et al., 1999; Kushida et al., 2002). HPMC remarkably enhances the water-solubility of drugs compared with other water-soluble carriers and/or prevents drugs from re-crystallizing in the dissolution medium (Sugimoto et al., 1982; Suzuki and Sunada, 1998; Kohri et al., 1999; Kushida et al., 2002).

HPMC is a water-soluble polymer and cannot be dissolved in alcohol alone, while HPMC can be easily dissolved in water, and the mixtures such as water and alcohol or alcohol and chlorohydrocarbon. Any method, in which a single solvent such as ethanol alone is employed, has not been reported for the preparation of SDF with HPMC by the solvent method. Usually, the mixed solvents with dichloromethane have been utilized for the preparation of SDF with HPMC by the solvent method (Ho et al., 1996; Yano et al., 1997; Jung et al., 1999; Kobayashi et al., 2001; Kushida et al., 2002). SDF of tacrolimus with HPMC was also prepared using the mixture of ethanol and dichloromethane (Honbo et al., 1987). Dichloromethane, commonly used in the solvent method for HPMC, is classified in Class 2 solvents of ICH harmonized tripartite guideline. Therefore, it is really expected that a preparation method for SDF without dichloromethane is established from the environmental point of view.

In this study, physicochemical properties of SDFs prepared with different water-soluble carriers, PEG 6000, PVP and HPMC, were evaluated to select an appropriate carrier for SDF of tacrolimus. Then, to establish the new preparation method for SDF of tacrolimus with HPMC, we tried to use a single solvent, ethanol, and prepared SDF by swelling HPMC with ethanol solution of tacrolimus. Furthermore, SDF prepared by the new method was compared with that prepared by the conventional solvent method in terms of the physicochemical and biopharmaceutical properties.

2. Materials and methods

2.1. Materials

Tacrolimus was provided by Fujisawa Pharmaceutical Co., Ltd. (Osaka and Tokyo, Japan). HPMC, PVP and PEG 6000 were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo), BASF Japan, Ltd. (Tokyo) and

Sanyo Chemical Industries, Ltd. (Kyoto, Japan), respectively. All other materials were of analytical regent grade.

2.2. Animals

Male beagle dogs (Japan Laboratory Animals, Inc., Tokyo) and male cynomolgus monkeys (Charles River Japan, Kanagawa, Japan), maintained at 23 °C and 55% humidity, were allowed free access to standard laboratory chow (Maruha Pet Food Co., Ltd., Tokyo; Oriental Yeast Co., Ltd., Tokyo) and water prior to the experiments. Dogs weighing 11.5–15.9 kg and monkeys weighing 6.2–7.0 kg were assigned randomly to each experimental group. Our investigations were performed after approval by our local ethical committee at Fujisawa Pharmaceutical Co., Ltd. and Okayama University.

2.3. Preparation of SDF

2.3.1. Solvent method

Three different water-soluble polymers, PEG 6000, PVP and HPMC, were used as the carrier of SDF. SDF was prepared by the solvent evaporation method (Chiou and Riegelman, 1971). Briefly, 5 g of tacrolimus and 5 g of each water-soluble polymer were accurately weighed and dissolved in the mixture of 50 ml of ethanol and 25 ml of dichloromethane. Then, the mixed solvent was evaporated under reduced pressure using a vacuum dryer at 40 °C. After drying, SDF was pulverized using an agate mortar and pestle. The pulverized powder was classified using the sieves (size: 60 and 330 mesh), and the particle size fraction of 45–250 μm was used for the study. A physical mixture of tacrolimus and each water-soluble polymer (1:1, w/w) was prepared by the pulverization with an agate mortar and pestle. The mixtures through the sieve (size: 60 mesh) were used for the study.

2.3.2. New preparation method

Five grams of tacrolimus and 5 g of HPMC were accurately weighed and tacrolimus was completely dissolved into 15 ml of ethanol. Then, HPMC was swollen by ethanol, in which tacrolimus is dissolved. Ethanol was evaporated under reduced pressure using a vacuum dryer at 40 °C. After drying, SDF was prepared and obtained by the same manner of the conventional solvent method described above.

2.4. Scanning electron microscope (SEM) study

The SEM pictures were obtained by a scanning electron microscope (Type S-800, Hitachi, Tokyo). The accelerating voltage is 10 kV at 100×.

2.5. Powder X-ray diffraction (XRD) measurement

XRD measurement was performed using a powder X-ray diffractometer (Multiflex, Rigaku, Tokyo) with Ni-filtered, Cu Kα radiation, a voltage of 40 kV and a current of 40 mA. The scanning rate was 4°/min over a 2θ range of 2.5–40° and with a sampling interval of 0.02°.

2.6. Differential scanning calorimetry (DSC) measurement

DSC curves were measured with a DSC instrument (SSC/560S, Seiko Instruments, Chiba, Japan). An aliquot of each sample corresponding to 2 mg as tacrolimus was placed in an aluminum pan. The heating rate was 10 °C/min and the heating ranging was 30–215 °C.

2.7. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analyses were performed using a FTIR spectrometer (Type FT-720, Horiba, Kyoto, Japan). Data were collected over a spectral region from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

2.8. Dissolution studies

The dissolution test was carried out in accordance with Japanese Pharmacopoeia (JP) 14 paddle method. The dissolution medium was 900 ml of JP first medium, pH 1.2, which was maintained at 37 ± 0.5 °C. The paddle rotation speed was 200 rpm. The excess amount of SDF was applied to the dissolution medium. Aliquots were withdrawn through the G4 glass filter at appropriate times, and equal volumes of fresh dissolution medium were replaced. The concentration of tacrolimus dissolved in the medium was

analyzed by high performance liquid chromatography (HPLC).

2.9. HPLC analysis

The HPLC analyses were performed using Waters HPLC system (Model 515 pump, Model 717 plus auto sampler, Model 486 UV detector) equipped with a 4.6 mm × 150 mm ODS column (TSK-Gel ODS 80TM, Tosoh, Tokyo). The mobile phase consisted of water, isopropyl alcohol and tetrahydrofuran (5:2:2, v/v/v). The flow rate was 1.0 ml/min, and the detection wavelength was 220 nm.

2.10. In vivo absorption studies

2.10.1. Comparison of oral absorption between crystalline powder and SDF with HPMC

The in vivo absorption studies of tacrolimus crystalline powder or SDF with HPMC prepared by the conventional solvent method were carried out using male beagle dogs ($n = 6$). Each dog was fasted overnight. The crystalline powder or SDF with HPMC containing 1 mg as tacrolimus was suspended with 20 ml of water, and then each suspension was orally administered. The blood samples were withdrawn into a heparinized syringe at 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h after oral administration. Those blood samples were kept under −20 °C until assayed. The concentrations of tacrolimus in the blood samples were analyzed by the enzyme immunoassay (EIA) method (Tamura et al., 1987).

2.10.2. Comparison of oral absorption between SDFs with two preparation method

The in vivo oral absorption study of SDFs prepared by the conventional solvent method and the new preparation method was performed using male cynomolgus monkeys ($n = 3$). Each monkey was fasted overnight. The SDF containing 5 mg as tacrolimus was filled into a hard gelatin capsule and then the capsule was orally administered with 20 ml of water. The blood samples were withdrawn into a heparinized syringe at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after oral administration. Those blood samples were kept under −20 °C until assayed. The concentrations of tacrolimus in the blood samples were analyzed by the EIA method (Tamura et al., 1987).

2.11. Pharmacokinetic analysis

The area under the blood concentration of tacrolimus versus time curve (AUC) and mean residence time (MRT) were calculated by the trapezoidal method.

2.12. Stability studies

The SDF of tacrolimus prepared by the new method was stored at 40 °C for 3 months. The content of tacrolimus in the SDF was measured by HPLC. XRD, DSC and the supersaturated dissolution studies were also performed.

2.13. Statistic analysis

Results are expressed as mean ± S.E. ANOVA was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Student's *t*-test.

3. Results and discussion

3.1. Selection of the appropriate water-soluble carrier for SDF

3.1.1. Solid state characterization

In general, it is well known that a drug in SDF often exists as an amorphous form. The amorphous form of a drug has a higher thermodynamic activity than its crystalline form. The higher thermodynamic energy level of the drug leads to the rapid dissolution property (Betageri and Makarla, 1995; Jung et al., 1999). In order to investigate the crystallinity of tacrolimus in SDF with PEG 6000, PVP or HPMC prepared by the conventional solvent method (tacrolimus:carrier = 1:1, w/w), XRD and DSC studies were carried out.

Fig. 2 shows the XRD patterns of tacrolimus, each water-soluble polymer, the physical mixture of tacrolimus with each carrier, and SDF of tacrolimus with each carrier. The XRD pattern of the physical mixture of tacrolimus with each water-soluble polymer was similar to the XRD pattern of tacrolimus crystalline powder alone. It was confirmed that the crystallinity of tacrolimus does not change in the physical mixtures with each carrier. On the other

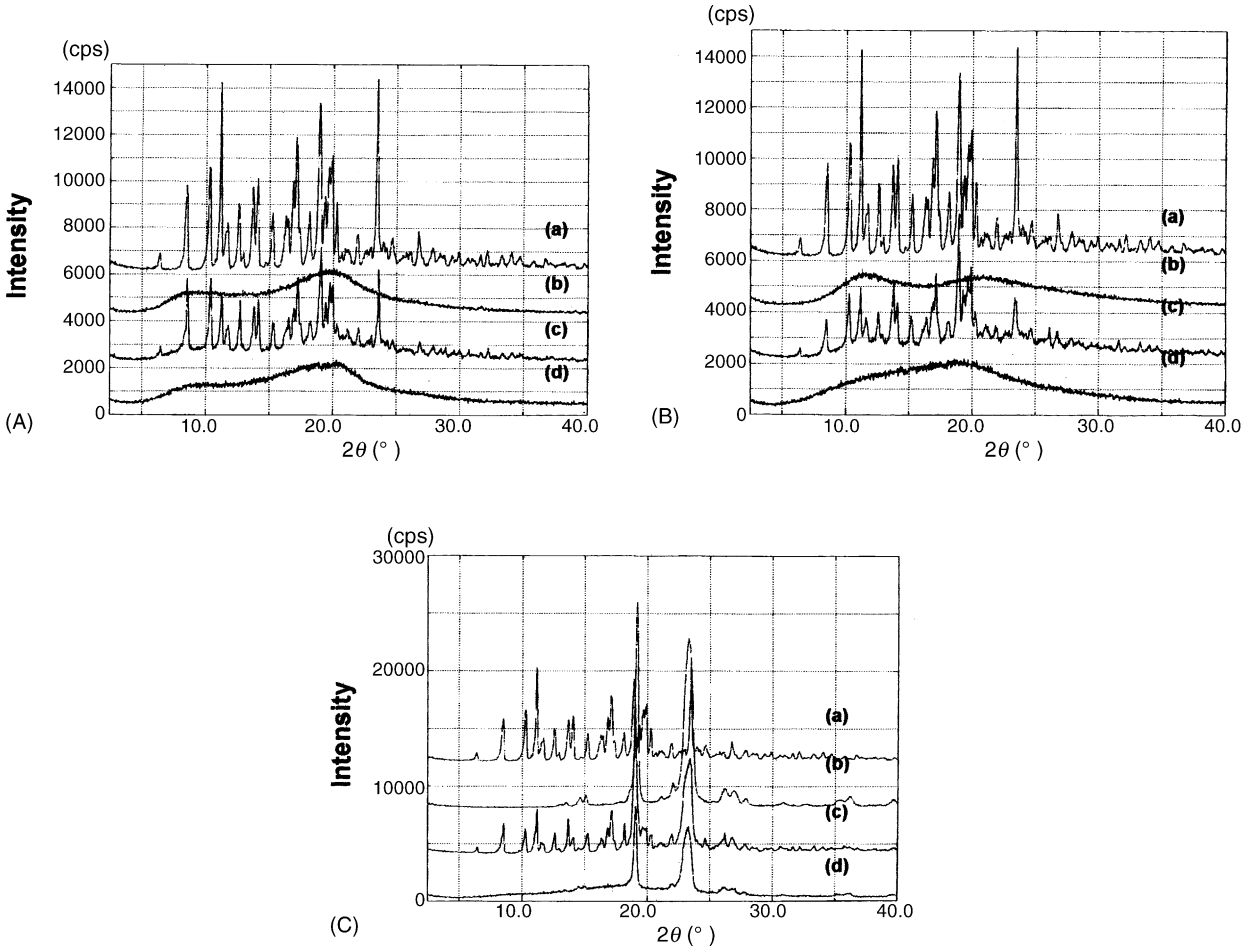

Fig. 2. XRD patterns for SDF of tacrolimus with HPMC (A), PVP (B) or PEG 6000 (C). (a) Tacrolimus crystalline powders; (b) carrier alone; (c) physical mixture of tacrolimus and a carrier; (d) SDF of tacrolimus with a carrier.

hand, no diffraction peak from tacrolimus was observed in all the SDFs investigated here, and the XRD pattern of each SDF was similar to that of the carrier itself used for each SDF. This result suggests that tacrolimus exists in an amorphous state in the SDF.

Fig. 3 shows the DSC thermograms of the physical mixture of tacrolimus and each carrier, and SDF of tacrolimus with each carrier. The physical mixture of tacrolimus and HPMC or PVP exhibited an endothermic peak at around 130 °C, which corresponds to the melting of tacrolimus. The physical mixture of tacrolimus and PEG 6000 showed no endothermic peak of tacrolimus, even though the peaks derived from tacrolimus were observed in XRD (Fig. 2C). It is speculated that tacrolimus is dissolved in melted PEG 6000 during DSC measurement, and only one endothermic peak at around 60 °C, which corresponds to the melting of PEG 6000, is observed. On the other hand, SDF of tacrolimus with each carrier exhibited no endothermic peak corresponding to tacrolimus, suggesting no crystalline of tacrolimus in each SDF. From the results of XRD and DSC studies, it was confirmed that tacrolimus exists in an amorphous state in every SDF with each of the three carriers investigated.

3.1.2. Dissolution study

To increase the oral absorption of poorly water-soluble drugs, it is very important to improve the

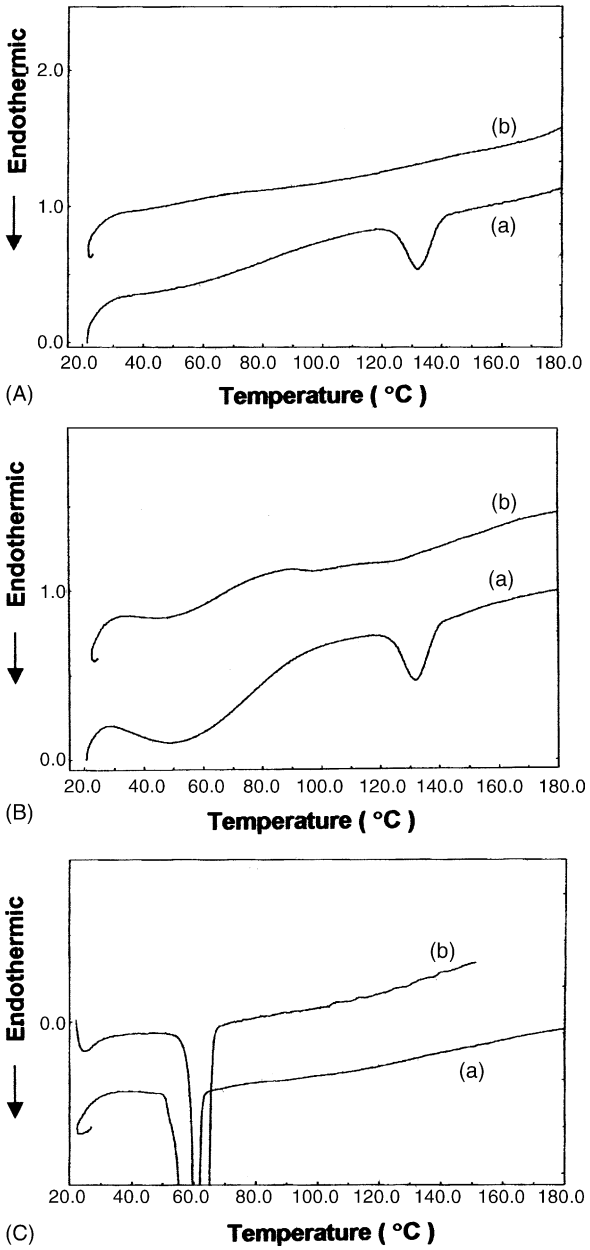

Fig. 3. DSC thermograms of SDF of tacrolimus with HPMC (A), PVP (B) or PEG 6000 (C). (a) Physical mixture of tacrolimus and a carrier; (b) SDF of tacrolimus with a carrier.

drug solubility in the gastrointestinal tract. Therefore, the dissolution of tacrolimus from SDF with each carrier, which was prepared by the conventional solvent method, was examined. Fig. 4 shows the dissolution profile of tacrolimus from SDF with each carrier together with that for tacrolimus crystalline powder. The supersaturation of tacrolimus for all SDFs was observed just after starting the dissolution test. The maximum supersaturated concentrations of tacrolimus from the three SDFs were almost similar and were about 25-fold higher (about 50 μg/ml) than the solubility of tacrolimus in JP first solution. However, the dissolution profile of tacrolimus from SDF with PEG 6000 showed that the supersaturated level of tacrolimus rapidly decreased and was only about 6 μg/ml after 24 h. Moreover, the supersaturated level of tacrolimus from SDF with PVP also decreased gradually to about 30 μg/ml after 24 h. The decrease of tacrolimus concentration in a supersaturated state could be due to the re-crystallization of tacrolimus as reported with regard to many other compounds (Sugimoto et al., 1980; Okimoto et al., 1997; Kohri et al., 1999). On the other hand, the extremely high concentration of tacrolimus in a supersaturated state was maintained up to 24 h in the case of SDF with HPMC, suggesting that the usage of HPMC as a carrier can prevent supersaturated tacrolimus from re-crystallizing. These results of the dissolution study clearly indicated that HPMC is the most appropriate

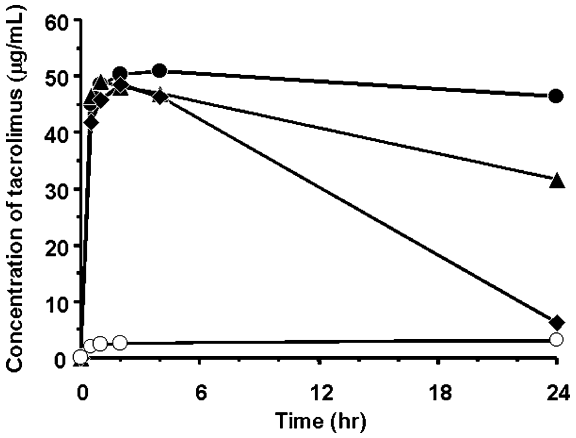

Fig. 4. Dissolution profiles of tacrolimus from SDFs. (●) SDF of tacrolimus with HPMC; (▲) SDF of tacrolimus with PVP; (◆) SDF of tacrolimus with PEG 6000; (○) tacrolimus crystalline powder. Each SDF corresponding to 70 mg as tacrolimus was dissolved in JP14 first fluid (pH 1.2). The test method was in accordance with JP14 paddle method; the rotation speed of paddle was 200 rpm and the dissolution medium was maintained at 37 ± 0.5 °C. Results are the mean of duplicate experiments.

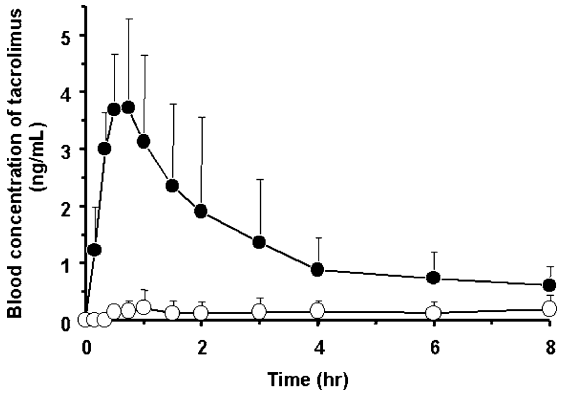

Fig. 5. Blood concentration of tacrolimus after oral administration of SDF with HPMC to beagle dogs. (●) SDF of tacrolimus with HPMC; (○) tacrolimus crystalline powders. Values are expressed as the mean with a vertical bar showing S.E. of six animals. Each dosage form was administered at the dose of 1 mg as tacrolimus.

carrier for SDF of tacrolimus among carriers examined in the present study.

3.1.3. In vivo oral absorption studies

To investigate whether the improved dissolution profile of tacrolimus by SDF with HPMC can be reflected in the in vivo oral absorption, the SDF was administered to dogs. Fig. 5 shows blood concentration–time profile of tacrolimus after oral administration of SDF with HPMC or crystalline powder of tacrolimus at the dose of 1 mg as tacrolimus. The pharmacokinetic parameters of tacrolimus are listed in Table 1. As shown in Fig. 5, when tacrolimus crystalline powder was administered orally, the tacrolimus levels in the blood were detected very low, and the C_{max} and $AUC_{0-8\,h}$ values were 0.4 ng/ml and 1.1 ng h/ml, respectively (Table 1). On the other hand, when the SDF with HPMC was administered orally, tacrolimus levels in the blood were markedly increased in comparison with the crystalline powder. The C_{max} and $AUC_{0-8\,h}$ values were 4.0 ng/ml and 10.9 ng h/ml, respectively (Table 1). Both AUC and C_{max} values for the SDF with HPMC were about 10 times higher than those of the crystalline powder, indicating that oral absorption of tacrolimus is extremely enhanced by its administration as SDF with HPMC. From the results of the physicochemical and pharmacokinetic studies, HPMC was selected as the most suitable water-soluble carrier for SDF of tacrolimus.

3.2. Establishment of new preparation method of SDF

To prepare SDF of tacrolimus with HPMC by the conventional solvent method, the mixed solvent systems including dichloromethane have been used (Ho et al., 1996; Yano et al., 1997; Jung et al., 1999; Kobayashi et al., 2001; Kushida et al., 2002; Honbo et al., 1987). However, since dichloromethane is classified in Class 2 solvents of ICH harmonized tripartite guideline, a new preparation method for SDF that does not need dichloromethane is really expected from the environmental point of view. Therefore, we tried to develop a new preparation method for SDF of tacrolimus with HPMC, in which dichloromethane is not used.

3.2.1. Observation of SDF by SEM

Fig. 6 shows SEM pictures of tacrolimus crystalline powder, HPMC, SDF of tacrolimus with HPMC prepared by the conventional solvent method and the SDF prepared by the new preparation method. Tacrolimus crystalline powder (Fig. 6A) and HPMC (Fig. 6B) showed the prismatic shape and the fibrous shape, respectively. The appearance of SDF prepared by the conventional solvent method was like a uniformed and homogeneously mixed mass (Fig. 6C). On the contrary, the appearance of SDF prepared by the new method showed the fibrous shape, and the unit shape of SDF is almost similar to that of HPMC (Fig. 6D). These results suggest that tacrolimus in SDF prepared by the new method is adsorbed into

Table 1
Pharmacokinetic parameters of tacrolimus after its oral administration to dogs as crystalline powders or SDF of tacrolimus with HPMC

Sample	$AUC_{0-8\,h}$ (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)	MRT (h)
Crystalline powders	1.1 ± 1.4	0.4 ± 0.3	3.1 ± 3.0	3.3 ± 0.9
SDF with HPMC	10.9 ± 6.1*	4.0 ± 1.2*	0.6 ± 0.2	2.7 ± 0.1

Each value represents the mean ± S.E. of six animals. Each dosage form was administered at the dose of 1 mg as tacrolimus.
* $P < 0.05$, compared to the corresponding parameter of crystalline powder.

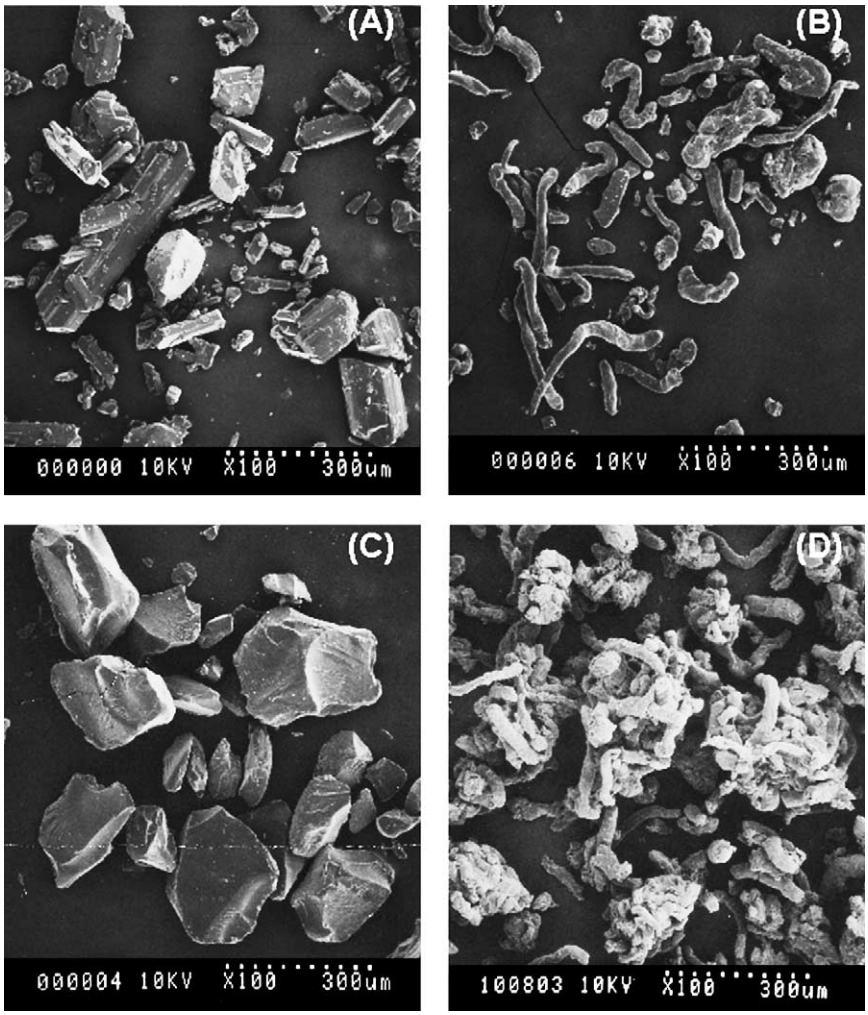

Fig. 6. SEM pictures of (A) tacrolimus crystalline powder, (B) HPMC, (C) SDF prepared by the conventional solvent method and (D) SDF of tacrolimus prepared by the new method.

HPMC swollen with ethanol and is homogeneously dispersed into HPMC at the molecular level.

3.2.2. Solid state characterization of SDF prepared by the new method

The crystallinity of tacrolimus in SDF with HPMC prepared by the new method was checked by XRD and DSC measurements. The XRD pattern and DSC thermogram are shown in Fig. 7A and B, respectively. No diffraction peaks were observed in the XRD pattern (Fig. 7A) and no endothermic peak corresponding to the melting of tacrolimus was observed in the DSC thermogram (Fig. 7B) for SDF prepared by the new method. These results suggest that tacrolimus exists in an amorphous state in SDF with HPMC prepared by the new method.

The FTIR studies were carried out to investigate the interaction between tacrolimus and HPMC. Fig. 8 shows the FTIR spectra of tacrolimus crystalline, HPMC, the physical mixture of tacrolimus and HPMC, and SDF with HPMC prepared by the new method. In the FTIR spectra of tacrolimus crystalline (Fig. 8A),

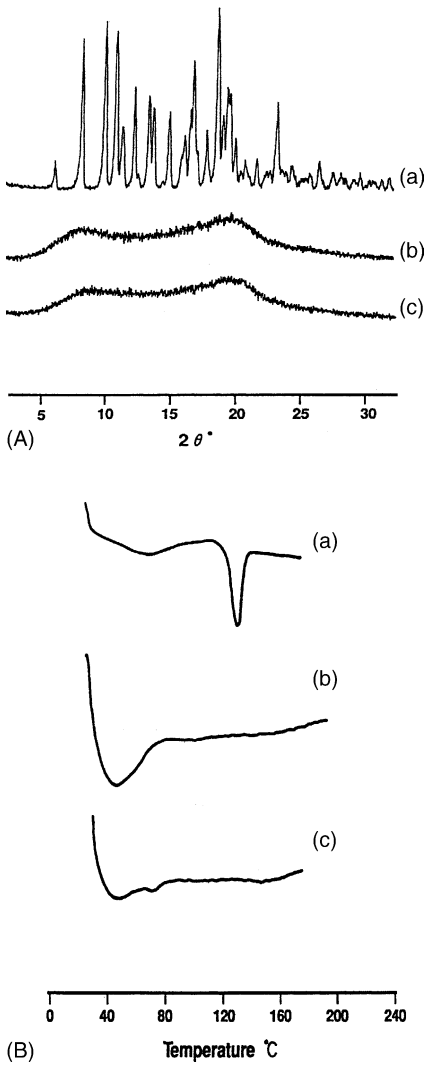

Fig. 7. Comparison of XRD patterns (A) and DSC thermograms (B) between SDFs of tacrolimus prepared by two different methods. (a) Tacrolimus crystalline powder; (b) SDF of tacrolimus prepared by the conventional solvent method; (c) SDF of tacrolimus prepared by the new method.

absorption bands of O–H stretching vibration at 3450 cm^{-1}, C=O (ester and ketone) stretching vibrations at 1740, 1725 and 1693 cm^{-1}, C=O (keto-amide) and C=C stretching vibration at 1637 cm^{-1}, C–O (ester) stretching vibration at 1194 cm^{-1}, C–O–C (ether) stretching vibrations at 1176 and 1094 cm^{-1} were observed (Hane et al., 1992). These bands were also observed for the physical mixture of tacrolimus and HPMC with the same absorbance (Fig. 8C). From these results, it was confirmed that there is no interaction between tacrolimus and HPMC in the physical mixture. In contrast, the bands due to the C=O stretching vibration of tacrolimus in SDF were different from those of physical mixtures, whereas the other stretching vibrations of tacrolimus were not affected. The absorption band attributed to C=O groups at 1725 cm^{-1} was disappeared, and the absorption bands at 1693 and 1637 cm^{-1} were shifted up to 1708 and 1647 cm^{-1}, respectively (Fig. 8D). These results suggest that the C=O functional groups and O–H of tacrolimus are interacted with the functional group of HPMC at the molecular level in SDF prepared by the new method (Cherng-Yih et al., 1998; Tantishaiyakul et al., 1999; Hirasawa et al., 1999; Kushida et al., 2002).

3.2.3. Dissolution property of tacrolimus from SDF prepared by the new method

As shown in Fig. 4, SDFs prepared by the conventional solvent method have a great difference in the dissolution profile of tacrolimus, even though they show almost the same maximal value of supersaturated concentration of tacrolimus. Therefore, it is very important to check the supersaturated dissolution profiles for the assessment of SDF potency. Fig. 9 shows the dissolution profiles of tacrolimus from SDFs prepared by the conventional solvent method and the new preparation method at four different amounts of SDF. Both SDFs showed the same supersaturated dissolution profiles and levels at each amount of SDF. The maximal value of the concentration of tacrolimus for both SDFs tended to increase as the amount of tacrolimus examined increased. The reason for this phenomenon remains to be clarified, but HPMC, of which the amount also increased, may improve the dissolution of tacrolimus a little bit more. Fig. 9C shows the statistically significant linear relationship in the equilibrium concentrations of tacrolimus between SDFs with HPCM prepared by the conventional and new methods ($r^2 = 0.99$). The slope of regression line was 1.024, indicating that the equilibrium (maximum) concentrations of tacrolimus from both SDFs are almost the same each other. From these results, it was confirmed that the supersaturated dissolution properties of tacrolimus from SDF with HPMC prepared by

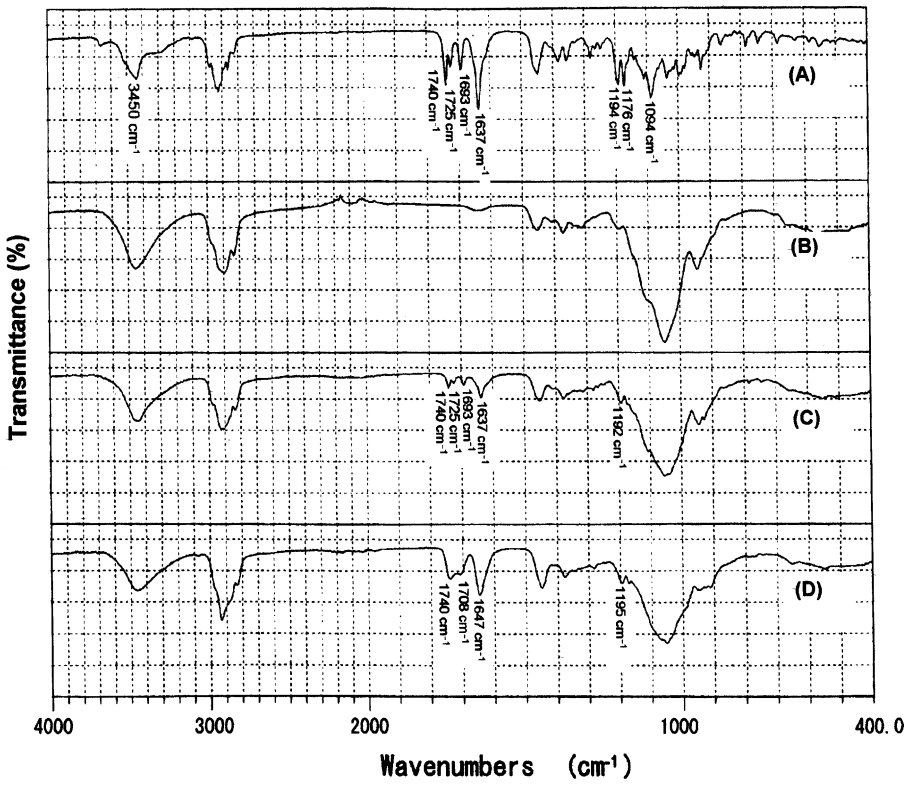

Fig. 8. FTIR spectra of (A) tacrolimus crystalline powder, (B) HPMC alone, (C) physical mixture of tacrolimus and HPMC and (D) SDF of tacrolimus prepared by the new method.

the new method were similar to that prepared by the conventional solvent method.

3.2.4. In vivo absorption study

Since SDF of tacrolimus with HPMC prepared by the new method showed the similar physicochemical properties to SDF prepared by the conventional solvent method, the in vivo oral absorption was compared between the two SDFs in monkeys (Fig. 10). The blood concentration profile of tacrolimus after dosing each of SDF was quite similar and the pharmacokinetic parameters such as AUC, C_{max}, T_{max} and MRT were not significantly different ($P > 0.01$) between the two SDFs (Table 2). These results of physicochemical and biopharmaceutical studies clearly show that an excellent SDF of tacrolimus with HPMC could be prepared not only by the conventional solvent method, but also the new preparing method.

3.2.5. Stability study

One of the problems that must be overcome for the commercial application of SDF is the stability is-

Table 2
Pharmacokinetic parameters of tacrolimus after oral administration of SDF prepared by the conventional solvent method or the new preparation method to monkeys

Sample	$AUC_{0-72\,h}$ (ng h/ml)	C_{max} (ng/ml)	T_{max} (h)	MRT (h)
Conventional solvent method	551.7 ± 88.6	31.7 ± 2.3	2.7 ± 0.8	21.6 ± 1.4
New preparation method	578.1 ± 57.2	38.7 ± 10.6	3.3 ± 0.8	20.5 ± 3.0

Each value represents the mean ± S.E. of three animals. Each dosage form was administered at the dose of 5 mg as tacrolimus.

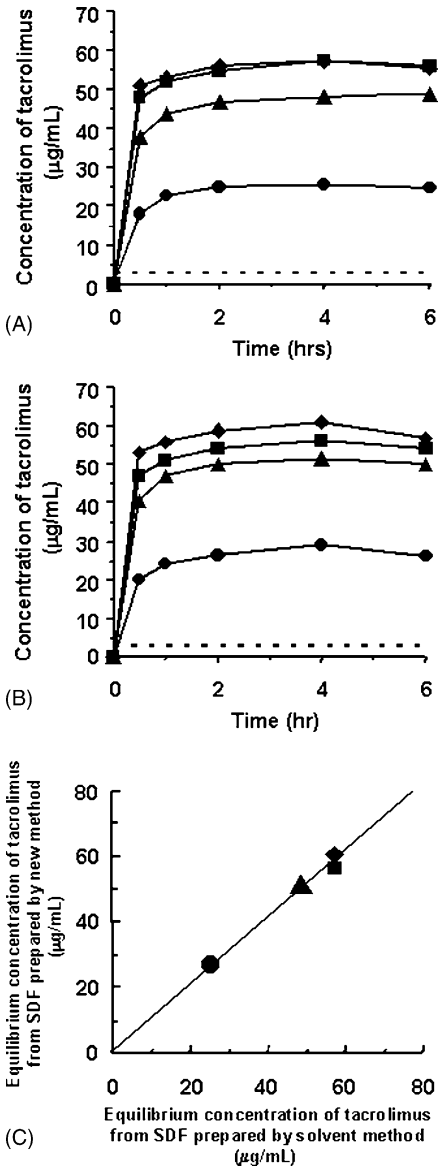

Fig. 9. Comparison of dissolution profiles between SDFs of tacrolimus prepared by two different methods. (A) and (B) represent dissolution profiles of SDFs of tacrolimus prepared by the conventional solvent method and the new method, respectively, at four different amounts applied, and the relationship between the equilibrium concentrations from the two SDFs are shown in (C). Applied amounts of SDF: (●) 25 mg as tacrolimus; (▲) 70 mg as tacrolimus; (■) 100 mg as tacrolimus; (◆) 200 mg as tacrolimus. The dotted line represents the solubility of tacrolimus in the dissolution medium. The test method was in accordance with JP14 paddle rotation method; the rotation speed of paddle was 200 rpm, and the dissolution medium (JP14 first fluid) was maintained at $37 \pm 0.5\,^{\circ}C$. Results are the mean of duplicate experiments.

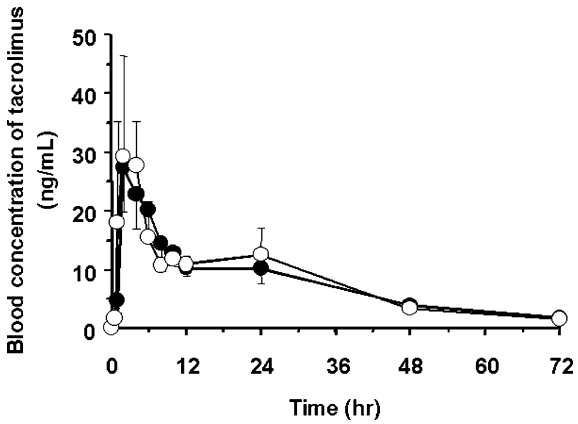

Fig. 10. Blood concentration of tacrolimus after oral administration of SDFs of tacrolimus prepared by two different methods to cynomolgus monkeys. (●) SDF prepared by the conventional solvent method; (○) SDF prepared by new method. Values are expressed as the mean with a vertical bar showing S.E. of three animals. Each dosage form was administered at the dose of 5 mg as tacrolimus.

sue of SDF, that is to say, the amorphous form may re-crystallize out on aging (Serajuddin, 1999). The re-crystallization of the drug results in the decrease of the supersaturated level. In fact, it was reported that griseofulvin was precipitated out from SDF with PEG 6000 during the storage (Chiou, 1977). Damian et al. (2002) reported that the dissolution rate of UC-781 from SDF with PEG 6000, Gelucire® 44/14 and PVP K30 decreased with time of the storage, which could be also attributed to the re-crystallization during the storage.

The stability study of SDF prepared by the new method was performed after the SDF was stored at 40 °C for 3 months. The amount of tacrolimus in the SDF was not decreased (101.5%) after the storage. The equilibrium concentration of tacrolimus in the supersaturated dissolution profile for the stored SDF was kept at 52.0 μg/ml, which is almost the same as that of SDF soon after the preparation (51.6 μg/ml). The supersaturated concentration of tacrolimus after 24 h for the stored SDF was the same as that of initial SDF. Furthermore, no diffraction peak of XRD and no endothermic peak of DSC were observed in SDF stored (data not shown). Thus, the amorphous state of tacrolimus in SDF prepared by the new method, was kept by storing at the accelerate condition. From these results, it

was confirmed that the SDF of tacrolimus with HPMC prepared by the new method is very stable.

4. Conclusion

From the physicochemical, biopharmaceutical and stability studies, it was clarified that SDF of tacrolimus with HPMC prepared by the new method, in which dichloromethane is not used and HPMC is just swollen with ethanol, has such an excellent property as SDF prepared by the conventional solvent method. Therefore, the new method for the preparation of SDF with HPMC could be very useful from the view-point of the environmental issue.

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