Enhanced Bioavailability of Probucol Following the Administration of Solid Dispersion Systems of Probucol–Polyvinylpyrrolidone in Rabbits

Yoshitada KUBO,* a Yuji TERASHIMA, a Naomi YAGI, a Hiromi NOCHI, b Koichi TAMOTO, b and Hitoshi SEKIKAWA a

* Department of Pharmaceutics, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido; 1757 Kamazawa, Ishikari-Tohetsu, Hokkaido 061–0923, Japan; and b Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University; 1314–1 Shido, Sanuki, Kagawa 769–2193, Japan. Received February 13, 2009; accepted September 4, 2009; published online September 7, 2009

Disks of probucol and solid dispersion systems of probucol–polyvinylpyrrolidone (PVP) in various weight ratios were prepared. Dissolution of probucol was markedly increased in the solid dispersion systems in J.P. XV disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8). The concentrations of probucol after the dissolution of the disks of solid dispersion systems showed supersaturation. Following the administration of disks of solid dispersion systems in rabbits, a marked increase in the area under the plasma concentration time curve (AUC) was observed. When the weight ratio of PVP to probucol was larger, a larger AUC was observed. When disks of the 1 : 9 solid dispersion system (weight ratio of probucol : PVP = 1 : 9) containing 50 and 100 mg probucol were respectively administered, AUC values were approximately proportional to the dose. AUC values following the administration of disks of the 1 : 9 solid dispersion systems containing 15 mg probucol (total weight: 150 mg) and 500 mg probucol were approximately equal. The mean half life (t1/2) was 12 h when disks of the 1 : 9 solid dispersion system were administered, whereas the t1/2 was 35 h when probucol disks were administered. The markedly increased dissolution of probucol in solid dispersion systems resulted in a marked increase in its bioavailability.

Key words probucol; solid dispersion system; rabbit; polyvinylpyrrolidone; bioavailability; dissolution

For poorly water-soluble drugs, the rate-limiting step in the absorption process is usually dissolution of the drug in the fluids of the gastrointestinal tract. Different products of the same drugs may have therapeutic inequivalence with regard to bioavailability even though the products meet existing official and compendium standards. Probucol, 4,4′-[(1-methylethylidene)bis(thio)]bis[2,6-bis-(1,1-dimethylethyl)] phenol, is a potent hypocholesterolemic agent. It lowers both low- and high-density lipoprotein cholesterol levels.

Yagi et al. reported the solubility and dissolution characteristics of probucol and of solid dispersion systems of probucol and polyvinylpyrrolidone (PVP). They found that the solubility of probucol in water at 25°C was only 5 ng/ml. Probucol could be one of the most insoluble drugs available today. However, several investigators have reported improved dissolution characteristics or bioavailability of probucol. Heeg et al. reported the bioavailability of [14C]probucol in an oil–water emulsion in rats. Shudo et al. reported on the pharmacokinetic profile of probucol after oral administration of various nanoparticles prepared by co-grinding probucol with PVP of various molecular weights and sodium dodecyl sulfate (SDS) in rats. Pongpeerap et al. reported on the mechanism of formation of colloidal nanoparticles obtained from a probucol/PVP/SDS ternary ground mixture. Thybo et al. reported on the characterization and physical stability of spray-dried solid dispersions of probucol and PVP K30.

We found that in solid dispersion systems of probucol and PVP prepared by the cocrystallization method, probucol dissolution was markedly increased. Although probucol has been used in the treatment of hypercholesterolemia, the pharmacokinetics and pharmacodynamics of the drug are still unknown. Blood levels vary greatly following the oral administration of probucol in humans. We speculated that the very low bioavailability of probucol could be due to poor absorption of the drug by the rate-limiting step of dissolution in the gastrointestinal tract.

In this study, we prepared disks of probucol and of solid dispersion systems of probucol and PVP, and compared probucol dissolution using these disks. We also studied the absorption of probucol following administration of the disks in rabbits.

MATERIALS AND METHODS

Materials Probucol powders were prepared by the method reported by Yagi et al. The mean diameter of the probucol powder, measured by optical microscopy (BH microscope, Olympus Co., Tokyo, Japan) was 119.7 ± 53.6 μm (Green diameter, mean ± S.D., n = 100). PVP K30 (average molecular weight of 40000) was obtained from Nacalai Tesque, Inc., Kyoto, Japan. Reagent grade pyrene was obtained from Wako Pure Chemical Inc., Ltd., Osaka, Japan. Acetonitrile of HPLC grade was obtained from Kanto Chemical Co., Inc., Tokyo, Japan. Sodium pentobarbital injection (Nembatal) was obtained from Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. All other chemicals were of reagent grade.

Preparation of Solid Dispersion Systems of Probucol–PVP The solid dispersion systems of probucol–PVP were prepared as follows: After dissolving probucol and PVP in a suitable weight ratio in ethanol, the solvent was removed in vacuo using a rotary evaporator at about 35°C. Then, the residue was dried in vacuo at room temperature for 24 h. The preparation was ground in a mortar, passed through a sieve (J.P. XV No. 100) and stored in a light-resistant tight container at room temperature.

Preparation of Disks of Probucol and Solid Dispersion Systems of Probucol–PVP The 1 : 6, 1 : 8 and 1 : 9 solid dispersion systems (weight ratio of probucol : PVP) were pre-
pared by the method described in a previous report. Probu-
col powder (250 mg), solid dispersion systems or 1 : 9 physical
mixtures (probucol : PVP in weight ratio, containing 50 mg probucol) were formed into disks (10 mm in diameter) using a press (Handpress, Iuchi Co., Osaka, Japan), and were then pressed again using a High Pressure Jack (Iuchi Co.). The thickness of the disks ranged from 3 to 9 mm. The mean hardness of the disks measured using a Montsant Hardmeter (Type 1441, Kayagaki Rika Kogyo Co., Tokyo, Japan) was 3.93 ± 0.144 kg (mean ± S.E.M., n = 3).

**Dissolution Studies** Dissolution of probucol from the powder or from disks in 500 ml of J.P. XV disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8) was measured at 37.0 ± 0.5 °C in a J.P. XV dissolution test apparatus (Toyama Sangyo Co., Osaka, Japan). The paddle was rotated at 150 rpm. Each sample powder or disk was directly transferred into the dissolution medium. A suitable aliquot was removed at an appropriate time using a syringe and was then filtered through a membrane filter (cellulose nitrate, pore size, 0.45 μm, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate (1 ml) was mixed with 0.5 ml internal standard solution and analyzed for probucol by HPLC.

**Animals** White male rabbits (Clea Japan, Inc., Tokyo, Japan) weighing 2.3 to 3.4 kg and maintained at the Center for Experimental Animals, Health Sciences University of Hokkaido, were used in this study. The animal experiments were performed in accordance with The Guidelines for Animal Experiments at the Health Science University of Hokkaido.

**Absorption Studies** Rabbits were used after a stomach-emptying-time controlling treatment. The rabbits were anesthetized with sodium pentobarbital (20 mg/kg) and administered the disks containing two disks of probucol (containing 500 mg probucol) or disks of solid dispersion systems (50 and 100 mg probucol) with 30 ml water. About 50 g of soft diet (Clea Japan, Inc., Tokyo, Japan) was fed after the rabbits woke up. Water was allowed ad libitum. Blood samples (1 ml) were collected from an ear vein using a heparinized syringe at appropriate times up to 120 h (5 d). Blood samples were centrifuged (3000 rpm, 10 min), and the plasma samples obtained were frozen and stored in the freezer until analysis. Rabbits were prevented from prophagy by fitting muzzles during the night.

**Analytical Procedure for Probucol** The analytical procedure for the dissolution study of probucol in aqueous solution was by the HPLC method reported by Yagi et al. The analytical procedure for probucol in plasma was as follows: Plasma (0.3 ml) was deproteinized by the addition of 1 ml of methanol–acetone (3 : 2) and was then centrifuged for 10 min at 3000 rpm. The supernatant was passed through a membrane filter (Columgard-LCR13, pore size 0.5 μm, Nihon Millipore Kogyo Co., Yonezawa, Japan). The filtrate (0.5 ml) was mixed with 0.2 ml of internal standard solution and 1.0 ml of n-hexane, shaken for 10 min, and centrifuged for 10 min at 3000 rpm. The hexane layer (0.6 ml) was dried by a stream of nitrogen gas. The residue was dissolved in 0.5 ml acetonitrile and injected into the HPLC system. The conditions of HPLC were the same as in the method reported by Yagi et al. except for using the mobile phase reported by Satonin and Coutant with some modifications. The mobile phase consisted of 90% acetonitrile, 3.5% hexane and 6.5% ammonium acetate. The retention times of probucol and the internal standard were 10.2 and 5.1 min, respectively. The detection limit of probucol was 10 ng/ml.

**Protection of Photolysis** During the study, all processes were carefully protected from the photolysis of probucol.

**Pharmacokinetic Analysis** The parameters of the area under the plasma concentration time curve (AUC), the maximum plasma concentration (C<sub>max</sub>), the time (T<sub>max</sub>), the absorption constant (k<sub>a</sub>) and the elimination constant (k<sub>e</sub>) were estimated by the one-compartment open model with absorption having a first-order process as reported by Yamaoka et al. These variables were measured using a microcomputer (model 9801, NEC Co., Tokyo, Japan). Statistical analysis was performed using Student’s t-test with p < 0.05% as the minimum level of significance.

**RESULTS**

**Dissolution Profiles of Probucol from Powders and Disks of Probucol–PVP Solid Dispersion Systems** Figure 1 shows the dissolution profiles of probucol from powdered probucol–PVP solid dispersion systems in J.P. XV disintegration media No. 1 (pH 1.2) and No. 2 (pH 6.8). When powdered probucol and physical mixture were dissolved, probucol concentrations were under the detection limit (10 ng/ml) and are not shown in the figure.

Figure 2 shows the dissolution profiles of probucol from disks of probucol–PVP solid dispersion systems in J.P. XV disintegration media No. 1 and No. 2. The dissolution of probucol from the disks could not be estimated because its concentration was under the detection limit (10 ng/ml) in the assay. Furthermore, the dissolution of probucol from the...
disks of the physical mixture could not be estimated. The dissolution of probucol from disks of the 1:9 (weight ratio of probucol:PVP) solid dispersion system showed excellent profiles in both media. Dissolution profiles of probucol from disks or powders21 were similar in both media. Disks of the 1:8 and 1:6 solid dispersion systems showed lower dissolution of probucol than their respective powders. When powders of the 1:6 solid dispersion systems were dissolved in both media, probucol concentration reached 8—10% of the dissolved amount. However, when disks of the 1:6 solid dispersion system having equal amounts of probucol were dissolved, probucol concentrations were 0.3—2.3%. Fine crystals of probucol were observed in the dissolution medium at 24 h.

**Pharmacokinetic Parameters of Probucol Following Oral Administration of Probucol and Solid Dispersion Systems in Rabbits**

Table 1. Pharmacokinetic Parameters of Probucol Following Oral Administration of Probucol (500 mg) and Solid Dispersion Systems (100 mg Probucol) in Rabbits

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg)</th>
<th>AUC (µg h/ml)</th>
<th>Cmax (µg/ml)</th>
<th>Tmax (h)</th>
<th>k1/2 (×10⁻² h⁻¹)</th>
<th>kEL (×10⁻² h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probucol Solid dispersion systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:9w</td>
<td>100</td>
<td>21.57 ± 4.03</td>
<td>0.52 ± 0.05</td>
<td>15.58 ± 1.07</td>
<td>7.92 ± 0.73</td>
<td>5.63 ± 0.83</td>
</tr>
<tr>
<td>1:8w</td>
<td>100</td>
<td>13.74 ± 4.16</td>
<td>0.30 ± 0.09</td>
<td>15.01 ± 1.14</td>
<td>10.57 ± 2.86</td>
<td>4.51 ± 1.00</td>
</tr>
<tr>
<td>1:6w</td>
<td>100</td>
<td>3.22 ± 0.72</td>
<td>0.05 ± 0.01</td>
<td>21.75 ± 1.06</td>
<td>4.77 ± 0.25</td>
<td>4.51 ± 0.24</td>
</tr>
<tr>
<td>1:9w</td>
<td>50</td>
<td>9.23 ± 0.08</td>
<td>0.26 ± 0.03</td>
<td>14.10 ± 2.13</td>
<td>8.49 ± 1.32</td>
<td>7.05 ± 1.48</td>
</tr>
</tbody>
</table>

a) Each data represents the mean ± S.E.M. of 5 rabbits. b) Each data represents the mean ± S.E.M. of 3 rabbits. c) Significantly different from probucol at p<0.05.

**Fig. 3. Plasma Concentrations of Probucol Following Oral Administration of Probucol (500 mg) and Solid Dispersion Systems (100 mg Probucol) in Rabbits**

- ○, probucol; △, 1:6 solid dispersion system; ▽, 1:8 solid dispersion system; ○, 1:9 solid dispersion system. Each point represents mean ± S.E.M. of three or five rabbits.

**Fig. 4. Plasma Concentrations of Probucol Following Oral Administration of 1:9 Solid Dispersion Systems in Rabbits**

- ○, 100 mg probucol; ▪, 50 mg probucol. Each point represents mean ± S.E.M. of three (50 mg) or five (100 mg) rabbits.
administration was 37 h. On the other hand, although the amount of probucol was 100 mg following the administration of disks of the 1:9, 1:8 and 1:6 solid dispersion systems, the AUC values were 8, 5 and 1.1 times the AUC values after the administration of disks containing 500 mg probucol. The values were 40, 25 and 5.5 times larger, respectively, if the dose of solid dispersion systems contained equal amounts of probucol. Following the administration of the disks of 1:9, 1:8 and 1:6 solid dispersion systems, $T_{\text{max}}$ values were observed at 16 h, 15 h and 22 h, respectively. The values were one third or one half of those found for probucol disks. Following the administration of the disks of 1:9 and 1:8 solid dispersion systems, $C_{\text{max}}$ values were 15 and 8 times higher, respectively, than those for probucol disks.

### Dose Dependence of 1:9 Solid Dispersion Systems on AUC of Probucol

Relation of the dose dependence of 1:9 solid dispersion systems and AUC is shown in Fig. 5. After the administration of one disk (50 mg probucol) or two disks (100 mg) in rabbits, $T_{\text{max}}$ was observed at 15.6 and 14.1 h, respectively. The values were significantly smaller ($p<0.05$) than those after probucol disks were administered. Values of $T_{\text{max}}$ were not significantly different between 50 mg and 100 mg of the 1:9 solid dispersion system. When disks of solid dispersion systems containing 50 mg probucol were administered, the mean AUC value (9.2 μg·h·ml⁻¹) was approximately half of that when disks of solid dispersion systems containing 100 mg probucol were administered (21.6 μg·h·ml⁻¹).

### DISCUSSION

Because of the low solubility of probucol in water, the absorption of probucol from the gastrointestinal tract is poor. Solid dispersion systems of probucol–PVP markedly improved its dissolution characteristics.³ Yagi et al. reported that dissolution of solid dispersion systems of probucol and PVP (1:9) in J.P. XII disintegration medium No. 1 (pH 1.2) and No. 2 (pH 6.8).³ PVP K25, K30 and K90 (average molecular weight of 25000, 40000 and 1200000, respectively) were used in the study. The dissolution of solid dispersion systems with PVP K30 showed highest concentration of probucol in both J.P. XII disintegration media No. 1 and No. 2. In the present study, we examined the dissolution and absorption of probucol from disks of solid dispersion systems or probucol itself. When powders of the solid dispersion systems were dissolved, the 1:9 and 1:8 systems showed a marked dissolution of probucol. The dissolution profiles of probucol were higher for the 1:9 systems than for the 1:8 systems. On the other hand, the 1:6 system showed increased dissolution; however, the concentrations were lower than those for the 1:9 or 1:8 systems. No crystalline probucol was found in the 1:9, 1:8 and 1:6 solid dispersion systems.³ Sekikawa et al. reported on the dissolution mechanisms of drugs from solid dispersion systems.³ Since PVP was coacervated by the addition of some electrolytes or aromatic compound.²⁶ When the solid dispersion systems were dissolved in water, drugs dispersed in PVP dissolved via small droplets of coacervates (micro-coacervates). They reported the dissolution process of coprecipitates (solid dispersion systems) of sulfisoxazole and PVP by photomicrographs. By addition of water to the coprecipitates, micro-coacervates were observed in the vicinity of the coprecipitates. They disappeared upon addition of a further amount of water. When concentrations of the drugs in the micro-coacervates were large, the dissolution rates of micro-coacervates were small.

The dissolution of probucol was less from the disks than from the powders. We assume that the specific surface area available for dissolution could be smaller in the disks. Penetration of water into the disks could be another reason for the lower dissolution. In the case of powders, interparticle void was considerably large. Water penetrated easily into void. Dissolution might occur after the hydration of the PVP molecule in the solid dispersion system. The interparticle void might be very small in the disks. Hydration might begin at the surface of the disks. As the viscosity of the surface might be large, water penetration into the disks seemed to be hard. Further penetration of water into the disks might occur by dissolution of hydrated PVP and probucol at the surface.

When preparing the solid dispersion systems of drug and PVP, PVP inhibits the crystallization of drugs during evaporating the solvent.²¹ In the dissolution study, concentrations of probucol exceeded the solubility. PVP in solution might inhibit its recrystallization.²¹ DiNunzio et al. reported the supersaturated dissolution in solid dispersion systems of itraconazole and cellulose acetated phthalate and polyvinyl acetate phthalate.²² Kohri et al. also reported the supersaturated solution by dissolution of the solid dispersion systems of albendazole with hydroxypropylmethylcellulose and hydroxymethylpropylcellulose phthalate.²³ In the absorption study, AUC values well reflected the results of the dissolution studies. The mean value of AUC following the administration of 1:9 solid dispersion system containing 100 mg probucol was eight times larger to that following the administration of 500 mg probucol. It meant 40 times increase of AUC if the equivalent doses were administered. Such increased AUC values were never reported when solid dispersion systems of drugs were studied. The reason might be due to the marked increased dissolution characteristics of probucol. Following the administration of a single dose of 3 g probucol to healthy human volunteers, mean peak plasma concentration of 3.9 μg/ml were observed at 24 h.²⁴ In that study, blood levels showed a diphasic elimination, with $t_{1/2}$ of about 1 and 23 d, and the values varied considerably. In the present study, when disks of the 1:9 solid dispersion system were administered in rabbits, the mean $T_{\text{max}}$ was found at 16 h (100 mg probucol equivalent) and 14 h (50 mg probucol equivalent), respectively. The mean $T_{\text{max}}$ was ob-
served at 37 h when disks of probucol were administered. The mean $t_{1/2}$ were found to be 12 h (100 mg probucol equivalent) and 10 h (50 mg probucol equivalent) when disks of the 1 : 9 solid dispersion system were administered. The mean $t_{1/2}$ of probucol was found to be 35 h when probucol disks were administered. The elimination of probucol itself could be equal when probucol or solid dispersion systems are administered. The larger value of the $t_{1/2}$ when probucol disks were administered could be the effect of continuous absorption of poorly water-soluble probucol in the gastrointestinal tract.

The present study showed that improvement of the dissolution characteristics of probucol by preparing solid dispersion systems with PVP resulted in an increase in the rate and extent of bioavailability with less variation. This method of increasing the dissolution characteristics of the poorly water-soluble drugs by preparing solid dispersion systems with PVP may be applicable in the processes for improving the bioavailability of other drugs.

Acknowledgements This work was supported by the High Technology Research Projects of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES