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# Enhancement of the dissolution rate and gastrointestinal absorption of pranlukast as a model poorly water-soluble drug by grinding with gelatin

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**Abstract**

The effect of grinding with gelatin on the dissolution behavior and gastrointestinal absorption of a poorly water-soluble drug was evaluated using the antiasthmatic agent, pranlukast, as a model poorly water-soluble drug. A ground pranlukast–gelatin mixture was prepared by grinding equal quantities of pranlukast and gelatin. In the dissolution testing, the dissolution rate of pranlukast in the suspension of the ground pranlukast–gelatin mixture under conditions of pH 3.0, 5.0 and 7.0 was markedly faster than that in the suspension of pranlukast. According to powder X-ray diffractometry (PXRD) and differential scanning calorimetry (DSC) analysis, the enhanced dissolution rate of pranlukast produced by grinding with gelatin was caused by changing the crystalline state of pranlukast into an amorphous state. In an animal experiment, the bioavailability of pranlukast following oral administration of the ground pranlukast–gelatin mixture to rats was threefold greater than that following administration of pranlukast. In the *in vitro* permeation experiment, the amount of permeated pranlukast through Caco-2 cell monolayers after application of the ground pranlukast–gelatin mixture was greater than that after application of pranlukast. These results suggest that the enhancement of the gastrointestinal absorption of pranlukast by grinding with gelatin is due to enhancement of the dissolution rate. Grinding a poorly water-soluble drug with gelatin is a useful method of enhancing its gastrointestinal absorption.
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*Keywords:* Gelatin; Poorly water-soluble drug; Pranlukast; Grinding; Dissolution rate; Gastrointestinal absorption

## 1. Introduction

Many drugs synthesized by procedure such as combinatorial chemistry have poor water solubility and unsatisfactory dissolution properties and this can give rise to low and erratic bioavailability and poor dose proportionality (Daumesnil, 2005; Lipinski, 2003). An improvement in the dissolution rate of poorly water-soluble drugs would be useful for enhancement of their gastrointestinal absorption following oral administration when the dissolution process is a rate-determining step in their gastrointestinal absorption (Horter and Dressman, 2001). Consequently, improvement in the dissolution rate of poorly water-soluble drugs is required for the development of oral formulations. Grinding with polymers is known to be one pharmaceutical approach to improve the dissolution rate of drugs (Yamada et al., 1999; Mura et al., 2001; Saito et al., 2002; Vippagunta et al., 2002) and may be useful for enhancement of the gastrointestinal absorption of poorly water-soluble drugs.

Gelatin, a water-soluble and biodegradable protein derived from collagen, has many applications in the food and pharmaceutical industries. In addition to conventional drug formulations, recently gelatin and gelatin derivatives have been investigated as novel systems designed for the controlled release of drugs (Cortesi et al., 1999; Fukunaka et al., 2002; Kantaria et al., 1999; Morimoto et al., 2000, 2001, 2005) and as a mucosal absorption enhancer of peptide and protein drugs (Seki et al., 2005, 2006; Wang et al., 2002). Gelatin improves the dissolution characteristics and gastrointestinal absorption of drugs (Imai et al., 1989, 1990; Kallinteri and Antimisiaris, 2001; Kimura et al., 1990). Thus, gelatin could be used as a pharmaceutical additive to improve the gastrointestinal absorption of drugs. Kallinteri and Antimisiaris (2001) have shown that the solubility of drugs in buffer containing gelatin is higher than that in buffer without gelatin. However, improvement of solubility in a solution system is not practical in the case of pharmaceutical preparations. Imai et al. (1989, 1990) and Kimura et al. (1990) have shown that the dissolution rate and gastroin-

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testinal absorption of poorly water-soluble drugs, acidic, basic and neutral drugs are enhanced in solid formulations containing gelatin, such as splay-dried powder, granules and tablets. Unfortunately, the preparations of these formulations are not easy to prepare while, in contrast, the grinding method is both simple and fast. Improvement of the dissolution rate of drugs is possible by reducing their crystallinity (Rasenack et al., 2003; Surana et al., 2004). Grinding of drugs with polymers is used as a simple method of reducing crystallinity (Yamada et al., 1999; Mura et al., 2002; Oguchi et al., 2003; Wongmekiat et al., 2003). Grinding with gelatin offers a useful pharmaceutical approach to improve the dissolution rate and gastrointestinal absorption of poorly water-soluble drugs, particularly in view of its rapidity and the well-known safety of gelatin as a pharmaceutical additive.

In the present study, an antiasthmatic agent, pranlukast, was chosen as a model poorly water-soluble drug, and a ground pranlukast–gelatin mixture was prepared by grinding equivalent amounts of pranlukast and gelatin, and then effects of grinding with gelatin on the dissolution behavior and gastrointestinal absorption following oral administration to rats were examined to examine whether grinding with gelatin could improve the gastrointestinal absorption of poorly water-soluble drugs. In addition, the solid physical properties of the ground pranlukast–gelatin mixture and the permeability of pranlukast through a Caco-2 cell monolayer were examined to study the improved mechanisms of solubility and gastrointestinal absorption.

## 2. Materials and methods

### 2.1. Materials

Gelatin (isoelectric point 5.0, $M_W$ 10 kDa) was purchased from Nitta Gelatin Co. Ltd. (Osaka, Japan). Pranlukast, shown in Fig. 1, was supplied from Ono Pharmaceutical Co. Ltd. (Osaka, Japan). All other regents were commercially available and of analytical grade.

### 2.2. Preparation of the ground pranlukast–gelatin mixture

The ground pranlukast–gelatin mixture was prepared as follows. Pranlukast (5.0 g) and gelatin (5.0 g) were mixed and ground at 18,000 rpm for 30 min using an electric grinding mixer (Nihonseiki Kaisha Ltd., Tokyo, Japan). Ground pranlukast was prepared by grinding pranlukast without additives by the same method.

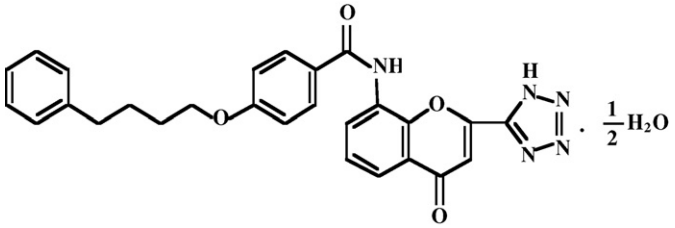


Fig. 1. The chemical structure of pranlukast.

Pranlukast in the ground mixture was extracted with $CH_3CN$/DMSO (3/1, v/v), and then centrifuged (8000 × *g* for 5 min) to determine the content of pranlukast in the ground mixture. The concentration of pranlukast in the centrifuged supernatant was determined by HPLC analysis (Ishido et al., 1993). The content of pranlukast in pranlukast–gelatin ground mixture was expressed as the content of pranlukast per gram mixture. The HPLC system (Shimadzu Co., Kyoto, Japan) consisted of a pump (LC-10AD), detector (SPD-10A), and integrator (CR-6A). An analytical column (LiChrosorb RP-18, 7 μm, 4.0 mm × 250 mm, Kanto Chemical Co. Inc., Tokyo, Japan) and mobile phase (20 mM $KH_2PO_4$/$CH_3CN$/$CH_3OH$ = 5/5/1, v/v/v) were used for the separation. The flow rate was 1.0 mL/min and the detector was operated at 260 nm.

The appearance of powders was visually monitored. The repose angle was obtained as follows (Parrott, 1981). The pile at a height of 2 cm was carefully built up by dropping the powders through a funnel until the tip of the funnel was reached and the radius at the bottom was then measured. The repose angle was calculated by the following equation:

$$\theta = \tan^{-1}\frac{h}{r} \quad (1)$$

where $\theta$, $h$ and $r$ are the repose angle, height (2 cm) and radius, respectively.

### 2.3. In vitro dissolution testing

Pranlukast (4 mg), ground pranlukast (4 mg) or ground pranlukast–gelatin mixture (4 mg as pranlukast) was dispersed in 25 mM phosphate buffer (8 mL; pH 3.0, 5.0 or 7.0) and then the suspension was incubated at 37 °C. At the indicated timepoints after incubation, a 0.5 mL aliquot of suspension was collected and immediately centrifuged (8000 × *g* for 5 min). The concentration of pranlukast in the centrifuged supernatant was determined by HPLC analysis as described above.

### 2.4. Powder X-ray diffractometry (PXRD) and differential scanning calorimetry (DSC)

Powder X-ray diffraction patterns were recorded using a powder diffractometer (M03XHF, Bruker AXS K.K., Tsukuba, Japan) with Cu Kα radiation, operated at a voltage of 40 kV and a current of 20 mA. The scanning speed was 2°/min with a scanning step 0.02° of 2$\theta$. The scanning range was from 5° to 40° of 2$\theta$. Calorimetric analysis was performed using a differential scanning calorimeter (DSC-50, Shimadzu, Kyoto, Japan). Samples were heated from 50 to 250 °C at a heating rate of 5 °C/min. A pranlukast–gelatin physical mixture, used as a comparison, was prepared by mixing equal amounts of pranlukast and gelatin using a soft spatula.

### 2.5. In vivo animal experiments

The animal experimental plan used was approved by the Committee of the Laboratory Animal Center, and conforms to

the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido Pharmaceutical University. Male SD rats (250 g body weight, Japan SLC, Shizuoka, Japan) were fasted for 18–20 h prior to the experiment. Pranlukast or the ground pranlukast–gelatin mixture was used to fill capsules (PCcaps$^{TM}$, Capsugel Japan Inc., Sagamihara, Japan) for oral administration to rats. Capsules were orally administered to rats using a specialized delivery device (PCcaps$^{TM}$-kit, Capsugel Japan Inc.) at a dose of 40 mg pranlukast/kg. At the indicated time-points after administration, blood (0.5 mL) was collected from the jugular vein under pentobarbital anesthesia. The plasma samples (200 μL) and ethanol (2 mL) were mixed, and centrifuged (2000 × *g* for 5 min). The centrifuged supernatant was evaporated and then the residue was dissolved in the mobile phase described above. The concentration of pranlukast in plasma was determined by HPLC analysis as described above. The area under the pranlukast plasma concentration–time curve from 0 to 8 h (AUC) was calculated by the trapezoidal rule and the bioavailability was calculated using the AUC following the intravenous administration of pranlukast.

### 2.6. In vitro permeation experiments

Caco-2 cells, used as model gastrointestinal epithelial cells, were obtained from the Riken Gene Bank (Tsukuba, Japan). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum, 40 μg/mL gentamicin and 1% non-essential amino acids, in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C. Cells from passage number 55–62 were seeded ($4.5 \times 10^5$ cell/cm$^2$) on polycarbonate filter inserts (pore size 0.4 μm, area 1.00 cm$^2$, Trans well, Coning, NY, USA) and cultivated in the medium for 23–28 days before starting the permeation experiments. The quality of the monolayers grown on the permeable membrane was assessed by measuring the transepithelial electrical resistances (TEER) using Millicell-ERS (Millipore, MA, USA). The TEER values were higher than 450 Ω cm$^2$.

Pranlukast (10 mg) or ground pranlukast–gelatin mixture (10 mg as pranlukast) was dispersed in 1 mL PBS containing 0.5% CMC-Na (pH 7.4) and the suspension (300 μL) was immediately applied to the apical side of Caco-2 cell monolayers. Hanks balanced salt solution (HBSS, pH 7.4, 600 μL) was used as the basolateral side solution. The HBSS was changed every 30 min for 2 h. The TEER was measured as described above before and after the permeation experiments. The concentration of pranlukast in the basolateral side solution was determined by HPLC analysis as described above. The data were expressed as the permeated amount of pranlukast. The apparent permeation clearance was calculated with dividing the permeation rate by saturated solubility in applied suspension to Caco-2 cell monolayers. The permeation rate was obtained from Fig. 6. The *in vitro* dissolution testing was preliminarily performed as described above and the concentration of pranlukast at 24 h was used as saturated solubility in applied suspension.

### 2.7. Storage stability testing

Ground pranlukast–gelatin mixture was stored at room temperature without protection from light and 20% relative humidity for 6 months. At indicated time points after starting storage, the content of pranlukast, its appearance and repose angle were recorded as described above. Powder X-ray diffraction patterns and DSC curves were also obtained as described above.

### 2.8. Statistics

Statistical analysis was performed by the Student's *t*-test and the Mann–Whitney *U*-test using Stat View software (Abacus Concepts Inc., Berkeley, CA, USA).

## 3. Results

### 3.1. Characteristics of ground pranlukast–gelatin mixture

The characteristics of the ground pranlukast–gelatin mixture are summarized in Table 1. The content of pranlukast did not decrease during the preparation process. Pranlukast and gelatin were quantitatively ground, and the content of pranlukast in the ground mixture was 0.495 g/g mixture. No decrease in the molecular weight of gelatin was produced by grinding according to electrophoresis (data not shown). No changes in appearance and repose angle by grinding were observed because the appearance and repose angle of the ground mixture were similar to those of pranlukast.

### 3.2. Dissolution characteristics of pranlukast

The effect of pH on the dissolution behavior of pranlukast in the suspension of pranlukast is shown in Fig. 2. The dissolution rate and saturated solubility of pranlukast in the suspension of pranlukast increased with an increase in pH. A comparison of the dissolution behavior of pranlukast in the suspension of pranlukast, ground pranlukast and the ground pranlukast–gelatin mixture is shown in Fig. 3. At each pH, the initial dissolution rate of pranlukast in the suspension of the ground pranlukast–gelatin mixture was markedly faster than that in the suspension of

Table 1
Characteristics of pranlukast and ground pranlukast–gelatin mixture

| | Content of pranlukast (g/g mixture) | Appearance | Repose angle (°) |
|---|---|---|---|
| Pranlukast ground | – | Pale yellow powder | 38.2 ± 0.7 |
| Ground pranlukast | – | Pale yellow powder | 34.2 ± 0.8 |
| Ground pranlukast–gelatin mixture | 0.495 ± 0.001 | Pale yellow powder | 39.0 ± 0.6 |

Each value represents the mean ± S.E. ($n = 4$).

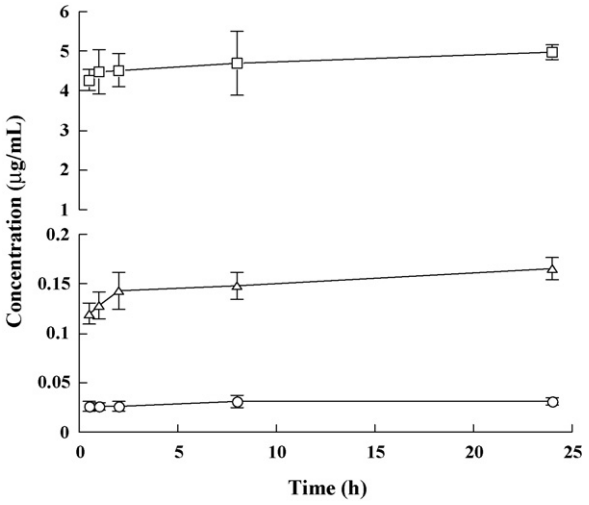


Fig. 2. The dissolution profiles of pranlukast in the suspension of pranlukast. Pranlukast was dispersed in phosphate buffer ((◯) pH 3.0; (△) pH 5.0; (□) pH 7.0) and then the suspension was incubated at 37 °C. At the indicated time-points after incubation, the concentration of pranlukast in suspension was determined. Each value represents the mean ± S.E. ($n$ = 3–5).

pranlukast. However, the concentrations of pranlukast in the suspension of ground pranlukast–gelatin mixture fell with an increase in the incubation time. The initial dissolution rate of pranlukast in the suspension of ground pranlukast tended to be slightly faster than that in the suspension of pranlukast.

### 3.3. PXRD and DSC analysis

The PXRD patterns are shown in Fig. 4A. The X-ray spectrum of pranlukast exhibited numerous sharp reflections indicative of its high degree of crystallinity. The X-ray spectrum of ground pranlukast was the same as that of pranlukast. The intensities of the PXRD peaks of the ground pranlukast–gelatin mixture were lower than those of pranlukast although there was no fall in those of the pranlukast–gelatin physical mixture. The DSC curves are shown in Fig. 4B. An endothermic peak due to melting point appeared at about 233 °C in the DSC curve of pranlukast. The DSC curve of ground pranlukast was same as that of pranlukast. The endothermic peak of the ground pranlukast–gelatin mixture at about 233 °C disappeared although that of the pranlukast–gelatin physical mixture did not. PXRD and DSC analysis indicate that grinding with gelatin caused a change in the crystalline state of pranlukast to an amorphous one.

### 3.4. Gastrointestinal absorption of pranlukast in rats

The time-courses of the concentrations of pranlukast in plasma after oral administration of pranlukast and pranlukast–gelatin mixture in rats are shown in Fig. 5. The initial absorption rate of pranlukast after administration of pranlukast–gelatin mixture was faster than that after administration of pranlukast. The concentration of pranlukast in plasma after administration of pranlukast–gelatin mixture was higher than that after administration of pranlukast at each time point. The pharma-

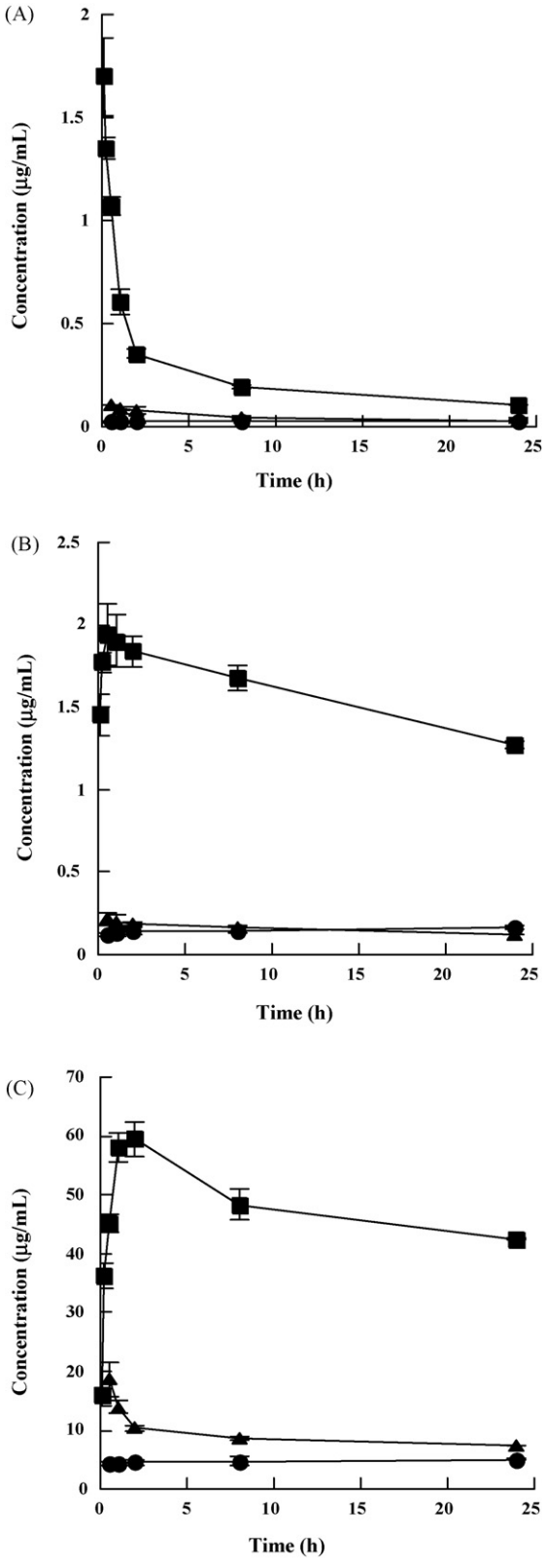


Fig. 3. The dissolution profiles of pranlukast in the suspension of pranlukast, ground pranlukast, ground pranlukast–gelatin mixture. Each powder sample was dispersed in phosphate buffer ((A) pH 3.0; (B) pH 5.0; (C) pH 7.0) and then the suspension was incubated at 37 °C. At the indicated time-points after incubation, the concentration of pranlukast in suspension was determined. (●) Pranlukast; (▲) ground pranlukast; (■) ground pranlukast–gelatin mixture. Each value represents the mean ± S.E. ($n$ = 3–5).

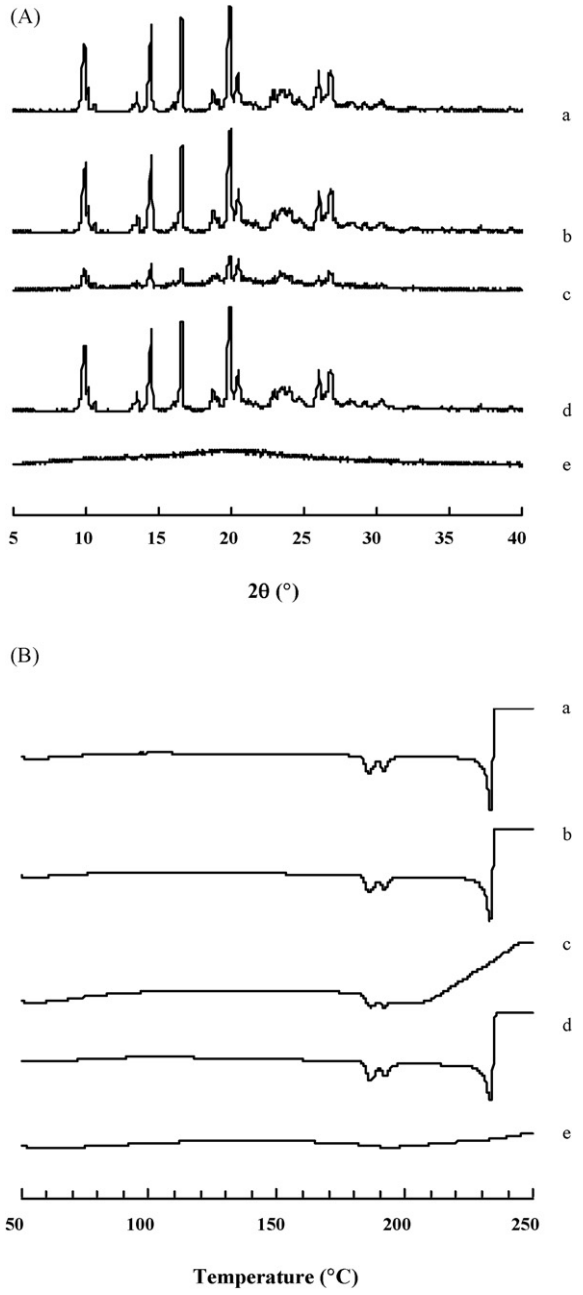


Fig. 4. The PXRD patterns (A) and DSC curves (B). (a) Pranlukast; (b) ground pranlukast; (c) ground pranlukast–gelatin mixture; (d) pranlukast–gelatin physical mixture; (e) gelatin.

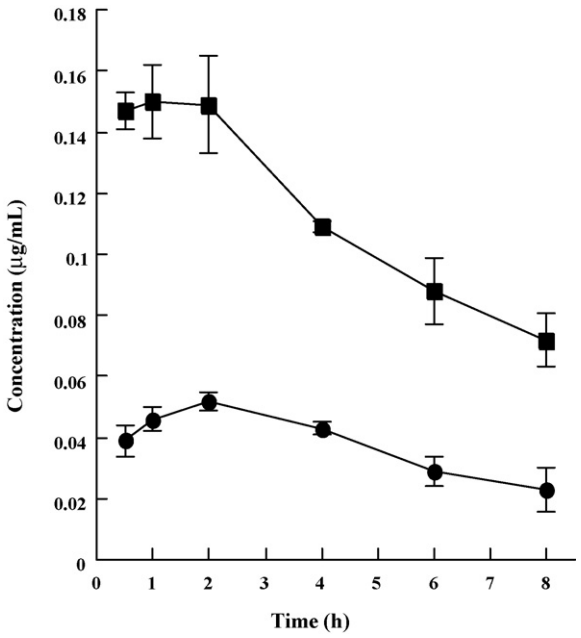


Fig. 5. The time-courses of concentrations of pranlukast in plasma after oral administration of pranlukast and ground pranlukast–gelatin mixture in rats. Capsules containing pranlukast or ground pranlukast–gelatin mixture were orally administered to rat using a delivery device. At the indicated time-points after administration, plasma was collected and the concentration of pranlukast in plasma was determined. (●) Pranlukast; (■) ground pranlukast–gelatin mixture. Each value represents the mean ± S.E. ($n = 4$).

cokinetic parameters of pranlukast following oral administration of pranlukast and ground pranlukast–gelatin mixture in rats are summarized in Table 2. The bioavailability of pranlukast following administration of pranlukast–gelatin mixture was threefold greater than that following administration of pranlukast.

### 3.5. Permeation of pranlukast through Caco-2 cell monolayers

The permeated amounts of pranlukast through Caco-2 cell monolayers after application of pranlukast and ground pranlukast–gelatin mixture are shown in Fig. 6. The permeated amount of pranlukast after application of ground pranlukast–gelatin mixture was greater than that after application of pranlukast at each time point. The apparent permeation clearance (4.1 μL/h) following application of ground pranlukast–gelatin mixture was similar to that (4.6 μL/h)

Table 2
Pharmacokinetic parameters of pranlukast following oral administration of pranlukast and ground pranlukast–gelatin mixture in rats

| | $C_{max}$ (μg/mL)[a] | $T_{max}$ (h)[b] | AUC (μg h/mL)[c] | BA (%)[d] |
|---|---|---|---|---|
| Pranlukast | 0.052 ± 0.003 | 2 | 0.299 ± 0.021 | 4.3 |
| Ground pranlukast–gelatin mixture | 0.150 ± 0.012* | 1 | 0.891 ± 0.055* | 12.9 |

Each pharmacokinetic parameters were obtained from data shown in Fig. 3. Each value represents the mean ± S.E. ($n = 4$).
[a] The maximum concentration of pranlukast in plasma.
[b] The time to reach $C_{max}$ after administration.
[c] AUC from time 0–8 h.
[d] The bioavailability calculated using AUC following intravenous administration.
* $p < 0.01$, significantly different from pranlukast in the Student's $t$-test.

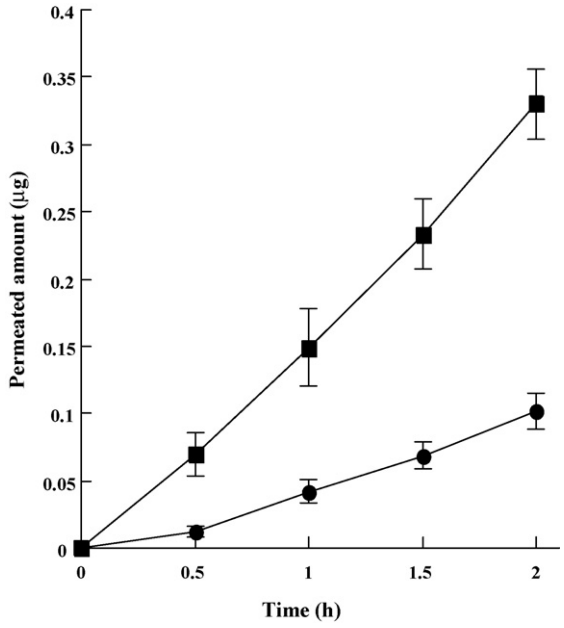


Fig. 6. The permeated amount of pranlukast through Caco-2 cell monolayers after application of pranlukast or ground pranlukast–gelatin mixture. A suspension of pranlukast or ground pranlukast–gelatin mixture was immediately applied to the apical side of Caco-2 cell monolayers and then incubated at 37 °C. At the indicated time-points after incubation, the concentration of pranlukast in the basolateral side solution was determined. (●) Pranlukast; (■) ground pranlukast–gelatin mixture. Each value represents the mean ± S.E. ($n = 4$–5). A significant difference was shown by the Mann–Whitney $U$-test ($p < 0.05$) for each time point.

following application of pranlukast. The TEER values of Caco-2 cell monolayers before and after permeation experiments are summarized in Table 3. The reduction of TEER following application of ground pranlukast–gelatin mixture was similar to that following application of pranlukast.

### 3.6. Storage stability of pranlukast–gelatin mixture

The storage stability of ground pranlukast–gelatin mixture at room temperature without protection from light and 20% relative humidity is shown in Fig. 7. Ground pranlukast–gelatin mixture was stable over a 6-month period. No changes were seen in the content of pranlukast in ground mixture and its repose angle. Also, the appearance of the ground pranlukast–gelatin mixture did not change from a pale yellow powder. The PXRD pattern and DSC curve of ground pranlukast–gelatin mixture at 180 days after preparation were the same as those immediately after preparation as shown in Fig. 4 (data not shown).

Table 3
TEER values of Caco-2 cell monolayers before and after permeation experiments

| | Before experiment ($\Omega\,cm^2$) | After experiment ($\Omega\,cm^2$) | Reduction (%) |
|---|---|---|---|
| Pranlukast | 524 ± 34 | 419 ± 10 | 19 ± 5 |
| Ground pranlukast–gelatin mixture | 549 ± 53 | 393 ± 18 | 26 ± 9 |

Each value represents the mean ± S.E. ($n = 4$–5).

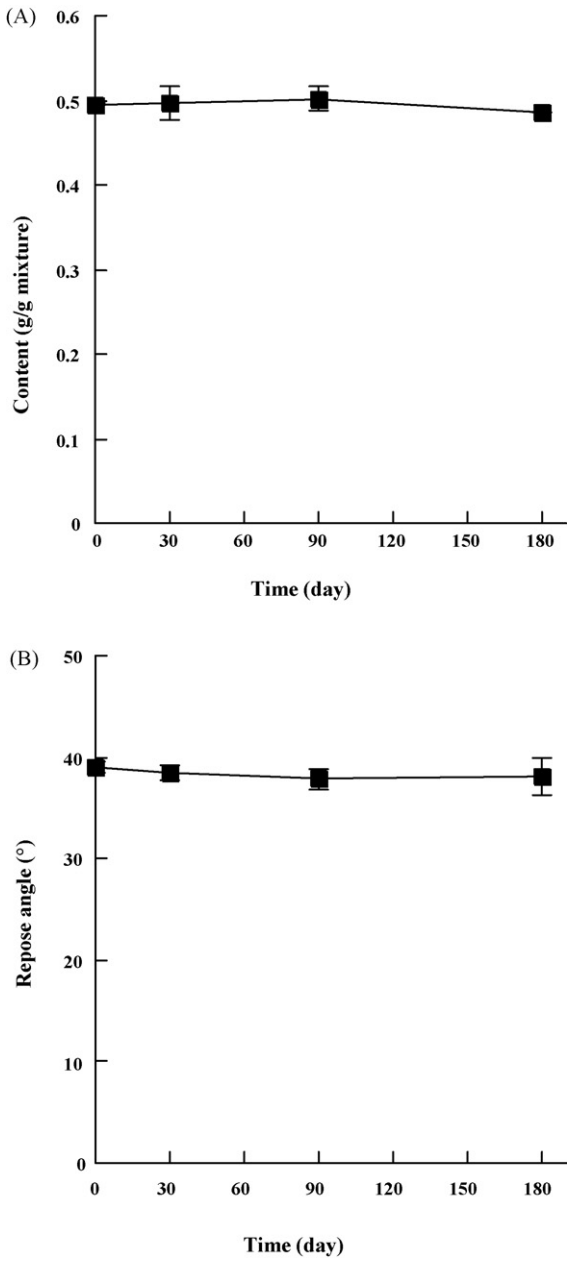


Fig. 7. The storage stability of ground pranlukast–gelatin mixture. Ground pranlukast–gelatin mixture was stored at room temperature without protection from light and a relative humidity of 20%. At the indicated time-points after starting storage, the content of pranlukast and the repose angle were determined. (A) The changes in the content of pranlukast in the ground pranlukast–gelatin mixture. (B) The changes in the repose angle of the ground pranlukast–gelatin mixture. Each value represents the mean ± S.E. ($n = 4$).

## 4. Discussion

Many drugs developed in recent years exhibit poor water solubility. Improvement of the dissolution rate of poorly water-soluble drugs is a useful approach for enhancement of their gastrointestinal absorption following oral administration. In the present study, a ground mixture of pranlukast, as a model poorly water-soluble drug, and gelatin was prepared and effect of grinding with gelatin on the dissolution behavior and gastrointestinal absorption of pranlukast was evaluated to provide

information to help with the development of novel oral formulations.

The *in vitro* dissolution behavior of pranlukast in phosphate buffer at each pH was examined. The dissolution rate and saturated solubility of pranlukast in the suspension of pranlukast increased with an increase in pH (Fig. 2). These findings suggest that pranlukast is an acidic drug and solubility of the ionic form increases with the release of $H^+$ from the tetrazole heterocycle on increasing the pH. According to a previous report about the release of $H^+$ from tetrazole heterocycles (Welz and Muller, 2002), the acid dissociation constant (p*K*a) of pranlukast is estimated to be approximately 5. The initial dissolution rate of pranlukast in the suspension of ground pranlukast–gelatin mixture was markedly faster than that in the suspension of pranlukast (Fig. 3). Because pranlukast exhibits nonlinearity, its absorption ratio decreases at high doses and its absorption ratio after a meal is higher than when fasting, it would appear that the site of absorption is in the upper part of the gastrointestinal tract (Ishido et al., 1993). Thus, the improvement in the initial dissolution rate at a low pH, such as pH 3.0 and 5.0, in the present study is especially significant for improvement of the gastrointestinal absorption of pranlukast. The concentration of pranlukast in the suspension of ground pranlukast–gelatin mixture was decreased with an increase in the incubation time (Fig. 3). After the *in vitro* dissolution testing for 24 h finished, a solid pranlukast–gelatin mixture dispersed in buffer was collected and analyzed to confirm that pranlukast crystallizes in buffer. The DSC curve of this solid corresponded to that of pranlukast shown in Fig. 4B (data not shown). This finding indicates that pranlukast changes into a crystalline form from an amorphous substance in buffer. Thus, a pharmaceutical technique to prevent the amorphous substance turning into the crystalline form in water will be required for the future design of pharmaceutical preparations. The PXRD patterns of the pranlukast–gelatin physical mixture were similar to those of pranlukast (Fig. 4A). This finding indicates that grinding of the drug with gelatin is important as well as the presence of gelatin to reduce the crystallinity of the drug. Gelatin shows a dissolution-supporting effect for several drugs in solution (Kallinteri and Antimisiaris, 2001), however, the solubility of pranlukast in buffer containing gelatin at 37 °C (31.8 ng/mL at pH 3.0; 3.3 μg/mL at pH7.0) was not higher than that in buffer without gelatin (32.3 ng/mL at pH 3.0; 3.5 μg/mL at pH7.0) in our preliminary experiments. A dissolution-supporting effect of gelatin for pranlukast may be poor although it is influenced by experimental conditions. To improve the dissolution rate of pranlukast by gelatin, grinding will play an important role. On the other hand, the initial dissolution rate of pranlukast in the suspension of ground pranlukast tended to be slightly faster than that in the suspension of pranlukast (Fig. 3). The temporary increase in the initial dissolution rate of pranlukast in the suspension of ground pranlukast may be due to alteration of surface structure by grinding. PXRD and DSC analysis were performed to check the crystallization status of pranlukast (Fig. 4). Although amorphous formation was not caused in grinding pranlukast alone, this indicates that the amount of pranlukast forming an amorphous substance increases in the presence of gelatin. The pranlukast-forming amorphous substance may be stabilized by contact with the gelatin surface. Although the crystallinity of pranlukast also decreased during grinding with hydroxypropylcellulose in our preliminary experiments, the degree of improvement in the dissolution rate was lower than that in the case of grinding with gelatin. These findings suggest that the some factors (e.g., dissolution property of polymer oneself) as well as the decrease of crystallinity of pranlukast may also involve in the dissolution behavior of pranlukast. Gelatin may have a greater ability to improve the solubility of pranlukast than hydroxypropylcellulose. However, interaction between pranlukast and gelatin is not clarified. Therefore, detailed investigation is required in the future.

According to the *in vitro* dissolution testing, because pranlukast returns to a crystalline form from an amorphous one in suspension and the concentration of dissolved pranlukast decreases during storage, a solid formulation not a suspension needs to be administered if efficacy as a pharmaceutical preparation is to be achieved. The gastrointestinal absorption of pranlukast following oral administration of capsules containing a pranlukast–gelatin mixture to rats was examined. The initial absorption rate of pranlukast after administration of pranlukast–gelatin mixture was faster than that after administration of pranlukast (Fig. 5). This result suggests that the improvement in the initial absorption of pranlukast by grinding with gelatin is due to an improvement in the initial dissolution rate in the gastrointestinal tract.

Gelatin and gelatin derivatives enhance mucosal absorption of peptide and protein drugs (Seki et al., 2005, 2006; Wang et al., 2002). To confirm that the improvement in the initial absorption of pranlukast following administration of ground pranlukast–gelatin mixture to rats is based on an improvement in the initial dissolution rate in the gastrointestinal tract and not to the enhancing effect of mucosal absorption, the permeability of pranlukast through Caco-2 cell monolayers following application of ground pranlukast–gelatin mixture was examined (Fig. 6 and Table 3). The apparent permeation clearance (4.1 μL/h) following application of ground pranlukast–gelatin mixture was not greater than that (4.6 μL/h) following application of pranlukast and the reduction in TEER following application of ground pranlukast–gelatin mixture was similar to that following application of pranlukast. These results suggest that the improvement in the gastrointestinal absorption of pranlukast following administration of ground pranlukast–gelatin mixture is based on an improvement in the initial dissolution rate in the gastrointestinal tract, not the enhancement of mucosal absorption depending on an alteration in the cell membrane and opening of intercellular spaces. The use of mucosal absorption enhancers is effective for improving the absorption of drugs, but this can frequently produce irritation and toxic effects on mucosal cells. On the other hand, the use of the ground mixture is effective and safe method for improvement of gastrointestinal absorption of drugs because its absorption-enhancing mechanism is improvement in solubility, not effects for mucosal cells.

It is desirable that pharmaceutical preparations remain stable during storage. The storage stability of ground pranlukast–gelatin mixture at room temperature when not protected from light and a relative humidity at 20% was examined (Fig. 7).

The results showed that ground pranlukast–gelatin mixture was stable for at least 6 months according to the measurement of the pranlukast content and the repose angle, observation of its appearance and analysis of its solid physical properties by PXRD and DSC. The present study indicates that ground pranlukast–gelatin mixture is an excellent pharmaceutical preparation having high storage stability and improving the gastrointestinal absorption of pranlukast.

## 5. Conclusion

In conclusion, the present study evaluated the effect of grinding with gelatin on the dissolution behavior and gastrointestinal absorption of pranlukast as a model poorly water-soluble drug. We have shown that grinding of pranlukast with gelatin improves its solubility and gastrointestinal absorption following oral administration. These findings suggest that grinding with gelatin is a useful pharmaceutical technique for improving the gastrointestinal absorption of poorly water-soluble drugs.

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## References

Cortesi, R., Esposito, E., Osti, M., Squarzoni, G., Menegatti, E., Davis, S.S., Nastruzzi, C., 1999. Dextran cross-linked gelatin microspheres as a drug delivery system. Eur. J. Pharm. Biopharm. 47, 153–160.

Daumesnil, R., 2005. How to fully exploit the market potential of poorly soluble actives? Case studies. In: Proceedings of the 27th Mini-Symposium & APSTJ Global Education Seminar East 3-05, September 2005, Tokyo, Japan.

Fukunaka, Y., Iwanaga, K., Morimoto, K., Kakemi, M., Tabata, Y., 2002. Controlled release of plasmid DNA from cationized gelatin hydrogels based on hydrogel degradation. J. Control. Release 80, 333–343.

Horter, D., Dressman, J.B., 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv. Drug Deliv. Rev. 46, 75–87.

Imai, T., Nishiyama, T., Ueno, M., Otagiri, M., 1989. Enhancement of the dissolution rates of poorly water-soluble drugs by water-soluble gelatin. Chem. Pharm. Bull. 37, 2251–2252.

Imai, T., Kimura, S., Iijima, T., Miyoshi, T., Ueno, M., Otagiri, M., 1990. Rapidly absorbed solid oral formulations of ibuprofen using water-soluble gelatin. J. Pharm. Pharmacol. 42, 615–619.

Ishido, M., Shibakawa, K., Nakao, Y., Sawada, M., Aishita, H., 1993. Studies on the metabolic fate of leikotriene antagonist ONO-1078 (1): absorption, distribution and excretion after single administration to rats. Xenobio. Metabol. Dispos. 8, 2–26.

Kallinteri, P., Antimisiaris, S.G., 2001. Solubility of drugs in the presence of gelatin: effect of drug lipophilicity and degree of ionization. Int. J. Pharm. 221, 219–226.

Kantaria, S., Rees, G.D., Lawrence, M.J., 1999. Gelatin-stabilised microemulsion-based organogels: rheology and application in iontophoretic transdermal drug delivery. J. Control. Release 60, 355–365.

Kimura, S., Nishiyama, T., Imai, T., Otagiri, M., 1990. Improved dissolution and absorption of drugs using low molecular gelatin. Acta Pharm. Nord. 2, 65–72.

Lipinski, C.R., 2003. Poor aqueous solubility-an industry-wide problem in ACME ascreening. In: Apsugel Symposium on Oral Delivery of Poorly Soluble Actives-From Drug Discovery to Marketed Products, June 2003, Tokyo, Japan.

Morimoto, K., Katsumata, H., Yabuta, T., Iwanaga, K., Kakemi, M., Tabata, Y., Ikada, Y., 2000. Gelatin microspheres as a pulmonary delivery system: evaluation of salmon calcitonin absorption. J. Pharm. Pharmacol. 52, 611–617.

Morimoto, K., Katsumata, H., Yabuta, T., Iwanaga, K., Kakemi, M., Tabata, Y., Ikada, Y., 2001. Evaluation of gelatin microspheres for nasal and intramuscular administrations of salmon calcitonin. Eur. J. Pharm. Sci. 13, 179–185.

Morimoto, K., Chono, S., Kosai, T., Seki, T., Tabata, Y., 2005. Design of novel injectable cationic microspheres based on aminated gelatin for prolonged insulin action. J. Pharm. Pharmacol. 57, 839–844.

Mura, P., Faucci, M.T., Parrini, P.L., 2001. Effects of grinding with microcrystalline cellulose and cyclodextrins on the ketoprofen physicochemical properties. Drug Dev. Ind. Pharm. 27, 119–128.

Mura, P., Cirri, M., Faucci, M.T., Gines-Dorado, J.M., Bettinetti, G.P., 2002. Investigation of the effects of grinding and co-grinding on physicochemical properties of glisentide. J. Pharm. Biomed. Anal. 30, 227–237.

Oguchi, T., Sasaki, N., Hara, T., Tozuka, Y., Yamamoto, K., 2003. Differentiated thermal crystallization from amorphous chenodeoxycholic acid between the ground specimens derived from the polymorphs. Int. J. Pharm. 253, 81–88.

Parrott, E.L., 1981. Thermal hardness coefficient of tablets. J. Pharm. Sci. 70, 328–329.

Rasenack, N., Hartenhauer, H., Muller, B.W., 2003. Microcrystals for dissolution rate enhancement of poorly water-soluble drugs. Int. J. Pharm. 254, 137–145.

Saito, M., Ugajin, T., Nozawa, Y., Sadzuka, Y., Miyagishima, A., Sonobe, T., 2002. Preparation and dissolution characteristics of griseofulvin solid dispersions with saccharides. Int. J. Pharm. 249, 71–79.

Seki, T., Kanbayashi, H., Nagao, T., Chono, S., Tomita, M., Hayashi, M., Tabata, Y., Morimoto, K., 2005. Effect of aminated gelatin on the nasal absorption of insulin in rats. Biol. Pharm. Bull. 28, 510–514.

Seki, T., Kanbayashi, H., Nagao, T., Chono, S., Tabata, Y., Morimoto, K., 2006. Effect of cationized gelatins on the paracellular transport of drugs through caco-2 cell monolayers. J. Pharm. Sci. 95, 1393–1401.

Surana, R., Pyne, A., Suryanarayanan, R., 2004. Effect of preparation method on physical properties of amorphous trehalose. Pharm. Res. 21, 1167–1176.

Vippagunta, S.R., Maul, K.A., Tallavajhala, S., Grant, D.J., 2002. Solid-state characterization of nifedipine solid dispersions. Int. J. Pharm. 236, 111–123.

Wang, J., Sakai, S., Deguchi, Y., Bi, D., Tabata, Y., Morimoto, K., 2002. Aminated gelatin as a nasal absorption enhancer for peptide drugs: evaluation of absorption enhancing effect and nasal mucosa perturbation in rats. J. Pharm. Pharmacol. 54, 181–188.

Welz, R., Muller, S., 2002. 5-(Benzylmercapto)-1*H*-tetrazole as activator for 2′-*O*-TBDMS phosphoramidite building blocks in RNA synthesis. Tetrahedron Lett. 43, 795–797.

Wongmekiat, A., Tozuka, Y., Oguchi, T., Yamamoto, K., 2003. Formation of fine drug particle by cogrinding with cyclodextrins. II. The influence of moisture condition during cogrinding process on fine particle formation. Int. J. Pharm. 265, 85–93.

Yamada, T., Saito, N., Imai, T., Otagiri, M., 1999. Effect of grinding with hydroxypropyl cellulose on the dissolution and particle size of a poorly water-soluble drug. Chem. Pharm. Bull. 47, 1311–1313.