Preparation and Characterization of Itraconazole- or Miconazole-Loaded PLGA Microspheres

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The purpose of this study was to prepare poly(lactide-co-glycolide) (PLGA) microspheres (MS) loaded with itraconazole (ITCZ) or miconazole (MCZ) under different evaporation temperatures (25 or 40°C) using an oil-in-water emulsion solvent evaporation method in order to evaluate the initial burst release of drug. Loading efficiencies were comparatively good and the diameters of prepared drug-loaded PLGA MS were around 20 µm in all formulations. The release rates of ITCZ-PLGA MS prepared at 40°C showed a significantly restricted release profile compared with the corresponding ITCZ-PLGA MS prepared at 25°C. This difference in release rate of ITCZ was thought to be caused by the self-healing effect of PLGA, as the glass transition temperature of PLGA is around 40°C. With respect to the MCZ-PLGA MS, the initial burst release was similar in formulations prepared at both 25 and 40°C. Scanning electron microscope results suggested that the initial burst release was due to the localization of MCZ on the surface of MCZ-PLGA MS at higher concentrations. Differential scanning calorimetry measurements suggested complete amor Physisation of MCZ in MCZ-PLGA MS, whereas crystalline ITCZ was detected in the ITCZ-PLGA MS. This complete amorphization of MCZ is considered to be one of the reasons for the initial burst release.

Keywords poly(lactide-co-glycolide); itraconazole; miconazole; self-healing; burst release; amorphization

Introduction

Itraconazole (ITCZ) is an extremely lipophilic, weakly basic, antifungal drug with a low aqueous solubility of <0.1 µg/mL. Miconazole (MCZ) is also a poorly soluble antifungal drug with an aqueous solubility of <1 µg/mL. A number of strategies have been tried for enhancing the release rates and bioavailabilities of oral dosage forms of these antifungal drugs. In injectable formulations, the use of cosolvents, such as ethanol, macrogl and propylene glycol, or surfactants, has been shown to successfully enhance the solubilization of the drugs. Recently, in order to achieve sustained-release delivery, an approach using biodegradable microspheres (MS) containing antimicrobial agents or other types of drug has been tried. ITCZ has a high clearance rate in humans and a low bioavailability when given orally. If we could incorporate this and other antifungal drugs with poor oral absorption into biodegradable MS for injection as controlled-release formulations, we could theoretically achieve 100% bioavailability for these drugs.

We recently proposed the use of self-healing ONO-1301-loaded poly(lactide-co-glycolide) (PLGA) MS to restrict the initial burst release of ONO-1301, a long-acting prostacyclin agonist with thromboxane synthase inhibitory activity, whose solubility is in a similar range to antifungal agents such as ITCZ or MCZ. Few groups previously reported ITCZ-loaded PLGA nanoparticle system for antifungal activity evaluating size dependency or characterization of prepared nanoparticles. In every article, initial burst release of ITCZ was observed or expected, whereas there has been no reports to try to restrict the burst release of MCZ in MCZ-loaded PLGA MS. Thus, to our knowledge, there has been no other reports to control the initial burst release from anti-fungal drugs loaded PLGA MS using self-healing effect.

In the present study, the versatility of the restriction effect on initial burst release using self-healing PLGA MS was investigated using PLGA MS containing ITCZ or MCZ (ITCZ-PLGA MS or MCZ-PLGA MS, respectively). MS were prepared using the oil-in-water (o/w) emulsion solvent evaporation method. Theoretical loading levels of drugs were set at 9.1 and 16.7%, and the evaporating temperature was set at 25 or 40°C. The surface morphology of PLGA MS was examined by scanning electron microscope (SEM) imagery, and amorphization of PLGA MS was investigated by differential scanning calorimetry (DSC), in order to characterize the release profile of each drug from PLGA MS.

Experimental

Materials Itraconazole was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and miconazole from Sigma-Aldrich (St. Louis, MO, U.S.A.). PLGA copolymer (75:25; PLGA 7505; average molecular weight 5000) was used as the substrate of the MS. Polyvinyl alcohol (PVA) (polymerization degree of 500, saponification degree of 86.5–89.0 mol%) and Tween 80 (both Nacalai Tesque Ltd., Kyoto, Japan) were used as dispersants for the preparation of PLGA MS. Organic solvents (dichloromethane, acetone) were purchased from Wako Pure Chemical Industries, Ltd., dimethyl sulfoxide (DMSO) from Nacalai Tesque Ltd. All other reagents were special reagent grade.

Preparation of ITCZ- or MCZ-PLGA MS Various formulations of ITCZ or MCZ-PLGA MS were prepared using an
hydrogen sulfate and (B) acetonitrile, respectively, and the solution was acetonitrile–water (60 : 40, v/v) and that for MCZ was (A) DMSO for the extraction of ITCZ or MCZ, respectively. The solution was homogenized using a sonicator and stirred at temperatures of 25 or 40°C for 6 h. After centrifugation and washing, ITCZ or MCZ were dissolved in dichloromethane. The organic phase was added to the PVA and stirred to form an o/w emulsion. The solvent was evaporated off by stirring at temperatures of 25 or 40°C for 6 h. After centrifugation and washing, ITCZ or MCZ-PLGA MS were isolated by lyophilization. PLGA MS for each drug were prepared at two different theoretical drug content, 9.1 and 16.7%. The preparative conditions are summarized as follows: an aqueous solution of PVA (a poor solvent of PLGA) was placed in a glass vessel. PLGA and ITCZ or MCZ were dissolved in dichloromethane. The organic phase was added to the PVA and stirred to form an o/w emulsion. The solvent was evaporated off by stirring at temperatures of 25 or 40°C for 6 h. After centrifugation and washing, ITCZ or MCZ-PLGA MS were isolated by lyophilization. PLGA MS for each drug were prepared at two different theoretical drug content, 9.1 and 16.7%. The preparative conditions are summarized in Table 1 (formulation Nos. 1–8).

In order to clarify the effect of the evaporation rate of dichloromethane on surface morphology of MS, two different formulations of PLGA MS were also prepared. PLGA MS formulation No. 9 was prepared with an evaporation temperature of 25°C for 12 h, and PLGA MS formulation No. 10 was prepared with an evaporation temperature of 25°C for 6 h, followed by 40°C for 6 h. The characteristics of these two formulations are given in Table 2.

**Drug Content and Encapsulation Efficiency** ITCZ or MCZ-PLGA MS (5 mg) were mixed with 25 mL of dimethyl sulfoxide (DMSO) for the extraction of ITCZ or MCZ, respectively. The solution was homogenized using a sonicator and the concentration of ITCZ or MCZ determined using HPLC. The HPLC methods used for ITCZ and MCZ were based on the Japanese Pharmacopoeia and a previous report.15) For HPLC, 10 µL was injected onto a chromatograph (LC-10AT VP, Shimadzu Corporation, Kyoto, Japan), equipped with a UV detector (SPD-10A VP, Shimadzu Corporation), an integrator (LC solution, Shimadzu Corporation) and reverse-phase column (CAPCELL PAK C18 UG120 S5: 150×4.6 mm i.d.; Shiseido Co., Ltd., Tokyo, Japan). The column temperature was kept at 30°C. The mobile phase composition for ITCZ was acetonitrile–water (60:40, v/v) and that for MCZ was (A) an aqueous solution of 2.7% (w/v) tetra-n-butyl ammonium hydrogen sulfate and (B) acetonitrile, respectively, and the flow rate was 1.0 mL/min. Run time for ITCZ was 10 min and for MCZ 25 min, with linear gradient elution: 0–4 min (20% B), 4–15 min (20–50% B), 15–16 min (50–20% B), 16–25 min (20% B). The ultraviolet detection wavelengths for ITCZ and MCZ were set at 261 and 221 nm, respectively.

**Release of Drug from ITCZ- or MCZ-PLGA MS** In vitro drug-release studies of MS (1 mg) were carried out using the paddle method at 37°C and 100 rpm using 500 mL of phosphate buffer containing 0.2% Tween-80 (pH 7.0). At various time intervals, 1 mL aliquots were withdrawn and replaced by the same volume of fresh medium. The drug concentration was analyzed by HPLC. From the slope of the plots obtained in the in vitro drug release study, the first-order rate constant was calculated, and the regression coefficient determined.

**Particle Size Measurement** The particle size was measured after lyophilization; the dry particles were resuspended in 0.2% (w/v) Tween-80 solution using a vortex mixer and measured using a Multisizer™3 Coulter Counter® (Beckman Coulter, Inc., U.S.A.).

**Scanning Electron Microscope (SEM)** The surface morphology of MS was examined in SEM images taken with a JEOL JSM-5600LV Scanning Microscope (JEOL, Tokyo, Japan).
Briefly, MS were fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in a vacuum, with a thin layer of gold. Images of the MS surfaces were taken at an excitation voltage of 10 kV.

**Differential Scanning Calorimetry (DSC)** The $T_g$ of ITCZ or MCZ-PLGA MS was measured in a differential scanning calorimeter (DSC6200, Seiko Instruments Inc., Chiba, Japan). PLGA MS samples were sealed in aluminum hermetic pans and thermograms were determined by first cooling the sample to 20°C, then heating to 100 or 200°C at a rate of 10°C/min under a purged nitrogen atmosphere.

**Fourier Transform Infrared Spectroscopy (FT-IR)** The interaction between chemical bonds of drug and polymer was examined in ITCZ-PLGA MS. FT-IR spectra were obtained on a Spectrum 100 (PerkinElmer, Inc.) in attenuated total reflectance mode. The range of acquisition was 4000–400 cm$^{-1}$ at a resolution of 2 cm$^{-1}$. Four scans were acquired at room temperature.

**Statistical Analysis** The results are expressed as means ± standard deviation (S.D.) ($n = 3$) with error bars. The release rate data were evaluated for statistical significance using the Tukey test for differences between groups while the significance was determined in repeated measures of two-way ANOVA. The statistical significance for the first-order rate constant was evaluated using $t$-test, A $p$-value of $<0.05$ was considered to be statistically significant.

**Results and Discussion**

The *in Vitro* Characterization of ITCZ- or MCZ-PLGA MS with Theoretical Drug Content 9.1 or 16.7% The characterization (encapsulation efficiency, particle size, etc.) of obtained ITCZ- or MCZ-PLGA MS prepared at different evaporation temperatures (25 or 40°C) is shown in Table 1. Experimental drug content and encapsulation efficiency were comparatively good, ranging between 80 and nearly 100% in every formulation. No difference was found between PLGA MS loaded with ITCZ or MCZ prepared at evaporation temperatures of 25 and 40°C with respect to experimental drug content or encapsulation efficiency. For both drugs, experimental drug content and encapsulation efficiencies for theoretical drug content 16.7%-loaded PLGA MS were over 90% and much higher than those for 9.1%-loaded PLGA MS. This was considered to be due to the low aqueous solubility of the two drugs, reported to be $<0.1$ and $<1$ mg/L, for ITCZ and MCZ, respectively, in a previous article. Thus, the aqueous solubilities of ITCZ and MCZ belong to U.S. Pharmacopoeia categories practically insoluble and insoluble, respectively. In general, drugs with aqueous water solubility are difficult to incorporate in PLGA MS by the o/w emulsion solvent evaporation method. However, drugs with solubilities in these ranges can be successfully encapsulated into PLGA MS with an average diameter of around 20 µm.

The release profiles for the ITCZ- and MCZ-PLGA MS prepared at different evaporation temperatures (25 and 40°C) are shown in Fig. 1. In Fig. 1A (theoretical drug loading 9.1%), ITCZ-PLGA MS prepared at 40°C showed a restricted release profile compared to those prepared at 25°C. In Fig. 1B (theoretical drug loading 16.7%), ITCZ-PLGA MS prepared at 40°C showed a significantly restricted release profile compared to those prepared at 25°C. For MCZ-PLGA MS, immediate release of MCZ was observed under both preparation
conditions (Figs. 1C, 1D).

Release kinetic parameters were calculated, based on the first-order model, for the eight formulations and the results are summarized in Table 3. On analysis based on the first-order model, drug release from PLGA MS seemed to belong to first-order release, as judged by the regression coefficient of correlation for the release model.

**Morphology by SEM of ITCZ- and MCZ-PLGA MS**

SEM photographs of ITCZ- and MCZ-PLGA MS with a theoretical drug content 16.7% prepared at an evaporation temperature of 25 or 40°C are shown in Fig. 2. No difference in the smoothness of the surfaces of ITCZ or MCZ-PLGA MS were observed in formulations prepared at 25 or 40°C. However, small particles attached to the spheres were observed in ITCZ-PLGA MS evaporated at 40°C while small surface pores were visible in MCZ-PLGA MS under both temperature conditions.

In our previous article, ONO-1301-loaded PLGA MS were prepared under different evaporation temperatures, 25 or 40°C; restriction of the initial burst release of ONO-1301 from the PLGA MS prepared at 40°C was superior to that from those prepared at 25°C.11 We previously explained this difference in release rate as follows: annealing of ONO-1301-loaded PLGA MS at 40°C, which is near the $T_g$ of the PLGA MS, leads to a self-healing effect of the PLGA MS. We suggest that a self-healing effect also occurred in the ITCZ-PLGA MS in the present study. However, in our o/w emulsion solvent evaporation method, MS are formed by evaporation of the dichloromethane from the dispersed oil drops (solution of drug, PLGA and dichloromethane) in the water phase. The dichloromethane evaporation rate changes depending on temperature and this may affect the morphology of the MS. Therefore, we believe that the pores of MCZ-PLGA MS appear during the solvent evaporation process and MCZ is distributed on the surface of the MS at high concentrations as a phase-separation phenomenon.

**Dependence of Self-Healing Effect of ITCZ-PLGA MS on Preparation Temperature**

In order to clarify the effect of the evaporation rate of dichloromethane on the surface morphology of ITCZ-PLGA MS, two additional formulations were prepared: formulation No. 9 was prepared by evaporation at 25°C for 12 h, and formulation No. 10 by evaporation at 25°C for 6 h followed by 40°C for 6 h. Characterization of the two formulations is given in Table 2. The surface morphology of the MS examined by SEM and the ITCZ release profiles of the two formulations are shown in Figs. 3 and 4, respectively. By contrast, in the case of MCZ-PLGA MS, pores were observed on MS surfaces under both temperature conditions, 25 and 40°C (formulations Nos. 6 and 8, shown in Fig. 2). An initial burst release of MCZ was observed for both these formulations (shown in Fig. 1D). This phenomenon was not observed for ITCZ-PLGA MS or ONO-1301-loaded PLGA MS,11 suggesting that the self-healing effect of PLGA annealing at 40°C did not occur in MCZ-PLGA MS. It has been reported that phase separation of drug within the polymer matrix and at the surface of MS can occur during preparation using the solvent evaporation method, and that in vitro release from drug-loaded MS is almost completely dependent on the surface phase separation of drug-rich domains.16 Therefore, we believe that the pores of MCZ-PLGA MS appear during the solvent evaporation process and MCZ is distributed on the surface of the MS at high concentrations as a phase-separation phenomenon.

### Table 3. In Vitro Release Kinetic Parameters of ITCZ- and MCZ-PLGA MS

| Microsphere formulation No. | First-order |
|----------------------------|------------|
|                            | $R^2$     | $K_1$ (h$^{-1}$) |
| 1                          | 0.99      | 0.27           |
| 2                          | 0.98      | 0.32           |
| 3                          | 1.00      | 0.18           |
| 4                          | 1.00      | 0.08***        |
| 5                          | 0.88      | 0.27           |
| 6                          | 0.98      | 0.31           |
| 7                          | 0.83      | 0.22           |
| 8                          | 0.83      | 0.25           |

*$R^2$ is the regression coefficient; $K_1$ is the release rate constants for first-order.

***$p<0.001$ versus 25°C ($t$-test).
formulation No. 9 (prepared at 25°C for 12h), as shown in Fig. 4. This suggests that the differences in both surface morphology and ITCZ release profile between the ITCZ-PLGA MS are not caused by different evaporation rates of dichloromethane, but by the self-healing effect of PLGA at 40°C. In the case of heat-sensitive drugs, it is possible to prepare the microspheres which have restriction both decomposition and initial burst release of drug by performing solvent evaporation process at 25°C and then annealing at 40°C.

Characterization of Amorphized Drug and Measurement of ITCZ- and MCZ-PLGA MS by DSC The drug in drug–polymer mixtures exists in a crystalline or amorphous form in the polymer. Several studies have reported amorphization of ITCZ in various polymers, such as cellulose acetate phthalate, polyvinyl acetate phthalate, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate succinate and polyvinylpyrrolidone.5-7) However, to our knowledge, no articles have yet been published on amorphization of ITCZ or MCZ in PLGA. Therefore, the existence of amorphized drug in ITCZ- and MCZ-PLGA MS was examined using DSC.

The DSC curves of PLGA, ITCZ, MCZ and ITCZ- or MCZ-PLGA MS are shown in Fig. 5. The MCZ-PLGA MS had thermal behavior indicating a glass transition at 37°C, attributed to PLGA, and complete amorphization of MCZ was observed. On the other hand, ITCZ-PLGA MS had thermal behavior indicative of glass transition at 45°C, corresponding to PLGA, and a small endothermic peak at ca. 150°C which was considered to be attributable to ITCZ crystals. Therefore, it was considered that the complete amorphization of MCZ in the PLGA MS may also be one cause of the initial burst release of MCZ-PLGA MS.

The \( T_g \) of ITCZ- and MCZ-PLGA MS prepared under various conditions was examined by DSC. The \( T_g \) of ITCZ- and MCZ-PLGA MS and the relationship between \( T_g \) and drug loading content is summarized in Fig. 6. The ITCZ-PLGA MS showed a tendency for increased \( T_g \) with increasing drug loading in the PLGA polymer matrix, whereas the MCZ-PLGA MS did not show a big change of \( T_g \). Generally, it has been reported that when low molecular weight compounds are added to the polymer matrix, the added compounds act as

![Fig. 3. SEM Images of ITCZ-PLGA MS (Formulation Nos. 9 and 10)](image)

![Fig. 4. In vitro Drug-Release Profile of ITCZ-PLGA MS (Formulation Nos. 9 and 10)](image)

![Fig. 5. DSC Curves of (A) PLGA, MCZ and ITCZ, and (B) MCZ-PLGA MS (Formulation No. 8) and ITCZ-PLGA MS (Formulation No. 4)](image)
observed at 1510 cm$^{-1}$ in the previous report. Therefore, the interaction between chemical and the carboxyl group of dicarboxylic acids, as described in a previous report, occurs between the triazole group of ITCZ and the carboxyl group of dicarboxylic acids, as described in a previous report. Therefore, the interaction between chemical bonds of ITCZ and PLGA in ITCZ-PLGA MS was examined by FT-IR.

**FT-IR Spectra of ITCZ, PLGA and ITCZ-PLGA MS**

FT-IR spectra of ITCZ, PLGA and ITCZ-PLGA MS in the region 1540–1480 cm$^{-1}$ are shown in Fig. 7. FT-IR spectrum of ITCZ showed a characteristic peak at 1510 cm$^{-1}$ corresponding to aromatic C=C stretch vibration. The peak of ITCZ observed at 1510 cm$^{-1}$ was shifted to 1512 cm$^{-1}$ in ITCZ-PLGA MS. This peak shift may be ascribed to interaction with the moieties of the PLGA polymer chain. Other peaks of ITCZ-PLGA MS seemed to be the sum of the peaks of ITCZ and PLGA, and a definite peak shift were not observed. In contrast, a peak shift corresponding to the MCZ peak was observed (FT-IR data not shown). Based on these results, it was suggested that the glass transition temperature of ITCZ-PLGA MS increased due to an interaction between PLGA and ITCZ in the ITCZ-PLGA polymer matrix.

**Conclusion**

In ITCZ-PLGA MS prepared at 40°C, the initial burst release of ITCZ was significantly restricted compared to ITCZ-PLGA MS prepared at 25°C. This is thought to be due to a self-healing effect of PLGA at a temperature (40°C) near to the $T_g$ of PLGA. In MCZ-PLGA MS, an initial burst release of MCZ was observed at all preparation temperatures. It is suggested that MCZ is distributed on the surface of MS at high concentrations and complete amorphization of MCZ occurs in the MCZ-PLGA MS. The present results suggest that the initial drug release rate from PLGA MS prepared using the o/w emulsion solvent evaporation method may be affected by physicochemical interactions between the drugs and PLGA and by the preparation temperature.

**Conflict of Interest**

The authors declare no conflict of interest.

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