Powder Coating Mixture of a Novel Anxiolytic, Y-23684: Dissolution Characteristics and Bioavailability

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A powder coating mixture was investigated with a view toward improving the dissolution property of the anxiolytic 2-(4-chlorophenyl)-5,6-dihydro-[1]benzo-thiepino-[5,4-*c*]-pyridazin-3(2*H*)-one 7-oxide (1), which was barely water soluble. The powder coating mixture in various ratios of 1 and cornstarch was prepared in an automated mortar. Among these mixtures, at the cptimum ratio of 1 and cornstarch (2:1, 67% drug content), the powder coating mixture gave a maximized effect for solubilizing 1 on the bases of stability and solubility. Conventional granules were made from the 67% powder coating mixture. The granules showed an excellent absorption profile in beagle dogs. The mechanism of the solubilizing effect resulting from a pharmaceutical process was also discussed.

Keywords powder coating; anxiolytic; solubilization; bioavailability; cornstarch

Introduction

A novel anxioselective anxiolytic, 2-(4-chlorophenyl)-5,6-dihydro-[1]benzothiepino[5,4-*c*]pyridazin-3(2*H*)-one 7-oxide (**1**: Y-23684) is a sparingly water-soluble compound (solubility: 0.001% (w/v) in water).[1] This characteristic suggested insufficient gastro-intestinal bioavailability of **1**. In preparing oral dosage forms of the compounds, therefore, some sorts of pharmaceutical techniques were required. There are many methods to modify this unfavorable property of poorly water-soluble compounds. The preparation of inclusion complex with cyclodextrins is one well-known technique.[2] However, a clinical dose of **1** was predicted to be in the range of 5 to 20 mg/d/man. These values seemed to restrict the availability of cyclodextrins as carriers for **1** because their enormous molecular weights might enlarge the volume of an unit dosage form of the drug. The preparation of a solid dispersion appeared to raise other problems such as stability, hygroscopicity, poor compressibility, and difficulty of solvent removal. These difficulties in the solubilization of **1** may be solved by enlarging the effective surface area of the compound. For such purposes, a drug is frequently micronized by means of an atomizer or a jet mill. Jimbo *et al.* reviewed the practical limitation, namely, the so-called limit of 3 μm, in reducing the particle size of a compounds by dry processes.[3] This unfavorable phenomenon is mainly caused by both a van der Waals interaction and an electrostatic force of attraction between the particles. Recently, Hersey *et al.* reported that specific mixed states were produced by the dry mixing of fine and coarse particles. When large differences in particle size and particle interaction existed between the two types of particles, fine particles adhered to coarse particle surfaces and specific mixed states were obtained.[4] By employing this phenomenon, the powder coating mixture technique was expected to be an alternative pharmaceutical method. The powder coating mixture, where fine particles of a drug adhere to the coarser granules of an inert carrier with an interparticle force strong enough to prevent the formation of aggregates and the breakdown of interacting units, would exhibit a significant solubilizing effect resulting from an increase in effective surface area.[5] The present report is focused on the application of the powder coating technique for improving the undesirable *in vitro* and *in vivo* characteristics of **1**.

Materials and Methods

Materials Compound **1** was synthesized in our laboratories, and was pulverized with a sample mill (K-II-1, Fuji Denki Kogyo Co., Ltd. Japan) to a powder (mean particle size: 14.1 μm). This powder was further ground, if necessary, with a jet mill (100-AS, Fuji Sangyo Co., Ltd., Japan), to a micronized particle (mean particle size: 3.8 μm). The other chemicals were purchased from commercial sources as follows: lactose (HMS, the Netherlands), hydroxypropylmethylcellulose (methocel 25, Shin-Etu Chemical Co., Ltd., Japan), cornstarch (Nihon Shokuhin Kako Co., Ltd., Japan), polysolvate 80 (Nikko Chemicals Co., Ltd., Japan), and polyethylene glycol (average molecular weight; 300, Nacalai Tesque, Inc., Japan).

Preparations Powder Coating Mixture: Corn starch was used as a carrier of the powder coating mixture system for **1**. For preparing the powder coating mixture, the drug and the carrier in various ratios (1:9, 1:4, 2:1, and 4:1 in weight, respectively) were weighed and applied to an automated mortar (ANM-200W, Nitto Kagaku Co., Ltd., Japan), to be ground in a dry process for appropriate time.

Conventional 1% Granules: Two kinds of granules were prepared from the 67% powder coating mixture (2:1 in the drug/carrier ratio, ground for 2 h; preparation A) or the physical mixture of the same composition as the former. The dosage forms were comprised of 1.5% preparation A or the physical mixture (corresponding to 1% of **1**, respectively), 97.5% lactose as a filler, and 1% of methocel-25 as a binder. An extruder (RG-5, Kikusui Seisakusho, Ltd., Japan) was used for granulation.

Measurement of Average Particle Size The mean particle diameters were determined according to the sedimentation method by using a centrifugal particle analyzer (APA-500, Horiba Ltd. Japan) with a tungsten-halogen lamp (wavelength: 530 nm). A 0.15% (w/v) aqueous polysorbate 80 solution was used as a susupending medium at a revolution speed of 500 rpm at 20°C.

Powder X-Ray Diffraction Pattern The extent of crystallinity of **1** was estimated with an X-ray diffractometer (XD-610, Shimadzu Corporation, Japan) by using Ni-filtered CuK_α ray and a graphite-monochrometer at the voltage of 50 kV and a current of 30 mA. The physical mixtures of the same compositions as the individual powder coating mixtures were supplied as the references.

Dissolution Studies The dissolution studies were carried out according to the paddle method by using an automated dissolution tester (NTR-VS, Toyama Sangyo Co., Ltd., Japan) under the following conditions: dissolution medium, 900 ml of JP XI 1st fluid or 900 ml of 0.01% (w/v) polysolvate 80 aqueous solution; temperature, 37±1 °C; and stirring speed, 100 rpm. The preparations containing 10 mg of the drug were continuously monitored according to the flow through cell method by using a ultraviolet spectrophotometer (UV-160, Shimadzu Corporation, Japan) at 322 nm.

Scanning Electron Microscopic Photograph The surface appearance of the powder coating mixture was observed using scanning electron microscopy (S-4000, Hitachi Instrument Engineering Co., Ltd., Japan).

Bioavailability Studies Six male beagle dogs weighing between 10.5—12.0 kg were fasted for 24 h before drug administration, but were allowed free access to water. The beagle dogs were divided into two groups of three dogs, and experiments using two kinds of 1% granules were carried out by using a latin-square cross-over design at 1-week intervals. A

500 mg aliquot of each dosage form (containing 5 mg of **1**) was encapsulated in a hard gelatin capsule. A dog orally received a single capsule. For determination of an oral absorption profile of **1**, the drug was dissolved in polyethylene glycol at the concentration of 2.5 mg/ml, and was administered intravenously to six beagle dogs at a dose 1 mg/kg. Venous blood samples (2.5 ml) were taken at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after each administration in heparinized tubes. The samples were centrifuged immediately at 3000 rpm for 15 min, and the plasma fraction was frozen for storage until assay.

Analytical Method The plasma concentration of **1** was determined by high performance liquid chromatography (HPLC). A mixture of 1 ml of the plasma sample, 0.1 ml of 1 N NaOH and 2 ml of *n*-hexane: chloroform (7 : 3, v/v) was shaken for 10 min. After centrifugation, a portion (1.8 ml) of the organic layer was taken and evaporated to dryness under reduced pressure at 25 °C. The residue was dissolved in 0.2 ml of the mobile phase described below, and 0.15 ml of the solution was supplied for the analysis. The HPLC system consisted of a pump (LC-6A, Shimadzu Corporation, Japan), a spectrophotometric detector (SPD-6A, Shimadzu Corporation, Japan), and an injector (7125, Rheodyne, CA) with a 200-μl loop. The chromatogram was recorded on an integrator (C-R3A, Shimadzu Corporation, Japan). A normal phase column packed with Nucleosil 50-5 (15 cm × 4.6 mm i.d., Chemco, Japan) was used as the stationary phase under ambient conditions. The mobile phase was *n*-hexane containing 40% (v/v) of ethanol, and was pumped at a rate of 1.2 ml/min. The wavelength was set at 240 nm.

Pharmacokinetic Analysis The plasma concentration of **1** was plotted against time, and both the peak concentration (C_{max} and the time required to attain the peak concentration were determined directly from the graph. The total area under the plasma concentration–time curve from 0 to 24 h after administration (AUC_{0-24h}) was calculated by means of the trapezoidal rule. Analysis of variance (ANOVA) was carried out for the pharmacokinetic parameters. The cumulative fraction of the drug absorbed as a function of time was calculated from intravenous (mean plasma concentration) and oral administration data (individual plasma concentration) by the deconvolution method.[5]

Result and Discussion

As shown in Fig. 1, the smaller was the particle size of **1**, the larger was the percent of the compound which dissolved. An increase in the effective surface area apparently contributed to modification of the dissolution characteristics of **1**.

In expecting an efficient improvement of bioavailability of the compound, however, the mean particle size (3.8 μm) of the micronized powder was observed to be insufficient. Therefore, it seemed advantageous to employ another method to enhance the solubility of **1**.

The solid dispersion of **1** in polyvinylpyrrolidone (20—50%) exhibited favorable dissolution profiles (a 500 mg aliquot of **1** was completely dissolved in 900 ml JP XI 1st fluid). However, the physicochemical stability of the dispersion was not good enough because of its marked hygroscopicity and deterioration of dissolution properties during storage under humidified conditions.

The solubility of **1** was not significantly improved under the co-existence of surfactants.

Finally, we examined the powder coating mixture system.[6,7] Cornstarch was chosen as a carrier because its

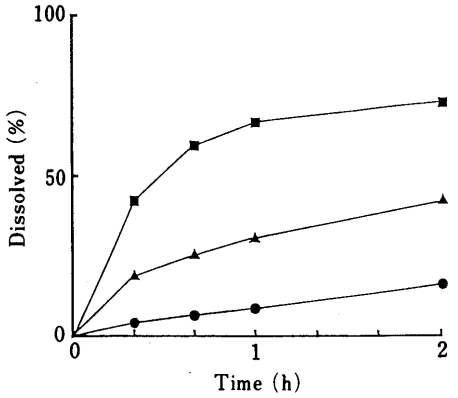

Fig. 1. Particle-Size Dependent Dissolution Profiles of Y-23684 Powder 10 mg in 900 ml of JP XI 1st Fluid at 37 °C (Paddle Method 100 rpm) $n=6$

●, no treatment; ▲, prepared by a sample mill ($d_p=14.1$ μm); ■, prepared by a jet mill ($d_p=3.8$ μm) ($n=6$). d_p; average particle diameter.

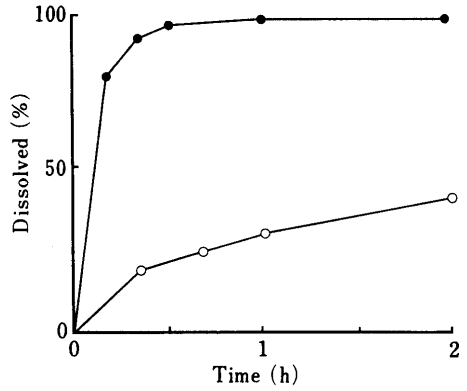

Fig. 2. The Dissolution Profiles of Preparation A and Physical Mixture Containing 10 mg of Y-23684 in 900 ml of JP XI 1st Fluid at 37 °C (Paddle Method 100 rpm) ($n=6$)

●, preparation A; ○, physical mixture.

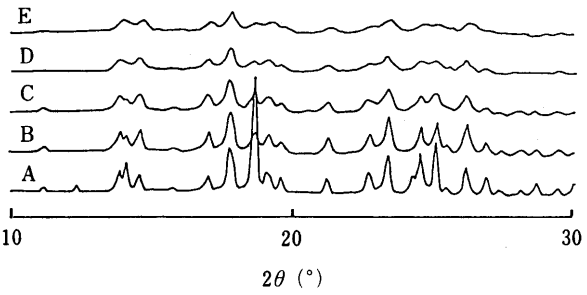

Fig. 3. Powder X-Ray Diffraction Pattern of the Preparation A at Various Sampling Times

A, no treatment; B, ground for 10 min; C, ground for 30 min; D, ground for 60 min; E, ground for 120 min.

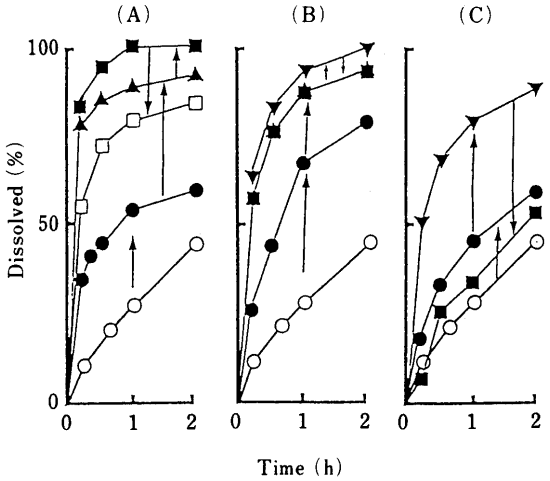

Fig. 4. Grinding Time-Dependent Dissolution Profiles of Various Ratios of Y-23684: Cornstarch Mixture Containing 10 mg of Y-23684 in 900 ml of JP XI 1st Fluid at 37 °C (Paddle Method 100 rpm) ($n=6$)

A, Y-23684: cornstarch = 1 : 9; B, Y-23684: cornstarch = 2 : 1; C, Y-23684: cornstarch = 4 : 1; grinding time: ○, 0 min; ●, 30 min; ▲, 60 min; ▼, 120 min; ■, 360 min; □, 480 min.

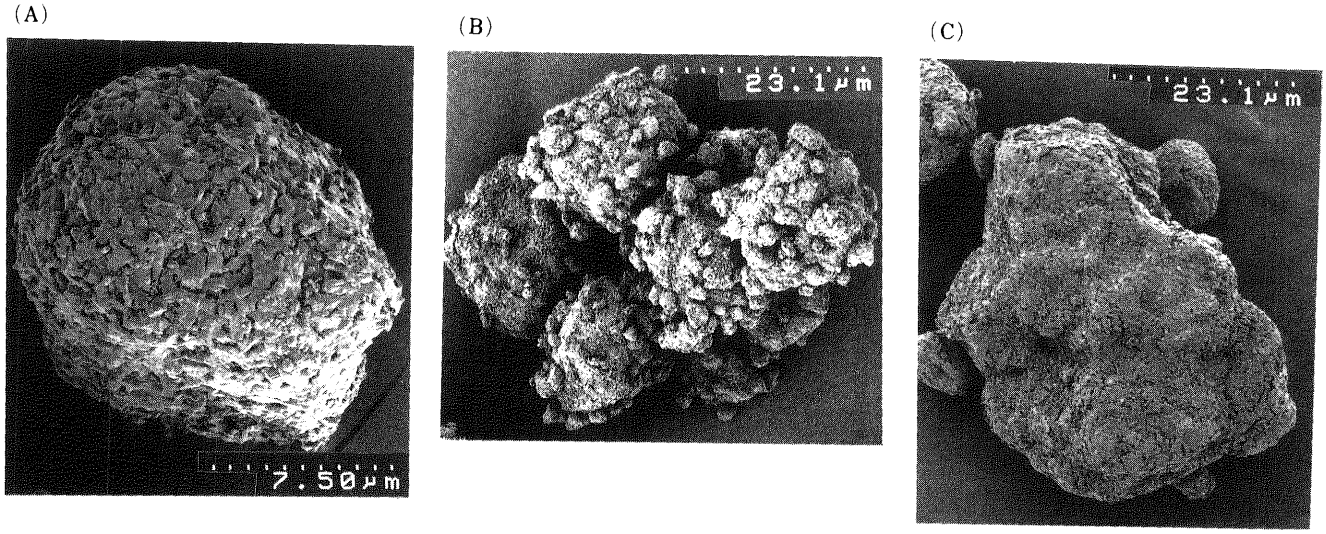

Fig. 5. Scanning Electron Microscopic Photograph under Various Conditions of Y-23684/Cornstarch Powder Coating Mixture

A, preparation A; B, Y-23684: cornstarch = 1 : 4; C, Y-23684: cornstarch = 4 : 1. Magnifications: A, ×4000; B, ×1300; C, ×1300. Each powder coating mixture was sampled out after grinding for 480 min.

particle size showed pretty narrow distribution compared to other kinds of starches. The powder coating mixtures of various proportions of **1** and cornstarch were prepared to evaluate their dissolution characteristics. As indicated in Fig. 2, compound **1** dissolved very quickly into the dissolution medium (JP XI 1st fluid) from a powder coating mixture up to 67% drug content (preparation A, ground for 2 h), and reached an equilibrium concentration at hour 2 of the test period. Compared to the atomizer milled raw material (average particle size: 14.1 μm), preparation A showed about 2.5 times higher drug concentration in the test medium at this time.

The powder x-ray diffraction pattern of preparation A suggested a grinding time-dependent reduction in the crystallinity of **1** (Fig. 3).

By excessive grinding (>6 h), both the powder coating mixture of relatively low drug content (10—40% drug content) and of high drug content (80% drug content) formed aggregated particles in the automated mortar to result in a lowering of the dissolution rate compared to preparation A. However, preparation A (grinding for 2 h) was free from such problems and gave excellent dispersion in the dissolution fluid regardless of grinding time (Fig. 4).

An elucidation of these physicochemical problems was furnished by examining the scanning electron microscopic photograph (Fig. 5). Thus, the surface of the carrier in preparation A was covered with a sufficient powder layer of **1** to prevent aggregation in the mortar. In the powder coating mixture with a drug content of 10 to 40%, a partially superimposed layer of **1** was observed on the surface of the carrier, which means the thickness of the drug layer was insufficient to prevent the aggregation of cornstarch particles in the automated mortar in comparison with preparation A. Grinding of cornstarch particles alone in the automated mortar also showed aggregation; however, individual particles retained their normal shape. In the case of the mixture of 80% drug content, the mixtures seemed ambiguous or irregular in shape.

Therefore, these phenomena were presumed to bring about a grinding time-dependent deterioration in dissolution properties of the incompletely layered and excessively

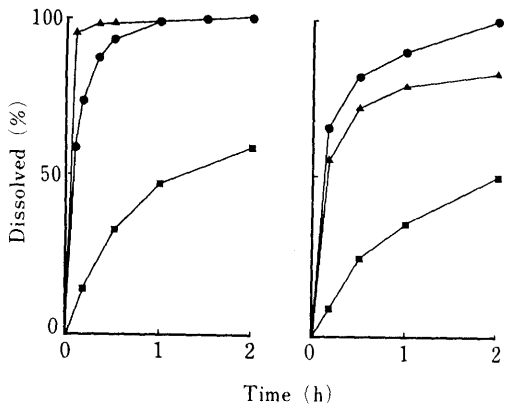

Fig. 6. Effect of Surfactant on the Dissolution of Aggregated Powder Coating Mixture (8 h Grinding; ▲, 1 : 9; ●, preparation A; ■, 4 : 1) Corresponding 10 mg of Y-23684 in 900 ml of 0.01% (w/v) Polysolvate 80 Aqueous Solution (Left) or JP XI 1st Fluid (Right) at 37 °C (Paddle Method 100 rpm) ($n = 6$)

layered mixture by different mechanisms. Preparation A, where the drug and the carrier were mixed in a ratio of 2 : 1 (67% drug content), was therefore screened as a candidate for further *in vivo* evaluation.

A relationship between the aggregation of the particles and the dissolution rate of **1** would be clarified by using an aqueous solution of a surfactant as the dissolution medium instead of the JP XI 1st fluid. Since surfactants are generally recognized as dispersing factors, the aggregated particles might be dissociated to individual particles particles in the medium containing surfactant. If the excessively ground mixtures were diminished in solubility by particle aggregation, the dissolution properties were assumed to be closer to preparation A in such a medium. As we expected, the amount of **1** dissolved after 2 h from the excessively ground mixtures (drug content 10 and 20%) showed nearly identical dissolution profiles to preparation A in a 0.01% (w/v) polysorbate 80 aqueous system, whereas the mixture of 80% drug content in the same medium didn't. Figure 6 shows typical examples of such cases (drug content 10, 67, 80%; 8 h grinding).

That means the aggregation of particles was the main

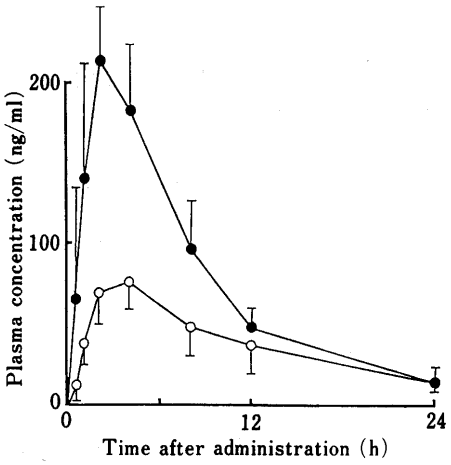

Fig. 7. Plasma Levels of Y-23684 after Oral Administrations of Two Types of 1% Granules Prepared from Preparation A or Physical Mixture in Dog

The results are expressed as the mean ± S.D. ($n=6$). ●, preparation A; ○, physical mixture.

TABLE I. Pharmacokinetic Parameters of Y-23684 after Oral Administration of Granules Prepared from Preparation A or Physical Mixture at a Dose of 5 mg/Dog ($n=6$)

	Preparation A	Physical mixture
T_{max} (h)	2.0±1.1	3.0±1.1
C_{max} (ng/ml)	223.2±34.5[a)]	81.0±1.1
AUC_{0-24h} (ng·h/ml)	1916.6±512.5[b)]	901.9±284.1

a) $p<0.01$, b) $p<0.05$.

factor in lowering the dissolution of **1** from the excessively ground powder coating mixture of 10 to 20% drug content in JPXI 1st fluid.

Two types of the conventional 1% granules of **1** were prepared from the preparation A and the physical mixture of the same composition as the former, and were compared to each other on their oral bioavailabilities by using beagle dogs. Both species of the granules were orally administered to the dogs, and the plasma level-time courses of **1** were monitored for 24 h by HPLC (Fig. 7). The pharmacokinetic parameters are shown in Table I. The granules from preparation A showed a significantly higher C_{max} ($p<0.01$) and greater AUC_{0-24h} ($p<0.05$) than those determined for the granules from the physical mixture.

Figure 8 shows the dissolution characteristics of the two types of granules.

Reflecting the *in vitro* results, the systemic absorption profile of granules from preparation A also exhibited a remarkable superiority over that of the physical mixture (Fig. 9).

An advantage of the powder coating mixture system was confirmed for **1** on the basis of the above mentioned *in vitro* and *in vivo* data. Thus, it is concluded that the optimal drug/cornstarch ratio of 2:1 would be a hopeful intermediate product for further pharmaceutical studies on the compound.

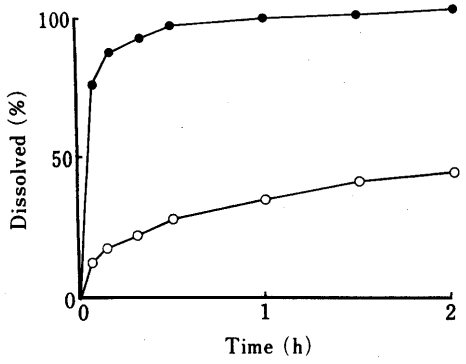

Fig. 8. Dissolution Profiles of 1% Granules Prepared from Preparation A or Physical Mixture Corresponding to 10 mg of Y-23684 in 900 ml of JP XI 1st Fluid at 37 °C (Paddle Method 100 rpm)

●, preparation A; ○, physical mixture.

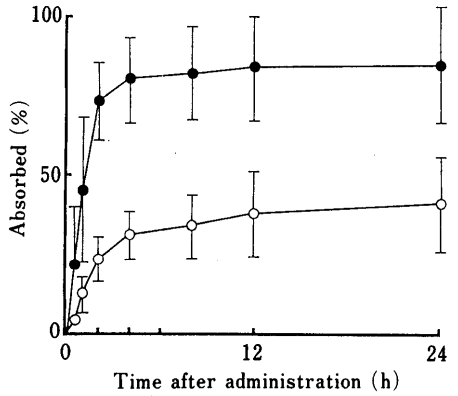

Fig. 9. Systemic Absorption Profiles of Granules Prepared from Preparation A or Physical Mixture

Calculations were carried out by means of the deconvolution method. ●, preparation A; ○, physical mixture.

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