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Dissolution Behavior and Gastrointestinal Absorption of Dicumarol from Solid Dispersion Systems of Dicmarol-Polyvinylpyrrolidone and Dicumarol-β-Cyclodextrin[1)]

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Solid dispersion systems of dicumarol–polyvinylpyrrolidone (PVP) and dicumarol–β-cyclodextrin (β-CD) were prepared by co-evaporation or freeze-drying of the drug-matrix mixture ammonium solution. Comparative studies were made on *in vitro* dissolution and *in vivo* absorption of the solid dispersion systems and dicumarol crystal powder. The dissolution rates of dicumarol were markedly increased in these solid dispersion systems in the pharmacopeial disintegration medium at pH 7.5. *In vivo* absorption studies were carried out in rabbits by measuring the plasma levels of dicumarol followng the oral administration of the solid dispersion systems and dicumarol crystal powder. The peak levels of the drug were observed at 4—6 h postadministration in the cases of the solid dispersion systems. On the other hand, they were observed at 2—12 h postadministration in the case of dicumarol crystal powder. It appears that the modification of the dissolution characteristics of dicumarol by preparing the solid dispersion systems results in increased bioavailability of dicumarol.

Keywords——dicumarol; bioavailability; dosage form; polyvinylpyrrolidone; β-cyclodextrin; dissolution rate; gastrointestinal absorption; solid dispersion system; anticoagulant

When a poorly water-soluble drug is administered orally, the rate-determining step in the absorption process is usually dissolution of the drug in the biological fluids of the gastrointestinal tract.[3)] Different preparations of such drugs may have a nonequivalent therapeutic response due to differences in bioavailability, even though these products meet the existing official compendial standard.[4)] As improvement of the dissolution characteristics of such poorly water-soluble drugs results in higher plasma peak levels or area under the blood concentration curve (AUC) of the drug following oral administration, it should be possible to decrease the dose to obtain effective therapeutic plasma levels of the drug with reduced side effects.[5)] In recent years, numerous methods to modify the dissolution characteristics of poorly water-soluble drugs have been investigated with the aim of obtaining better bioavailability.[6~10)] The authors were successful in improving the dissolution characteristics of some poorly water-soluble drugs by coprecipitation[11)] with polyvinylpyrrolidone (PVP), and obtained improved bioavailability of the drugs following oral administration of the coprecipitates.[12)]

Dicumarol, a long-acting anticoagulant, has been shown to be poorly absorbed,[13)] and its bioavailability varies widely, although the preparations are chemically equivalent.[14)] The authors therefore attempted to modify the dissolution characteristics of dicumarol by preparing solid dispersion systems using PVP and β-cyclodextrin (β-CD)[15)] to obtain better bioavailability of the drug with less variation following oral administration. Gastrointestinal absorptions of dicumarol from the solid dispersion systems and the drug alone were compared and evaluated in rabbits.

Materials and Methods

Materials——Dicumarol (U.S.P. grade) was obtained from Abbott Laboratories, North Chicago, Ill. (lot No. 825—7074). The mean particle size of dicumarol, as measured under an optical microscope, was 9.9±3.8 μm (Green diameter). The melting point of a dicumarol sample was 288°C. PVP K-15 (average molecular weight of 10000) was obtained from Daiichi Pure Chemicals Co., Tokyo. β-CD was obtained from Ando Kaseihin Co., Tokyo. All other chemicals were of reagent grade.

Preparation of Solid Dispersion Systems using PVP and β-CD——Solid dispersion systems were prepared as follows.

a) Co-evaporation Method (Method I): After dissolving both dicumarol and PVP or β-CD in a minimum amount of ammonium solution (25%), the solvent was removed *in vacuo* in a rotary evaporator at about 40°C. The residue was collected and dried *in vacuo* at room temperature for about 30 h in a vacuum oven.

b) Freeze-drying Method (Method II): Both dicumarol and PVP or β-CD were dissolved in a minimum amount of ammonium solution, and the solution was freeze-dried. The preparation was stored *in vacuo* at room temperature in a vacuum oven. Dicumarol–PVP and dicumarol–β-CD physical mixture were prepared by blending both dicumarol and PVP or β-CD powder in a mortar with a spatula. Residual ammonia in the solid dispersion systems was checked by the use of Nessler's reagent.

Dissolution Rate Studies——Dissolution rates of dicumarol from the preparations in 1 l of J.P.IX disintegration medium No. 2 (pH 7.5) were measured at 37.0±0.1°C in a constant-temperature water-bath (Taiyo, Thermounit, HM Type). The beaker had a 1 l capacity and was 105 mm in diameter. A stainless steel three-bladed propeller (40 mm in diameter and about 2 cm^2 in area of each blade) was immersed in the beaker at a depth of 30 mm from the bottom, and was rotated at 60 rpm. The rate of rotation was checked occasionally by using a hand tachometer (Teclock Co.). The amount of the preparation used was 100 mg dicumarol equivalent. Each preparation was added directly to the dissolution medium. Suitable aliquots were removed at the specified times with a syringe, then filtered quickly through a membrane filter (TM-4, pore size 0.2 μm, Toyo Scientific Co.). The same volume of fresh medium was added to the beaker. Sample solutions were diluted appropriately with 0.1 N NaOH prior to assay for dicumarol at 314 nm using a Hitachi 100-20 spectrophotometer. No significant absorbance of PVP and β-CD was found over the range used for the drug analysis.

X-Ray Diffraction Patterns——X-Ray powder diffraction patterns were obtained with a Rigaku Denki D-9C X-ray diffractometer.

Plasma Levels of Dicumarol in Rabbits——White male rabbits, 2.5—3.9 kg, were used in this study following stomach-emptying-time controlling treatment.[16,12b] Dicumarol (30 mg/kg) and the solid dispersion systems containing 30 mg/kg dicumarol equivalent in capsules (J.P.IX, No. 2) were orally administered to three rabbits in each group with about 50 g of special soft diet (Nihon Clea Co., Tokyo), and water was allowed *ad libitum*. Blood specimens were taken by cardiac puncture using a heparinized syringe at appropriate times up to 48 h. Blood samples were centrifuged (3500 rpm, 20 min) and the plasma samples were frozen and stored in a refrigerator until assayed. Rabbits were prevented from indulging in coprophagy by being fitted with a muzzle during the night.

Analytical Procedure for the Determination of Dicumarol in Plasma Samples——The concentration of dicumarol in the plasma samples was assayed by ultraviolet spectrophotometry as reported by Nagashima *et al.*[17] with some modifications. The procedure is as follows; to 1 ml of plasma sample in a glass-stoppered test tube (20 ml) were added 0.5 ml of pH 3.0 citrate buffer, 10 ml of *n*-heptane, and 0.5 g of sodium chloride. The tube was shaken for 30 min at 250 rpm, then centrifuged at 3500 rpm for 20 min. Six ml of the organic layer was transferred into another glass-stoppered test tube (20 ml) and 4 ml of 2.5 N NaOH was added. The test tube was shaken for 20 min, and centrifuged for 10 min (2500 rpm), then 3 ml of the aqueous layer was diluted to 10 ml with distilled water. The absorbance at 314 nm was determined. The determination of a plasma blank[17] was not necessary by this method. Linear calibration plots were obtained from 0 to 200 μg/ml dicumarol concentration (Fig. 1).

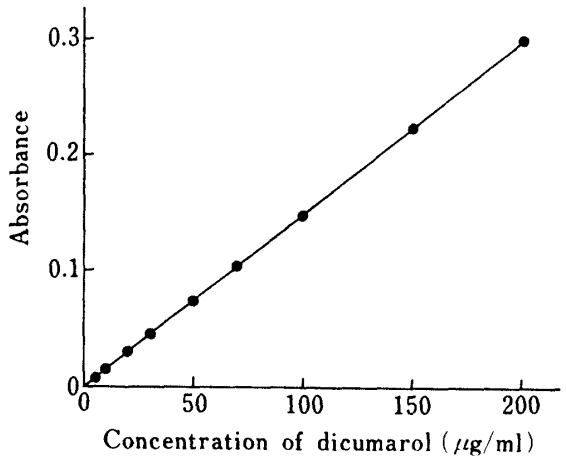

Fig. 1. Calibration Curve for Dicumarol in Plasma

Results and Discussion

The Properties of Solid Dispersion Systems of Dicumarol-PVP and Dicumarol-β-CD

The X-ray diffraction patterns of dicumarol, dicumarol–PVP solid dispersion systems and the physical mixture are shown in Fig. 2. In solid dispersion systems of 1 : 5 drug-to-PVP weight ratio, prepared by method I and method II, sharp diffraction peaks attributed to dicumarol crystals were no longer apparent and only the halo, similar to that of PVP powder alone, was observed in the X-ray diffraction spectra. Sharp diffraction peaks still remained in the simply blended physical mixture. Similar observations were also made with sulfisoxazole–PVP, phenytoin–PVP, tolbutamide–PVP, and sulfamethoxazole–PVP coprecipitates prepared by co-evaporation of the drug-PVP mixture ethanolic solution in a rotary evaporator.[12] On the other hand, in the preparation with 1 : 3 drug-to-PVP weight ratio, diffraction peaks still remained in the spectra of the sample prepared by method I. This result indicates that dicumarol in the sample still remained partially in the crystalline state. Diffraction peaks were not observed in the spectra of the 1 : 3 drug-to-PVP sample prepared by method II. The NH_3 adduct[18] of dicumarol was not formed by the evaporation of ammonium solution containing dicumarol alone, and polymorphism of dicumarol was not apparent. X-ray diffraction peaks and the melting point of dicumarol crystal powder prepared by evaporation of its ammonium solution were consistent with those of the original dicumarol crystal powder.

Well-defined coprecipitate[12a] was not formed when a sample was prepared by the conventional coprecipitating procedure using ethyl alcohol as the solvent, at 1 : 5 or 1 : 10 drug-to-PVP weight ratio. The apparent stability constant of formation of the possible complex between dicumarol and PVP in absolute ethanol at 37°C was large (37.5 M^{-1}, as vinylpyrrolidone equivalent); however, the amount of dicumarol solubilized by PVP was so small ($4.14\ M \times 10^{-3}$, by 1 M vinylpyrrolidone equivalent) that it might not be sufficient to inhibit the recrystallization of dicumarol during the solvent removing process from the dicumarol-PVP mixed solution.[19]

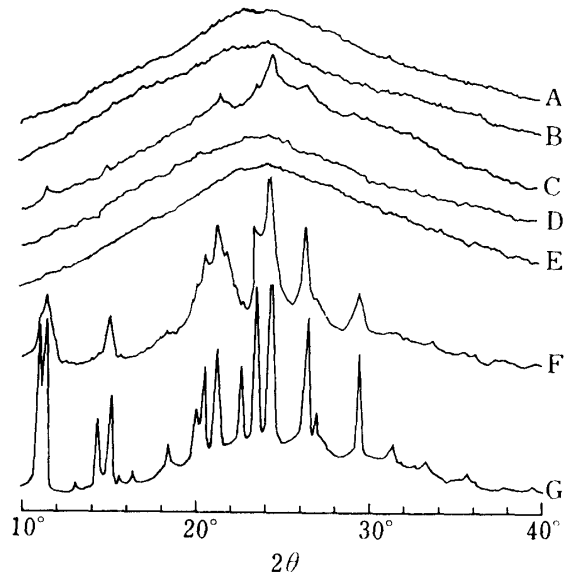

Fig. 2. Powder X-Ray Diffraction Patterns of Samples of Dicumarol-PVP Systems

A, PVP powder;
B, dicumarol: PVP=1: 5 (weight ratio) prepared by method I;
C, dicumarol: PVP=1: 3 prepared by method I;
D, dicumarol: PVP=1: 5 prepared by method II;
E, dicumarol: PVP=1: 3 prepared by method II;
F, dicumarol: PVP=1: 5 physical mixture;
G, dicumarol crystal powder.

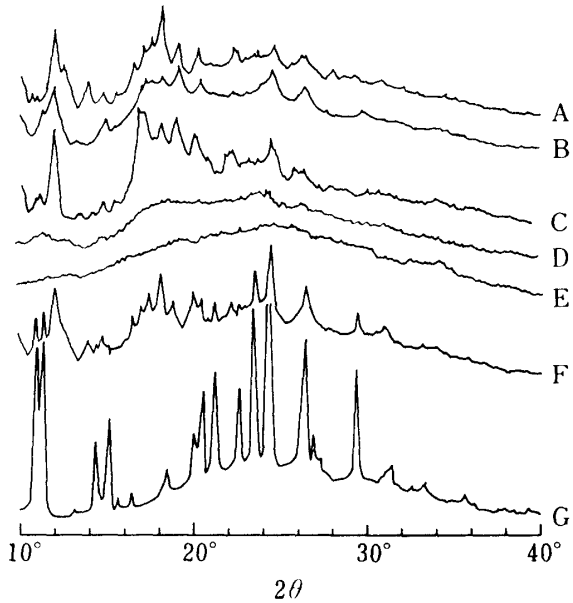

Fig. 3. Powder X-Ray Diffraction Patterns of Samples of Dicumarol–β-CD Systems

A, β-CD crystal powder;
B, dicumarol: β-CD=1: 2 (molar ratio) prepared by method I;
C, dicumarol: β-CD=1: 1 prepared by method I;
D, dicumarol: β-CD=1: 2 prepared by method II;
E, dicumarol: β-CD=1: 1 prepared by method II;
F, dicumarol: β-CD=1: 2 physical mixture;
G, dicumarol crystal powder.

Figure 3 shows the X-ray diffraction patterns of the dicumarol–β-CD dispersion systems, physical mixture and β-CD crystal powder.

In 1: 1 dicumarol-to-β-CD molar ratio systems prepared by method I, diffraction peaks attributable to both dicumarol crystals and β-CD crystals still remained. In 1: 2 drug-to-β-CD molar ratio systems, however, the peaks attributable to dicumarol crystals were negligible, and the peaks attributable to β-CD were observed. In 1: 2 molar ratio physical mixiture, peaks attributable to dicumarol crystals were significant. This indicated that dicumarol in 1: 2 molar ratio systems might have lost its crystalline structure, or at least that the crystalline size had fallen below the limit of the instrumental analysis, during the solvent removing process. The systems prepared by method II showed diffraction peaks attributable to both dicumarol and β-CD crystals. Diffraction peaks were not observed in the samples prepared by method II at 1: 1 and 1: 2 drug-to-β-CD molar ratios.

No residual ammonia was detected in the preparations.

Dissolution Studies

1. **Dicumarol-PVP Systems**——The dissolution behavior of dicumarol alone, solid dispersion systems and the physical mixture in J.P.IX disintegration medium No. 2 (pH 7.5) at 37°C is shown in Fig. 4.

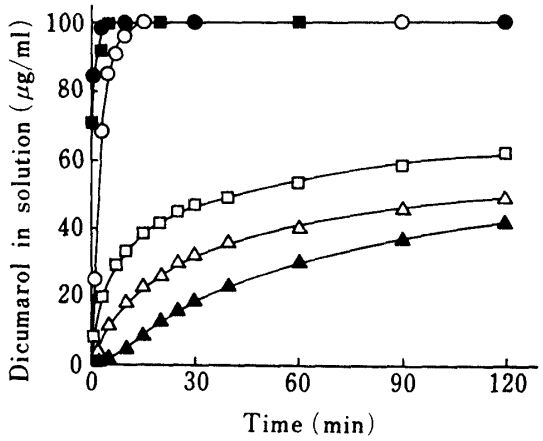

Fig. 4. Dissolution Profiles of Dicumarol from Test Preparations containing 100 mg Dicumarol Equivalent in 1 l of J.P. IX Disintegration Medium No. 2

○, dicumarol: PVP=1: 5 prepared by method I; ●, dicumarol: PVP=1: 5 prepared by method II; ■, dicumarol: PVP=1: 3 prepared by method II; □, dicumarol: PVP=1: 3 prepared by method I; △, dicumarol: PVP=1: 5 physical mixture; ▲, dicumarol crystal powder.

Each point represents the mean of three determinations.

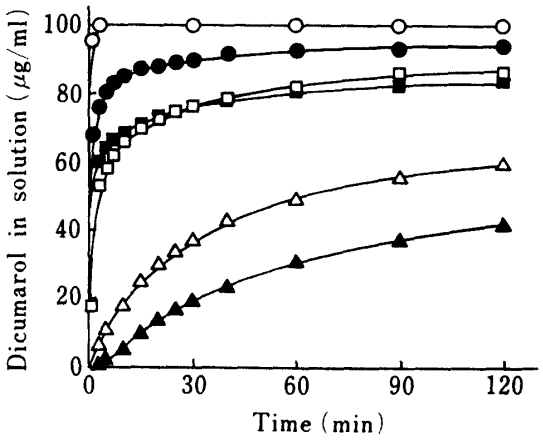

Fig. 5. Dissolution Profiles of Dicumarol from Test Preparations containing 100 mg Dicumarol Equivalent in 1 l of J.P. IX Disintegration Medium No. 2

○, dicumarol: β-CD=1: 2 prepared by method I; ●, dicumarol: β-CD=1: 2 prepared by method II; ■, dicumarol: β-CD=1: 1 prepared by method II; □, dicumarol: β-CD=1: 1 prepared by method I; △, dicumarol: β-CD=1: 2 physical mixture; ▲, dicumarol crystal powder.

Each point represents the mean of three determinations.

The solid dispersion systems prepared by method I and method II both exhibited faster drug dissolution than dicumarol alone or the physical mixture. The faster dissolution rates of the drug from the solid dispersion systems might be attributed to the presence of non-crystalline dicumarol dispersed in the systems and to co-dissolution with the water-soluble PVP molecule.[20] The sample of 1: 3 drug-to-PVP weight ratio might not be a complete solid dispersion system, because it contained some crystalline form. Its dissolution rate was inferior to those of other dispersion systems.

2. **Dicumarol-β-CD Systems**——The dissolution patterns of the systems of dicumarol–β-CD are shown in Fig. 5. In 1: 1 drug-to-β-CD weight ratio samples, the dissolution patterns were almost concordant, in spite of the difference of X-ray diffraction spectra. In 1: 2 samples,

the dissolution profile of dicumarol was rather superior in the sample prepared by method I to that in the sample prepared by method II. This phenomenon is in conflict with the X-ray diffraction data, because the potency of dissolution is generally considered to be higher in the noncrystalline state. Further investigation is clearly necessary.

In Vivo Absorption Studies of Dicumarol in Rabbits

Dicumarol in the solid dispersion systems was expected to show good bioavailability, because of the increased drug dissolution rates in aqueous solution. These solid dispersion systems and dicumarol alone were orally administered to gastric-emptying-time-controlled rabbits to evaluate their absorption characteristics.

1. **Dicumarol-PVP Systems**——Figure 6 shows the mean plasma levels of dicumarol following the oral administration of 1 : 5 weight ratio dicumarol–PVP solid dispersions and dicumarol alone to rabbits. The peak plasma levels were observed at 2—12 h (average at 8 h) postadministration in the case of dicumarol alone, and at 4—6 h postadministration in the cases of solid dispersion systems. The average plasma dicumarol levels following the administration of dispersion systems of 1 : 5 drug-to-PVP weight ratio prepared by methods I and II were higher than those of the control (dicumarol alone) after 2 h. Significant differences were observed at 4 and 24 h, and at 4 h postadministration for the systems prepared by methods I and II, respectively. Average AUC (0—48 h) values of dicumarol following the administration of the dicumarol–PVP solid dispersion systems were 3.31 times ($p<0.05$) and 1.54 times ($p>0.05$) that of the control (Table I).

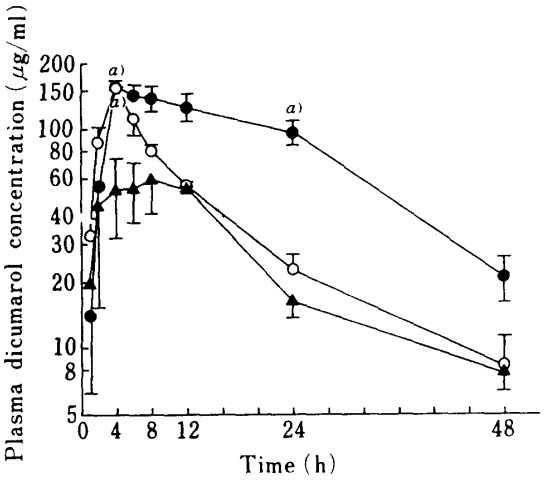

Fig. 6. Plasma Levels of Dicumarol following the Oral Administration of Dicumarol (▲) and Dicumarol–PVP Solid Dispersion Systems prepared by Method I (○) and Method II (●)

Each point represents the mean ± S.E. of three rabbits.
a) $p<0.05$ (*versus* dicumarol alone).
The weight ratio of dicumarol: PVP was 1 : 5.

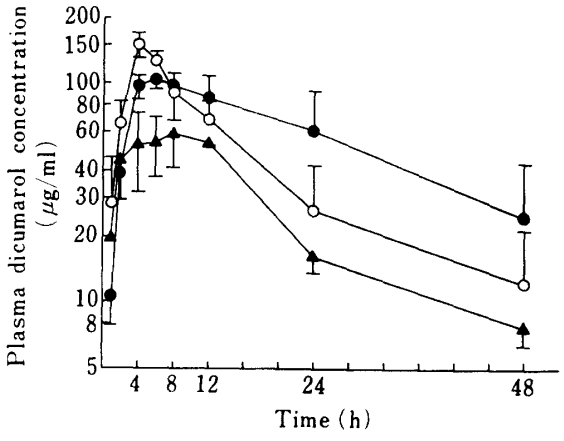

Fig. 7. Plasma Levels of Dicumarol following the Oral Administration of Dicumarol (▲) and Dicumarol–β-CD Solid Dispersion Systems prepared by Method I (○) and Method II (●)

Each point represents the mean ± S.E. of three rabbits.
The molar ratio of dicumarol: β-CD was 1 : 2.

2. **Dicumarol-β-CD Systems**——Figure 7 shows the plasma levels of dicumarol following the administration of dispersion systems of 1 : 2 drug-to-β-CD molar ratio prepared by methods I and II. The peak plasma levels of dicumarol were found at 6 and 4 h postadministration for dicumarol–β-CD solid dispersion systems prepared by methods I and II, respectively. The mean plasma levels were higher than those of the control from 4 to 48 h (method I) and from 1 to 48 h (method II), but the differences between these values and those of the control were not significant ($p>0.05$). Average AUC values (0<48 h)of dicumarol following the administration

TABLE I. Comparison of $AUC_{0\text{-}48h}$[a] Values following the Oral Administration of Dicumarol and Solid Dispersion Systems

Sample	$AUC_{0\text{-}48}$ h[b] (h·μg/ml)	Compared by the *t*-test (*versus* dicumarol alone)
Dicumarol alone	1236 ± 104	
Dicumarol-PVP (Method I)	4098 ± 456	$p<0.05$
Dicumarol-PVP (Method II)	1900 ± 53	$p<0.05$
Dicumarol-β-CD (Method I)	2698 ± 217	$p<0.05$
Dicumarol-β-CD (Method II)	2131 ± 488	N.S.[c]

a) Calculated by the trapezoidal method.
b) Each valus is the mean ± S.E. of three rabbits.
c) Not significantly different.

of the dicumarol–β-CD solid dispersion systems were 2.18 ($p<0.05$) and 1.72 (not significantly different) times that of the control (Table I).

General Discussion

From the *in vitro* dissolution behavior of the solid dispersion systems of dicumarol–PVP and dicumarol–β-CD prepared by methods I and II, and the *in vivo* absorption data, the following hypothesis may be proposed. After oral administration of the solid dispersion systems, dicumarol may dissolve rapidly in the gastrointestinal tract. Dicumarol in the fluid may be absorbed from the gastrointestinal tract rapidly. Following the administration of dicumarol alone, however, the drug may dissolve slowly and incompletely in the gastrointestinal tract. This poor dissolution may be the rate-determining step in the absorption process in individual subjects, and may depend on physiological conditions, resulting in large variations in bioavailability.

The present study showed that the use of solid dispersion systems of poorly water-soluble drugs may be effective in improving the bioavailability of such drugs.

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References and Notes

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