Preparation and in Vivo Ocular Absorption Studies of Disulfiram Solid Dispersion

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Disulfiram, a dimer of diethylthiocarbamate (DDC) which is a strong radical scavenger, is known to pre-
vent cataract development. However, disulfiram is hardly absorbed from the cornea and its bioavailability is ex-
tremely low. In this study, we attempted to prepare disulfiram solid dispersion for the improvement of ocular
bioavailability.

Solid dispersions of disulfiram were prepared by either an evaporation method or a spray-drying method,
using polyvinylpyrrolidone (PVP) as a carrier. Preparations were analyzed by scanning electron microscopy,
powder X-ray diffractometry and differential scanning calorimetry, and confirmed to be a solid dispersion. The
particle size of the solid dispersion prepared by the spray-drying method was smaller than the preparation by
the evaporation method (spray-drying: 3.3±0.04 μm, evaporation: 34.3±18.0 μm). An in vivo ocular absorption
experiment was conducted by instilling solid dispersions to rabbit eye and measuring the DDC in the aqueous
humor. After instillation of disulfiram and PVP physical mixture, DDC was not detected in the aqueous humor.
On the other hand, DDC appeared in the aqueous humor after the instillation of a solid dispersion. Maximal
concentration and the area under the aqueous humor concentration–time curve were greater in the solid disper-
sion prepared by the spray-drying method than the preparation by the evaporation method.

Disulfiram solid dispersion, especially prepared by the spray-drying method, improved ocular bioavail-
bility.

Key words solid dispersion; ocular absorption; anti-cataract drug; spray-drying; polyvinylpyrrolidone; powder X-ray diffraction

Cataracts are a major cause of blindness, particularly in developed countries, and are among the most common
diseases in aged people.1,2) Many investigations have been done to clarify the mechanism of lens opacifica-
tion. To date, it is considered that oxidative stress strongly participates in cataract formation.3) Highly reactive oxygen species which are generated mainly by ultraviolet waves in sunlight, react with proteins, lipids and the DNA of lens cells, and those cause irreversible damage, leading to lens opacification.3)

Disulfiram has long been used for the treatment of alco-
holic syndrome without severe inconvenience.4) As illus-
trated in Fig. 1, disulfiram is a diethylthiocarbamate (DDC). DDC is a powerful antioxidant since it scav-
enges reactive oxygen species such as hydroxyl radicals, su-
peroxide and peroxynitrite, and it chelates metal ions.5) We
previously reported that disulfiram is converted to DDC in the cornea and aqueous humor, and also reduces the risk of
lens opacification.6)

To achieve effective ophthalmic therapy, adequate amounts of active ingredient must be delivered and sustained at its site of action within eye. The eye is a highly protective organ from exogenous compounds, including the cornea, sclera and conjunctiva epithelial barriers. Of those of barriers, the cornea is thought to be the most effective, excluding instilled drugs from the internal eye.7) Consequently, limited instilled ophthalmic drugs penetrate the cornea and its bioavailability is very low. Many attempts have been made to improve oph-
thalmic drug absorption from the cornea, such as using lipo-
somes, mucoadhesive gel and implanted devices.8–11)

Solid dispersions have been introduced to improve the ab-
sorption of poorly water-soluble drugs from the gastrointesti-
nal tract by using a water-soluble inert carrier.12–18) Many
successes in the preparation of solid dispersions of drugs,

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(tacrolimus),15) have been reported. Several polymers such as polyethylene glycol, hydroxypropylmethylcellulose and polyvinylpyrrolidone (PVP) have been used to form solid dispersions.12–18) PVP is a widely used nontoxic carrier and is known to prevent drug crystallization in solid dispers-
ions.16–18) In this study, we prepared a solid dispersion of disulfiram using PVP as a carrier by two different methods, evaporation and spray-drying, and compared their ocular absor-
sorption characteristics.

MATERIALS AND METHODS

Materials Disulfiram was obtained from Ouchi Shinko

Chemical (Tokyo, Japan). PVP (K = 30, molecular weight

25000–30000) was purchased from Kanto Chemical

(Tokyo, Japan), bovine serum albumin was obtained from

Wako Pure Chemical Industries (Osaka, Japan). All other

chemicals used were of the highest purity available.

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\begin{align*}
\text{C}_6\text{H}_5 & \quad \text{N} \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{C}_6\text{H}_5 \\
\text{C}_6\text{H}_5 & \quad \text{N} \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{C}_6\text{H}_5 \\
\text{C}_6\text{H}_5 & \quad \text{N} \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{C}_6\text{H}_5 \\
\end{align*}
\]

Fig. 1. Structures of Disulfiram and DDC and Scheme of Disulfiram De-

livery in the Eye

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Preparation of Solid Dispersion by Evaporation Method  
Ten grams of disulfiram were dissolved in 50 ml of acetone, and 10.0 g of PVP was dissolved in 50 ml of ethanol. After mixing both solutions, there was no precipitate in the mixture, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40 °C. Then, the preparation was dried under reduced pressure for 24 h. Preparations were ground by an agate mortar and pestle and were sieved to a particle diameter of less than 75 μm, because JPXIII defines that the particle size of an ophthalmic suspension must be less than 75 μm. To test the effect of the drug-to-polymer ratio, the solid dispersion was prepared with changes in the disulfiram and PVP weight ratios. The total weight of disulfiram and PVP was set at 20.0 g.

Preparation of Solid Dispersion by Spray-Drying Method  
The same solution as that for the evaporation method was fed into a spray-dryer (Model SD-1, Tokyo Rikakiki, Tokyo, Japan). The temperature at the inlet of the drying chamber was maintained at 60 °C. The physical mixture was prepared by thoroughly mixing disulfiram and PVP in an agate mortar and passing it through a 75 μm sieve to confirm that the preparation met the requirements of JPXIII.

Physicochemical Analysis of Solid Dispersion  
The solid dispersions were observed with a scanning electron microscope (Model S-700, Hitachi, Tokyo, Japan) to examine their shape and surface characteristics. The particle diameter of the solid dispersion was determined by a Coulter N4S laser diffraction scattering particle analyzer (Coulter, FL, U.S.A.) or a micrometer with an optical microscope. Powder X-ray diffraction was carried out with a RINT 2000 (Rigaku Denki, Tokyo, Japan) under the conditions of CuKα, 40 kV, 80 mA. The scanning rate was 3°/min over a 2θ range of 10–40° and with a sampling interval of 0.02°. A differential scanning calorimetric study was performed using a DSC5240D (Rigaku Denki, Tokyo, Japan) at a heating rate of 3 K/min.

In Vitro DDC Release Experiment  
Prepared disulfiram solid dispersion (1%) and bovine serum albumin (3.5%) were incubated at 37 °C in sodium phosphate buffer (0.35 m Na₂HPO₄, 0.01 m EDTA, pH 9.5). Aliquots of the incubation mixture were ultrafiltrated by Centrisart 1 (cut off molecular weight 10000, Sartorius, Göttingen, Germany), and the DDC released in the filtrates was measured by HPLC using indomethacin as an internal standard. The conditions of HPLC are as follows: column, Wakosil 3C8, 4.6×150 mm (Wako Pure Chemical Industries, Osaka, Japan); mobile phase, 0.1% trifluoroacetic acid in 60% acetonitrile; detection, 215 nm.

In Vivo Absorption Experiment  
Prepared disulfiram solid dispersions were suspended immediately before instillation in a solution containing 5.60 g of sodium dihydrogenphosphate, 2.84 g of disodium hydrogenphosphate, 0.26 g of methyl p-hydroxybenzoate and 0.14 g of n-propyl p-hydroxybenzoate in 1000 ml of distilled water. Fifty microliters of 1% solid dispersion suspension were instilled in the eye of male Japanese albino rabbits (2 kg). After 5 to 60 min of instillation, aqueous humor samples were taken by a 27-gauge needle. The concentration of DDC in the aqueous humor samples was measured by HPLC using indomethacin as an internal standard. The conditions of HPLC were the same as in the in vitro DDC release experiment. The area under aqueous humor concentration-time curve (AUC) was calculated by the trapezoidal rule up to the last measured aqueous humor concentration. The area under the first moment curve (AUMC) was also calculated by the trapezoidal rule and mean residence time (MRT) was calculated as AUMC/AUC. AUCID was calculated by dividing the AUC by the disulfiram amount in the instilled solid dispersion suspension.

RESULTS AND DISCUSSION

Physicochemical Properties of Disulfiram Solid Dispersion  
Figure 2 shows scanning electron micrographs of prepared disulfiram solid dispersions. The particle diameter of the solid dispersion (disulfiram : PVP=1 : 1) prepared by the spray-drying method was smaller than that by the evaporation method (spray-drying, 3.28±0.04; evaporation, 34.3±18.0, μm, mean±S.D. of 5 measurements). Particle diameters were also different in preparations by the spray-drying method using different weight ratios of disulfiram to PVP (1 : 1, 3.28±0.04; 1 : 2, 1.24±0.21; 1 : 5.7, 2.39±0.27, disulfiram : PVP, μm, mean±S.D. of 3—5 measurements). Solid dispersion prepared by the spray-drying method using the disulfiram-to-PVP ratio of 1 : 2 had the smallest particle diameter in our preparations.

Figure 3 depicts the powder X-ray diffraction patterns of physical mixture and solid dispersion prepared by the evaporation or spray-drying method using a disulfiram-to-PVP weight ratio of 1 : 1. While the diffraction pattern of the physical mixture showed sharp peaks due to crystal, small peaks were observed in the diffraction patterns of solid dispersions. The degree of crystallinity was estimated based on the X-ray diffraction intensities at 2θ=14.2°, and the crystallinity of pure drug was set to 100%. Disulfiram crystallinity was de-
creased in the solid dispersion prepared by the vaporization method (46.7%) and by the spray-drying method (14.1%). Figure 4 shows the differential scanning calorimetric thermograms of physical mixture and solid dispersion prepared by a spray-drying method using a disulfiram-to-PVP weight ratio of 1:1. While the thermogram of the disulfiram and PVP physical mixture showed a sharp peak at about 75°C (Fig. 4A), a small peak was found in the thermogram of the solid dispersion (Fig. 4B). Figure 5 summarizes enthalpy changes in the physical mixture and solid dispersions. The physical mixtures at any ratios of disulfiram to PVP did not affect the ΔH value. However, the ΔH values of solid dispersions were decreased as the ratio of disulfiram to PVP decreased, suggesting a decrease in crystallinity.

By microscopy, powder X-ray diffractometry and thermal analysis, we confirmed that solid dispersions could be obtained by evaporation or spray-drying methods. The shape and particle diameter of the preparations by the two methods were quite different. Solid dispersions by the spray-drying method had a spherical shape and small diameter, whereas preparations by the evaporation method had a rough shape and large diameter (Fig. 2).

It is considered that the particle diameter of an ophthalmic drug plays an important role in the irritation potential on the eye. To minimize potential irritation to the eye, the particle size should be less than 10 μm. Increasing the size significantly beyond this value may increase the elimination of the instilled drug through patient discomfort and tearing, and the rapid drainage could reduce bioavailability. The shape of the drug is an additional factor that affects irritation and discomfort to the eye. From these viewpoints, disulfiram solid dispersion prepared by the spray-drying method would be better suited to ophthalmic application.

**In Vitro DDC Release from Disulfiram Solid Dispersion**

It is reported that DDC is produced by the interaction of disulfiram with protein which has a free-SH group, such as albumin. The *in vitro* DDC release characteristics from disulfiram solid dispersions were investigated using bovine serum albumin. In the absence of albumin, neither DDC nor disulfiram were detected in the water phase excluded by ultracentrifugation. On the other hand, in the presence of albumin, DDC was released from disulfiram solid dispersions (Fig. 6). The apparent DDC release rate was calculated from the slope in Fig. 6. The DDC release rate from disulfiram solid dispersion prepared by the evaporation method (0.48 μg/min) was about 40-fold higher than that of the physical mixture (0.013 μg/min). In solid dispersions prepared by two methods, the apparent DDC release rate was greater in the preparation created by the spray-drying method (1.07 μg/min). It is considered that disulfiram was first dissolved from solid dispersion, then the dissolved disulfiram reacts with albumin, and free DDC is released. Therefore, the higher DDC release from the solid dispersion prepared by the spray-drying method would mean higher disulfiram dissolution compared with preparation by the evaporation method.

**In Vivo Absorption Characteristics of Disulfiram Solid**

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**Fig. 3.** Powder X-ray Diffraction Patterns of Disulfiram and PVP Physical Mixture (A), Disulfiram Solid Dispersion Prepared by the Evaporation Method (B) or the Spray-Drying Method (C) Using a Disulfiram-to-PVP Weight Ratio of 1:1

**Fig. 4.** Differential Scanning Calorimetric Thermograms of Disulfiram and PVP Physical Mixture (A) and Disulfiram Solid Dispersion Prepared by the Spray-Drying Method (B) Using a Disulfiram-to-PVP Weight Ratio of 1:1

Both analyzed preparations contained equal amounts of disulfiram (1.7 mg).

**Fig. 5.** Effect of Disulfiram Content in Solid Dispersion on Enthalpy Change

Δ, physical mixtures of disulfiram and PVP; ○, solid dispersions prepared by the evaporation method; ●, solid dispersions prepared by the spray-drying method.

**Fig. 6.** Released DDC Concentrations from Physical Mixtures of Disulfiram and PVP (Δ), Solid Dispersions Prepared by the Evaporation Method (○) or Solid Dispersions Prepared by the Spray-Drying Method (●) Using a Disulfiram-to-PVP Weight Ratio of 1:1

Each point represents the mean ± S.D. of 3—6 determinations.
Fig. 7. DDC Concentrations in Aqueous Humor after the Instillation of Disulfiram Solid Dispersions Using a Disulfiram-to-PVP Weight Ratio of 1:1

Δ, physical mixtures of disulfiram and PVP; ○, solid dispersions prepared by the spray-drying method; •, solid dispersions prepared by the spray-drying method. Each point represents the mean±S.D. of three animals.

Table 1. In Vivo Absorption Characteristics of Disulfiram Solid Dispersion

<table>
<thead>
<tr>
<th>(Disulfiram : PVP)</th>
<th>Cmax (μg)</th>
<th>AUC (ms·min)</th>
<th>AUC/D (ms·min/mg)</th>
<th>MRT (min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Evaporation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(1:1)</td>
<td>13.1±0.9</td>
<td>0.58±0.20</td>
<td>2.07±0.7</td>
<td>33.4±0.1</td>
</tr>
<tr>
<td>Spray-drying</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1:1)</td>
<td>33.4±4.4</td>
<td>1.24±0.11</td>
<td>4.97±0.4</td>
<td>28.6±1.6</td>
</tr>
<tr>
<td>(1:2)</td>
<td>44.7±4.5</td>
<td>1.56±0.05</td>
<td>9.37±0.3</td>
<td>25.4±0.8</td>
</tr>
<tr>
<td>(1:5:7)</td>
<td>20.1±7.0</td>
<td>0.57±0.11</td>
<td>7.63±1.5</td>
<td>24.2±0.4</td>
</tr>
</tbody>
</table>

Data represent the mean±S.D. of three animals.

Dispersion The in vivo absorption characteristics of prepared disulfiram solid dispersion were investigated using rabbits. First, to investigate how differences in the in vitro DDC release rates from physical mixture or solid dispersions would affect the in vivo absorption characteristics, we compared the in vivo ocular absorption of physical mixture and solid dispersions prepared by two methods using a disulfiram-to-PVP weight ratio of 1:1. Neither DDC nor disulfiram were detected in the aqueous humor after instillation of the disulfiram and PVP physical mixture (Fig. 7). On the other hand, DDC appeared in the aqueous humor after instillation of solid dispersions (Fig. 7). The DDC concentration reached a maximum at 10 min after instillation of the solid dispersion prepared by the spray-drying method, which was faster than that of the evaporation method. The in vivo absorption characteristics of disulfiram solid dispersions are summarized in Table 1. The Cmax and AUC values of solid dispersion prepared by the spray-drying method were higher than those of the evaporation method. Higher DDC release rate from the solid dispersion prepared by the spray-drying method resulted in a large improvement of the rate and extent of ocular bioavailability. Large differences between the solid dispersion and physical mixture in vivo ocular absorption also would be due to the great differences of their in vitro DDC release rates. However, the possibility that factors besides solid dispersion, such as the viscosity of the preparations, would affect the in vivo absorption characteristics, were not fully ruled out at this time.

The in vivo absorption characteristics of disulfiram solid dispersions prepared by the spray-drying method using different disulfiram-to-PVP weight ratios were also investigated (Fig. 8). The Cmax and AUC values were highest in preparations using a disulfiram-to-PVP ratio of 1:2. However, it is difficult to compare their ocular bioavailabilities from Cmax and AUC values, because the disulfiram amounts were different in each preparation. Therefore, we calculated and compared the AUC/D (ms·min/mg disulfiram in instilled solid dispersion suspension, Table 1). The AUC/D value was highest in the preparation using a disulfiram-to-PVP ratio of 1:2, and was lowest with a disulfiram-to-PVP ratio of 1:1. Particle diameter and crystallinity differed in the prepared solid dispersions when different disulfiram-to-PVP weight ratios were used. These differences would affect the in vivo ocular bioavailability.

The results shown in this study suggest that a solid dispersion has great possibilities in selective ophthalmic drug delivery and improved bioavailability. A solid dispersion can be easily and inexpensively prepared from nontoxic materials. However, there are many further aspects to be studied for their routine use. For example, studies concerning the sterility and stability of solid dispersions should take place.

In conclusion, a disulfiram solid dispersion prepared by a spray-drying method using a disulfiram-to-PVP ratio of 1:2 has a spherical shape and small particle size, and its bioavailability is highest in our preparations. Disulfiram solid dispersion thus has great potential as an anti-cataract agent.

REFERENCES