Research Article

Effect of Excipients on the Particle Size of Precipitated Pioglitazone in the Gastrointestinal Tract: Impact on Bioequivalence

Masaru Sugita,^{1,3} Makoto Kataoka,² Masahisa Sugihara,¹ Susumu Takeuchi,¹ and Shinji Yamashita²

Received 14 April 2014; accepted 2 July 2014; published online 29 July 2014

Abstract. This study sought to understand the reasons for the bioinequivalence of a newly developed generic product of pioglitazone hydrochloride and to improve its formulation so that it is equivalent to that of the reference listed drug (RLD). In this clinical study, despite a similar *in vitro* dissolution profile, the new oral product exhibited a lower plasma concentration of pioglitazone compared to the RLD. The strong pH-dependency of pioglitazone solubility as a weak base indicates that pioglitazone would precipitate in the small intestine after being dissolved in the stomach. Thus, *in vitro* experiments were performed to investigate the effect of excipients on the particle size distribution of precipitated pioglitazone. Then, the impact of particle size on *in vivo* absorption was discussed. The precipitated pioglitazone from the RLD showed a peak for small particles (less than 1 μm), which was not observed in the precipitate from the new product. As an excipient, hydroxypropyl cellulose (HPC) influenced the particle size of precipitated pioglitazone, and the amount of HPC in the formulation was increased to the same level as that in the RLD. The precipitate from this improved product showed approximately the same particle size distribution as that of the RLD and successfully demonstrated bioequivalence in the clinical study. In conclusion, for drugs with low solubility, this type of analysis of the particle size distribution of precipitated drugs, in addition to the dissolution test, may help to obtain a better *in vitro-in vivo* correlation for oral absorption and to develop a bioequivalent product.

KEY WORDS: bioequivalence study; hydroxypropylcellulose; particle size distribution; pioglitazone; precipitation.

INTRODUCTION

For class II drugs in the Biopharmaceutical Classification System (BCS) (1,2) having low solubility and high permeability, the solubility and/or dissolution rate in the gastrointestinal (GI) tract are considered the principal factors limiting oral absorption (3). Various technologies are used in the formulation of such poorly soluble drugs to improve absorption by enhancing the dissolution rate or solubility. These technologies include micronization, salt formation, co-crystallization, solid dispersion and the use of solubilizers or lipid-based formulations. To determine how these formulation technologies work, a dissolution profile of the drug from each formulation is observed *in vitro* before advancing to an *in vivo* study.

In the bioequivalence study of oral drug products, an *in vitro* dissolution test is also widely used to determine the dissolution profile of an active ingredient. For a generic product, to demonstrate the bioequivalency of a newly developed formulation with a reference listed drug (RLD),

similarities in the drug dissolution profile are first confirmed *in vitro* and then a human clinical study is performed (4). This process in the regulation of oral drug products arises from the concept that the oral absorption profile of the drug reflects its dissolution profile in the GI tract.

The *in vitro-in vivo* correlation (IVIVC) for drug absorption is used to predict the oral absorption of drugs from their *in vitro* dissolution profiles. However, various case studies have been reported in which only a poor correlation was found between the dissolution of a drug *in vitro* and its absorption *in vivo*. Poor IVIVC in drug absorption may derive from the fact that *in vitro* conditions for dissolution tests do not necessarily reflect the conditions *in vivo* in the human GI tract (5). Although the use of biorelevant dissolution media that mimic the composition of human GI fluid (e.g., fasted or fed state-simulated intestinal fluid, FaSSIF or FeSSIF, respectively) can significantly improve the predictability (6–9), differences in other conditions, such as the fluid volume and agitation force, remain as causes of poor IVIVC. As an advanced system to predict the oral absorption of poorly soluble drugs, the *in vitro* integration system of drug dissolution with membrane permeation was developed for the possibility of achieving a better IVIVC (10–18).

Salt, co-crystallization or amorphous formation (solid dispersion) can dissolve drugs at a higher concentration compared to their thermodynamic equilibrium solubility.

¹ Sawai Pharmaceutical Co., Ltd., 5-2-30 Miyahara, Yodogawa-ku, Osaka, Osaka 532-0003, Japan.

² Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan.

³ To whom correspondence should be addressed. (e-mail: m.sugita@sawai.co.jp)

This supersaturated state is now recognized as one of the critical factors for improving the absorption of poorly soluble drugs, and extensive efforts have been made to maintain a higher concentration longer in the GI tract (19–22). However, once the nucleation reaction is initiated, supersaturation causes the precipitation of drugs. The precipitated drugs are then re-dissolved in the GI tract to be absorbed into the systemic circulation; otherwise, precipitated drugs are not available for absorption. If this precipitation and re-dissolution process significantly contributes to the extent and rate of drug absorption, an *in vitro* test in which only the drug dissolution profile is observed may not be sufficient to predict the *in vivo* absorption. In other words, the total performance of an oral drug product in the GI tract, not only the dissolution but also the precipitation and re-dissolution processes, should be evaluated to achieve a better IVIVC.

Pioglitazone-HCl is an oral antidiabetic drug that is categorized as a BCS class II weak base ($pK_a=5.8$ and 6.8 , $\log P=2.3$). The solubility of pioglitazone-HCl extensively depends on pH and was shown to be 4.4 mg/mL (pH 1.2), 0.042 mg/mL (pH 3.0), 0.005 mg/mL (pH 4.0), 0.0005 mg/mL (pH 5.0) and 0.0003 mg/mL (pH 6.8) (23). Therefore, after oral administration and once dissolved in the stomach under low pH conditions, pioglitazone is likely to precipitate in the small intestine or achieve supersaturation. In this report, based on our experience in the bioequivalence study for a generic product of pioglitazone-HCl, the impact of the precipitation and re-dissolution process on drug absorption was investigated, focusing on the particle size distribution of precipitated pioglitazone.

MATERIALS AND METHODS

Chemicals and Reagents

Pioglitazone hydrochloride (pioglitazone-HCl) was purchased from Tokuyama Corporation (Tokyo, Japan). Hydroxypropyl cellulose (HPC) was purchased from Nippon Soda Co., Ltd. (Tokyo, Japan). Low-substituted hydroxypropyl cellulose (L-HPC) was purchased from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). D-mannitol was purchased from Mitsubishi Shoji Foodtech Co., Ltd. (Tokyo, Japan). Methanol, acetonitrile, acetic acid and ammonium formate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Drug Products

ACTOS® tablets 30 (ACT30) and ACTOS® OD tablets 30 (ACTOD30) were purchased from Takeda Pharmaceutical Co., Ltd (Osaka, Japan). The products 560T4SC704 (SC704) and 560T4SH906 (SH906) were generics for ACTOD30 developed by Sawai Pharmaceutical Co., Ltd (Osaka, Japan).

These products contain 30 mg of pioglitazone per tablet. The excipients in SC704 included D-mannitol, ethyl cellulose, hydroxypropyl methylcellulose (HPMC), HPC, triacetin, L-HPC, aspartame, thaumatin, crospovidone, light anhydrous silicic acid and sodium stearyl fumarate. The excipients in SH906 included D-mannitol, HPC, L-HPC, aspartame, sodium L-tartrate, crospovidone, light anhydrous silicic acid and magnesium stearate. The excipients in ACT30 included lactose hydrate, calcium carboxymethyl cellulose, HPC and

magnesium stearate. The excipients in ACTOD30 included D-mannitol, lactose hydrate, crystalline cellulose, calcium carboxymethyl cellulose, HPC, aspartame, sodium chloride, yellow ferric oxide, crospovidone and magnesium stearate.

In Vitro Dissolution Study

The dissolution profiles of pioglitazone-HCl from ACT30, ACTOD30, SC704, and SH906 were evaluated using the paddle method defined in Japanese Pharmacopoeia (24). Briefly, the time course of the dissolved drug concentration was observed in a dissolution medium (900 mL per vessel) with a paddle revolution speed of 50 rpm at $37.0\pm0.5^\circ\text{C}$. JP1 fluid for pH 1.2 (Japanese Pharmacopoeia Dissolution Test Fluid No. 1) and JP2 fluid for pH 6.8 (Japanese Pharmacopoeia Disintegration Test Fluid No. 2) were used as dissolution media. The samples were filtered through a 0.45- μm filter, and the filtrate was assayed by high-performance liquid chromatography (HPLC).

Bioequivalence Clinical Study

Bioequivalence clinical studies were performed in accordance with the Declaration of Helsinki and the guidelines for Good Clinical Practice. The first study was conducted by Doujin Memorial Meiwa Hospital (Tokyo, Japan), and the second study was conducted by NITTAZUKA Medical Welfare Center Fukui General Hospital (Fukui, Japan). These studies were approved by the Institutional Review Boards at each trial site.

The plasma concentration of pioglitazone after the oral administration of the test product (SC704 or SH906) was compared to that of the RLD (ACT30 or ACTOD30). The study design was a two-way, crossover, single-dose, fasting over 12 h and randomized study with a 7-day washout period. Crossover studies were performed in 20 or 24 healthy male volunteers after they had been informed of the purpose, protocol and risks of the study. The subjects did not take any other medication for at least 7 days prior to and during the study.

After an oral administration of a test or RLD product containing 30 mg of pioglitazone, blood samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 34 (or 32)h. The samples were centrifuged, and the plasma were separated and stored at -20°C until analysis. The plasma samples were diluted with 1% with methanol to precipitate the protein, and the drug concentration was determined by liquid chromatography tandem mass spectrometry (LC/MS/MS). The pharmacokinetic parameters were calculated and statistically compared using the computer software BESTS (version 3.0.4, CAC Corporation, Tokyo, Japan), which conforms to the guideline for the bioequivalence test of generic drugs in Japan. The bioequivalence between the test and RLD was accepted if the 90% confidence intervals (90% CI) of the mean ratio (test/reference) of the log-transformed $C_{\max}$ and AUC_t values fell within 80–125%.

Preparation of Pioglitazone-HCl Immediate-Release Granules

By shaking in a plastic bag, 16.5 g of pioglitazone-HCl, 24.5 g of D-mannitol and 7.5 g of L-HPC were mixed.

Subsequently, 15.0 g of a binding solution was added, and granulation was conducted. Two types of binding solutions containing 1% HPC and 10% HPC aqueous solutions (*w/w*) were prepared. The granules prepared with 1% HPC and 10% HPC aqueous solution (*w/w*) were abbreviated as “Granules-1” and “Granules-10”, respectively. The uniformly granulated products were dried in an oven at 60°C for 1 h. The obtained granules were screened through a 710- μ m sieve.

Preparation of the Suspensions Containing Precipitated Particles

The suspensions containing precipitated particles were obtained using the following procedures. ACTOD30, SC704, SH906, Granules-1 or Granules-10 (each containing 30 mg of pioglitazone) was dissolved in 5 mL of JP1 fluid, after which 1 mL of JP1 fluid was added to 200 mL of McIlvaine buffer (pH 6.5). At 10 or 30 min after mixing, the obtained suspensions were used for the particle size measurement.

In the experiments with Granules-1 and Granules-10, the time course of the dissolved concentration of pioglitazone in McIlvaine buffer was monitored. After adding 1 mL of JP1 fluid to McIlvaine buffer, aliquots were collected at 1, 15, 30, 45, 60, 90, 120, and 240 min. The samples were immediately filtered through a 0.45- μ m filter, and the filtrate was assayed by LC/MS/MS.

Measurement of the Particle Size Distribution in Solutions Containing Precipitated Particles

The Mastersizer® 2000 (Malvern Instruments Ltd., Worcestershire, UK), a laser diffraction particle size analyzer, was used to measure the size distribution of precipitated particles in the above suspensions. With this equipment, it is possible to measure the size of particles in the range of 0.01 to 2,000 μ m based on a laser diffraction technique. Regarding the optimized conditions for particle size measurement, a 300-rpm pump speed was used without sonication to avoid the disintegration of precipitated particles during the measurement. The particle size distribution profile was obtained by averaging the results of five measurements of one sample.

In Situ Absorption Study Using a Rat Intestinal Closed Loop

All of the animal experiments were approved by the Ethical Review Committee of Setsunan University and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

To evaluate the influence of HPC on the intestinal absorption of pioglitazone, the suspensions containing precipitated particles that were obtained from Granules-1 and Granules-10 were applied to a rat intestinal tract using the *in situ* closed loop method. Male Wistar rats (SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) weighing 200–250 g (7–8 weeks old) were fasted for 24 h before the experiment but had free access to water. After the abdominal cavity was opened, an intestinal loop (10 cm long) was made at the proximal jejunum by cannulating a silicone tube (3 mm i.d.), and the intestinal contents were then removed by a slow infusion of saline and air. A suspension containing precipitated drug particles was prepared using the same method

described above and stored for 10 min, after which point approximately 0.5 mL (2 mL/kg of body weight) of suspension was introduced into the intestinal loop, and both ends of the loop were ligated. Blood samples were taken from the jugular vein at 15, 30, 60, 90, 120, 180, and 240 min after introduction. The samples were centrifuged, and the plasma was separated and stored at –20°C until analysis. The plasma samples were diluted by 10% with methanol to precipitate the protein and then analyzed by LC/MS/MS.

Drug Concentration Determination

The concentrations of pioglitazone in the samples were determined using an LC/MS/MS system. The LC system consisted of two gradient pumps (LC10Avp), an autosampler (SIL-HTC) and a column oven (CTO10Avp) from Shimadzu Corporation (Kyoto, Japan). The analytical column was a Cadenza CD-C18 (30 $\times$ 2.0 mm i.d., Imtakt Corporation Kyoto, Japan). The mobile phases consisted of 5 mM ammonium formate solution and a mixture of acetonitrile and acetic acid (1,000:1). Quantitation was achieved by MS/MS detection in positive ion mode using a triple quadrupole mass spectrometer API 3000 (AB SCIEX MA, USA). The ion was detected in the multiple reaction-monitoring (MRM) mode, with the transition pairs of pioglitazone at the *m/z* 357 precursor ion to the *m/z* 134 product ion.

RESULTS

In Vitro Dissolution Study

The dissolution profiles of pioglitazone-HCl from the test product (SC704) and the RLD (ACT30) were evaluated in JP1 and JP2 media. The results are shown in Fig. 1.

The dissolution profiles of pioglitazone-HCl strongly depended on its solubility, and we obtained values of 4.4 and 0.0003 mg/mL at pH 1.2 and 6.8, respectively (23). In JP1 media, pioglitazone-HCl was nearly completely dissolved from both products within 15 min because the solubility of pioglitazone-HCl is adequately high at pH 1.2. When the dissolution rate during the first 10 min was compared, SC704 showed a significantly higher rate compared to ACT30. This difference in the dissolution rate may have been because SC704 is an oral disintegration dosage form that is designed to be quickly disintegrated in the oral cavity.

In contrast, in JP2 media, the fraction of dissolved pioglitazone-HCl was less than 10% in both of the products because of its very low solubility at pH 6.8. From these *in vitro* dissolution profiles, these products were evaluated as “similar” based on the Japanese guidelines for bioequivalence studies of generic products.

Bioequivalence Study in Humans

Because the *in vitro* dissolution test showed “similar” dissolution profiles for both of the products, a clinical study was performed to confirm the “bioequivalence” of the test product with the RLD. Figure 2 shows the plasma concentration–time course of pioglitazone after the oral administration of SC704 or ACT30 to healthy volunteers.

Fig. 1. Dissolved drug-time profiles with USP apparatus II dissolution test in JP1 medium (pH 1.2) and in JP2 medium (pH 6.8). Each data point represents mean $\pm$ S.D. ($n=12$)

In Fig. 2, using an average of 20 subjects, the oral administration of SC704 gave a lower plasma concentration of pioglitazone than that of ACT30 over 34 h. As a result, both the AUC_t and C_{max} were higher for ACT30 than for SC704. According to the Japanese guidelines, when the 90% CI of the mean ratios (test/reference) of the log-transformed AUC_t and C_{max} are within the predefined bioequivalence acceptance limits (80–125%), the test compound and RLD can be considered bioequivalent. However, in this study, because the 90% CI of AUC_t and C_{max} were 68.3–83.6% and 70.5–88.8%, respectively, the SC704 was considered bioinequivalent with ACT30.

Distribution of the Particle Size of the Precipitates

To clarify the reason for this bioinequivalence in the human bioequivalence study, we focused on the precipitation process of pioglitazone in the GI tract because the dissolution process was considered similar in the *in vitro* dissolution test. Suspensions containing precipitated particles were prepared

with ACT30, SC704, or SC704_(placebo), the latter of which does not contain pioglitazone. The distribution of the particle size in those suspensions was observed by laser diffraction and is shown in Fig. 3.

As shown in Fig. 3, different distribution profiles of particle size were observed between the suspensions that were prepared with ACT30 and SC704. In the case of ACT30, three peaks were detected in the profile of the particle size distribution at approximately 100, 4, and 0.2 μm . In contrast, SC704 produced only one peak at approximately 70 μm and one shoulder peak at approximately 6 μm , but the peak for smaller particles (less than 1 μm) disappeared. However, because these two products are different formulations including different excipients and agents, it was difficult to identify the differences in the particle size distribution of the precipitated pioglitazone. The particle size distribution profile shown in Fig. 3 may also include the peak of the insoluble ingredients and their agglomerations, such as disintegrating agents.

Fig. 2. Plasma concentration–time profiles of pioglitazone after an oral administration of a product in healthy male volunteers. Each data point represents mean $\pm$ S.D. ($n=20$)

Fig. 3. The particle size distributions of the precipitated drug from ACT30 (a), SC704 and SC704 (placebo) (b). Each data line represents mean $\pm$ S.D. ($n=5$)

Effect of HPC on the Particle Size Distributions of Precipitated Pioglitazone

In terms of the ingredients of these formulations, we hypothesized that the difference in HPC content might affect the particle size distribution of the precipitates. HPC was included in both of the products as a binding agent, although the amount was different. The amount of HPC in ACT30 was estimated as 3.0 mg from the patent information (25). In the SC704 formulation, an aqueous dispersion of polymers that contained 0.3 mg HPC, 0.3 mg HPMC, and 6.0 mg ethyl cellulose was sprayed on 33.1 mg of pioglitazone-HCl (as 30.0 mg of pioglitazone) to prepare the coated fine particles before granulation. Then, the fine particles and other excipients were granulated with the aqueous solution containing 0.45 mg of HPC as a binder.

To evaluate the effect of HPC on the particle size distributions of the precipitates, two types of granules, Granules-1 and Granules-10, were prepared that contained the same amount of pioglitazone-HCl and excipients, with the exception of HPC [HPC/pioglitazone (w/w)=1/100 for Granules-1 (this ratio was based on the most intimate HPC amount on pioglitazone-HCl in SC704) and=10/100 for Granules-10 (this ratio was based on the patent information of ACT30)].

Suspensions containing precipitated particles were prepared with Granules-1 and Granules-10, and the distribution of the particle size was observed. As shown in Fig. 4, only for Granules-10, a clear peak was observed at approximately 0.2–0.3 μm of particle size. The profile of the particle size distribution for Granules-10 was similar to that for ACT30, as shown in Fig. 3, while Granules-1 generated a similar profile as that for SC704. These results shown in Figs. 3 and 4 clearly indicate that the differences in the amount of HPC caused the differences in the particle size distribution profiles of the precipitates. Because the HPC that is included in the granules is soluble in the medium, we assumed that the small particle of less than 1 μm in diameter that was observed for Granules-10 was derived from the precipitated pioglitazone.

After dissolving Granules-1 or Granules-10 in 5 mL of JP1 medium, 1 mL of the solution was added to 200 mL of McIlvaine buffer (pH 6.5), after which point the dissolved concentration of pioglitazone was monitored to investigate the effect of HPC on the dissolved concentration of pioglitazone at pH 6.5 (Fig. 5). Because pioglitazone-HCl was completely dissolved in JP1 medium from both Granules-1 and -10, the dissolved concentration of pioglitazone in JP1 medium was 6 mg/mL (30 mg/5 mL). Therefore, the initial concentration of dissolved pioglitazone in McIlvaine buffer should theoretically be equal to 30 μg/mL (diluted by 0.5%). At the first sampling time (1 min after the dilution), the dissolved concentration of pioglitazone in McIlvaine buffer was only 1.2 μg/mL and gradually decreased to approximately 0.2 μg/mL at 240 min. The differences in the HPC content (Granules-1 and -10) caused no large differences in the dissolved concentration of pioglitazone.

Effect of the HPC Content on the Intestinal Absorption of Pioglitazone

An intestinal absorption study was performed using the *in situ* rat intestinal closed loop method to determine whether differences in the particle size distribution of the precipitates would affect the absorption of pioglitazone. The suspensions containing precipitated particles and agglomerations were prepared with Granules-1 and -10 and then applied to a rat intestinal loop to observe the absorption of pioglitazone into the blood circulation. Before applying the suspensions to the rat intestine, the concentration of dissolved pioglitazone was determined by HPLC. No significant differences were detected in the concentration of pioglitazone (680.3 ng/mL for Granules-10 and 704.9 ng/mL for Granules-1).

Figure 6 shows the plasma concentration–time course of pioglitazone after applying the suspensions containing precipitates. The AUC_t and C_{max} were calculated and are summarized in Table I. Although significant differences were not observed for either AUC_t or C_{max} , on average, the plasma concentration of pioglitazone after applying the

Fig. 4. The particle size distributions of the precipitated drug from Granules-1 (a) and Granules-10 (b). Each data line represents mean $\pm$ S.D. ($n=5$)

suspension that was prepared from Granules-10 was higher than that from Granules-1. In addition, when applying the suspension that was prepared from Granules-10, the plasma concentration increased rapidly to reach $C_{\max}$ at 1 h. However, in the case of Granules-1, the plasma concentration gradually increased until 3 h after the suspension was applied.

Because the dissolved concentrations of pioglitazone in both of the suspensions were approximately the same, the differences in the plasma concentration may have arisen from differences in the rate of re-dissolution of precipitated pioglitazone. The particle size distribution profile shown in Fig. 3 might support this hypothesis because the small particles (less than 1 μm) of precipitated pioglitazone were found only in the solution prepared from Granules-10. It is thus reasonable to consider that those small particles quickly re-dissolved in the intestine to be absorbed into the blood circulation.

New Oral Product of Pioglitazone-HCl with a High Content of HPC

Based on the results with Granules-1 and -10, a new product of pioglitazone-HCl (SH906) was developed. In SH906, pioglitazone-HCl was not coated before granulation. The aqueous solution containing 3.0 mg of HPC was used as a binder for granulating pioglitazone-HCl with other excipients. In addition, to identify the particle size of the precipitated pioglitazone, a placebo of SH906 and SH906_(placebo), which does not include pioglitazone, was prepared. The suspensions containing precipitated particles were prepared using SH906, SH906_(placebo), and the RLD (ACTOD30) to observe the particle size distribution by laser diffraction.

As shown in Fig. 7, SH906 showed a similar particle size distribution profile as that of ACTOD30, demonstrating a

Fig. 5. Dissolved pioglitazone concentration–time profiles in McIlvaine buffer added from Granules-1 or Granules-10 solution in the acid condition. Each data point represents mean $\pm$ S.D. ($n=3$)

Fig. 6. Rat plasma concentration–time profiles of pioglitazone after an introduction of the precipitated solution. Each data point represents mean $\pm$ S.D. ($n=6$)

peak at approximately 0.2–0.3 μm . The same profile was observed at 10 and 30 min after the suspension was prepared. In contrast, SH906_(placebo) produced no peak in the range of less than 1 μm . Furthermore, smaller particles (less than 1 μm) were not observed in either SC704 or SC704_(placebo), confirming that a peak at approximately 0.2–0.3 μm was derived from the precipitated pioglitazone but not from other excipients, including HPC. This result suggests the possibility of the bioequivalence of the new product containing a high amount of HPC (SH906) with the RLD (ACTOD30). Then, the bioequivalence clinical study was performed, and the results are shown in Fig. 8.

Both of the pioglitazone-HCl products demonstrated similar plasma concentration profiles of pioglitazone; thus, no differences were found in the AUC_t and C_{max} . The 90% CIs of the mean ratios (test/reference) of the log-transformed AUC_t and C_{max} were 95.7–111.6% and 96.6–113.6%, respectively, which were within the acceptance limits for bioequivalence.

DISCUSSION

In general, to demonstrate the bioequivalence of a newly developed oral drug product to the RLD, similarities in the dissolution profiles are first determined *in vitro*, after which point a human clinical study is performed. However, in this study, although the new oral product of pioglitazone-HCl showed a similar dissolution profile to the RLD, the human

study failed to demonstrate the bioequivalence of the new product due to the lower blood level of pioglitazone after oral administration.

Pioglitazone is a poorly water-soluble drug that is classified into BCS class II. Because pioglitazone is a weak base, its HCl salt dissolves rapidly under acidic conditions in JP1 medium but has limited solubility at pH 6.8 in JP2 (Fig. 1). From these results of the *in vitro* dissolution test, it is reasonable to consider that, after oral administration, pioglitazone-HCl completely dissolves in the stomach, then pioglitazone is precipitated in the small intestine. Precipitated pioglitazone should be re-dissolved in the small intestine to be absorbed into the systemic circulation.

The dissolution rate of pioglitazone-HCl from our new product (SC704) was faster than that from the RLD (ACT30) under acidic conditions. Therefore, the result of the clinical bioequivalence study (Fig. 2), in which SC704 exhibited a lower plasma concentration of pioglitazone, cannot be explained without assuming the effect of excipients on the process of drug precipitation or subsequent re-dissolution. Because the most distinct differences in the components of SC704 and ACT30 were related to the amount of HPC, we focused on the effect of HPC on the absorption of pioglitazone.

Water-soluble polymers, such as HPMC and HPC, maintain the state of supersaturation of poorly soluble drugs and enhance their oral absorption (20). These polymers prevent the nuclei reaction of drug molecules in the supersaturated solution (20–22,26). If this is the case for pioglitazone, a higher amount of HPC in ACT30 may have led to the higher absorption of pioglitazone by preventing precipitation in the small intestine. However, as shown in Fig. 5, the differences in HPC content did not cause significant differences in the time profile of the dissolved concentration (precipitation rate) of pioglitazone in pH 6.5 medium. Furthermore, 1 min after the addition of the dissolved pioglitazone solution (JP1) to the pH-6.5 medium, the dissolved concentration of pioglitazone was only 1.2 $\mu\text{g/mL}$. Because the initial dissolved concentration of pioglitazone was theoretically 30 $\mu\text{g/mL}$, this result indicates that approximately 96% of the dissolved pioglitazone was precipitated within 1 min after addition of the solution to the pH-6.5 medium. Therefore, in the case of pioglitazone, it is

Table I. Pharmacokinetic Parameters of Granules-10 and Granules-1 with the Mean Ratio (Granules-10/Granules-1) in Rat Intestine *In Situ* Closed Loop Method

Pharmacokinetic parameters	Formulation (mean $\pm$ S.D.)		
	Granules-10	Granules-1	Ratio (10/1)
AUC_t (ng hr/mL)	236.0 $\pm$ 83.7	165.0 $\pm$ 54.4	1.43
C_{max} (ng/mL)	83.8 $\pm$ 27.5	61.2 $\pm$ 19.9	1.37

AUC_t area under the plasma concentration–time curve till the last sampling time ($n=6$); C_{max} maximal plasma concentration

Fig. 7. The particle size distributions of the precipitated drug solutions of ACTOD30 (a), SH906 and SH906_(placebo) (b). Each data line represents mean $\pm$ S.D. ($n=5$)

reasonable to consider that the effect of HPC on supersaturation was not the main cause of the bioequivalence.

The suspensions containing precipitated particles prepared with Granules-1 and -10 showed similar profiles of particle size distribution as those observed for SC704 and ACT30, respectively (Figs. 3 and 4). In both of these cases, products including a higher amount of HPC yielded small particles less than 1 μm , suggesting that HPC worked to prevent the aggregation of particles in the solution. We performed an additional experiment in which HPC was added to McIlvaine buffer solution in advance at pH 6.5; then, JP1 solution, which dissolved SC704, was added to the buffer. The total amount of HPC that was added to the buffer was the same as that contained in ACT30. As a result, this procedure had no effect on the particle size distribution profile of SC704 (data not shown), indicating that the HPC molecules that were added to the solution failed to prevent the aggregation of pioglitazone particles. From these results,

the following was proposed as a possible mechanism for the effect of HPC on the particle size of precipitated pioglitazone.

During the precipitation process, HPC included in the formulation is thought to form a polymer layer on the surface of the precipitated particles. When the particles contact each other and the surface layers overlap, overlap repulsion energy is generated between the particles. If the repulsion energy is greater than the van der Waals attraction, further aggregation will not take place (27). This was the case for ACT30 and Granules-10. In contrast, if there is less HPC, the polymer layers become thin, the van der Waals attraction becomes stronger than the repulsion energy, and aggregation of the particles progresses, as observed for SC704 and Granules-1.

Generally, as the particle size decreases, the surface area increases, and the rate of dissolution increases. Because the oral bioavailability of pioglitazone-HCl is greater than 80% in animals (28), the intestinal permeability of pioglitazone is sufficient to complete the absorption if the dissolution rate is

Fig. 8. Plasma concentration–time profiles of pioglitazone after an oral administration of a product in healthy male volunteers. Each data point represents mean $\pm$ S.D. ($n=24$)

fast. In other words, the oral absorption of pioglitazone might be limited by the dissolution rate, and thus, faster dissolution in the intestinal tract can promote absorption. As shown in Fig. 6, the suspensions including precipitated particles that were prepared with Granules-10 showed higher and faster absorption from rat intestines than those with Granules-1.

Finally, based on the results obtained in this study, we improved the product of pioglitazone-HCl by increasing the amount of HPC (SH906) and successfully demonstrated its bioequivalence with the RLD (ACTOD30). Because the precipitated solution of SH906 contained a fraction of small particles less than 1 μm and the profile of the particle size distribution was nearly identical to that of ACTOD30, we confirmed that the particle size of the precipitated drug significantly affected its absorption and improved the pioglitazone-HCl product to reach bioequivalence.

CONCLUSION

In this study, it was clearly demonstrated that the amount of HPC in the oral formulation of pioglitazone-HCl affected the particle size distribution of precipitated pioglitazone. For drugs with low solubility in which precipitation in the intestinal tract may occur, an analysis of the resulting particle size of the precipitated drug may provide insight into the *in vivo* performance.

REFERENCES

- Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm Res*. 1995;12:413–20.
- Löbnerberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system: new scientific approaches to international regulatory standards. *Eur J Pharm Biopharm*. 2000;50:3–12.
- Lipinski CA, Lombardo F, Dominy BW, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug delivery and development setting. *Adv Drug Deliv Rev*. 2001;46:3–26.
- Guideline for Bioequivalence Studies of Generic Products. Minister of Health, Labor and Welfare, 2012 [http://www.nihs.go.jp/drug/be-guide\(e\)/Generic/GL-E_120229_BE.pdf](http://www.nihs.go.jp/drug/be-guide(e)/Generic/GL-E_120229_BE.pdf). Accessed 20 Mar 2014.
- Cascone S, De Santis F, Lamberti G, Titomanlio G. The influence of dissolution conditions on the drug ADME phenomena. *Eur J Pharm Biopharm*. 2011;79(2):382–91.
- Klein S. The use of biorelevant dissolution media to forecast the *in vivo* performance of a drug. *AAPS J*. 2010;12(3):397–406.
- Galia E, Nicolaides E, Horter D, Lobenberg R, Reppas C, Dressman JB. Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs. *Pharm Res*. 1998;15(5):698–705.
- Jantravid E, Janssen N, Reppas C, Dressman JB. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. *Pharm Res*. 2008;25(7):1663–76.
- Sheng JJ, McNamara DP, Amidon GL. Toward an *in vivo* dissolution methodology: a comparison of phosphate and bicarbonate buffers. *Mol Pharm*. 2009;6(1):29–39.
- Kataoka M, Masaoka Y, Yamazaki Y, Sakane T, Sezaki H, Yamashita S. *In vitro* system to evaluate oral absorption of poorly water-soluble drugs: simultaneous analysis on dissolution and permeation of drugs. *Pharm Res*. 2003;20:1674–80.
- Kataoka M, Masaoka Y, Sakuma S, Yamashita S. Effect of food intake on the oral absorption of poorly water-soluble drugs: *in vitro* assessment of drug dissolution and permeation assay system. *J Pharm Sci*. 2006;95(9):2051–61.
- Buch P, Langguth P, Kataoka M, Yamashita S. IVIVC in oral absorption for fenofibrate immediate release tablets using a dissolution/permeation system. *J Pharm Sci*. 2009;98(6):2001–9.
- Buch P, Holm P, Thomassen JQ, Scherer D, Kataoka M, Yamashita S, *et al*. IVIVR in oral absorption for fenofibrate immediate release tablets using dissolution and dissolution permeation methods. *Pharmazie*. 2010;65(10):723–8.
- Kataoka M, Isubata S, Masaoka Y, Sakuma S, Yamashita S. *In vitro* dissolution/permeation system to predict the oral absorption of poorly water-soluble drugs: effect of food and dose strength on it. *Biol Pharm Bull*. 2011;34(3):401–7.
- Buch P, Holm P, Thomassen JQ, Scherer D, Kataoka M, Yamashita S, *et al*. IVIVR in oral absorption for fenofibrate immediate release tablets using dissolution and dissolution permeation methods. *Pharmazie*. 2011;66(1):11–6.
- Kataoka M, Yokoyama T, Masaoka Y, Sakuma S, Yamashita S. Estimation of P-glycoprotein-mediated efflux in the oral absorption of P-gp substrate drugs from simultaneous analysis of drug dissolution and permeation. *Eur J Pharm Sci*. 2011;44(4):544–51.
- Kataoka M, Sugano K, da Costa Mathews C, Wong JW, Jones KL, Masaoka Y, *et al*. Application of dissolution/permeation system for evaluation of formulation effect on oral absorption of poorly water-soluble drugs in drug development. *Pharm Res*. 2012;29(6):1485–94.
- Kataoka M, Yano K, Hamatsu Y, Masaoka Y, Sakuma S, Yamashita S. Assessment of absorption potential of poorly water-soluble drugs by using the dissolution/permeation system. *Eur J Pharm Biopharm*. 2013;85(3 Pt B):1317–24.
- Bevernage J, Forier T, Brouwers J, Tack J, Annaert P, Augustijns P. Excipient-mediated supersaturation stabilization in human intestinal fluids. *Mol Pharm*. 2011;8(2):564–70.
- Brouwers J, Brewster ME, Augustijns P. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? *J Pharm Sci*. 2009;98(8):2549–72.
- Gao P, Shi Y. Characterization of supersaturable formulations for improved absorption of poorly soluble drugs. *AAPS J*. 2012;14(4):703–13.
- Bevernage J, Brouwers J, Brewster ME, Augustijns P. Evaluation of gastrointestinal drug supersaturation and precipitation: strategies and issues. *Int J Pharm*. 2013;453(1):25–35.
- Application materials for Pioglitazone Tablet 30 mg Sawai; Sawai Pharmaceutical Co., Ltd.
- The Japanese Pharmacopeia, 16th ed.; The Society of Japanese Pharmacopeia: Tokyo, Japan, 2011. <http://jpd.nihs.go.jp/jp16e/>. Accessed 20 Mar 2014.
- Patent application laid-open disclosure number 2002–360666; Japan Patent Office.
- Raghavan SL, Trividic A, Davis AF, Hadgraft J. Crystallization of hydrocortisone acetate: influence of polymers. *Int J Pharm*. 2001;212(2):213–21.
- Bagchi P. Theory of spherical colloidal particles by nonionic polymers. *J Colloid Interface Sci*. 1974;47:86–99.
- Maeshiba Y, Kiyota Y, Yamashita K, Yoshimura Y, Motohashi M, Tanayama S. Disposition of AD-4833(HCl), a new antidiabetic agent, in animals. *Jpn Pharmacol Ther*. 1996;24(12):83–103.