Pharmaceutical Properties of Freeze-Dried Formulations of Egg Albumin, Several Drugs and Olive Oil

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The freeze-dried ternary formulations of meclizine (MZ, an anti-motion sickness drug), prednisolone (PRED, an anti-inflammatory drug) and norfloxacin (NFLX, an anti-microbial drug) which are poorly water-soluble and are low bioavailability drugs, were prepared using egg albumin and olive oil. The powder X-ray diffractions, the dissolution rate and the bioavailabilities in vivo of these formulations were studied in comparison with each drug alone. By forming ternary formulations of these drugs, the dissolution rates of the drugs from the formulations were significantly improved compared with each drug alone. The results of their powder X-ray diffraction measurements showed that these drugs in the ternary formulations presented in an amorphous form, indicating increased dissolution rates. On the other hand, the plasma concentrations of these drugs increased significantly after oral administration in formulations to rats, except for the NFLX formulation, and the areas under the concentration–time curves (AUC) of the ternary formulations of MZ, PRED and NFLX were 2.1, 1.6 and 1.3 times those of the drugs alone, respectively. From these results, it was proven that formulations consisting of egg albumin, olive oil and poorly water-soluble drugs were useful preparations for improving the drug’s disadvantageous pharmaceutical properties.

Key words formulation; meclizine; prednisolone; norfloxacin; egg albumin; olive oil

In the process of the development of a new drug, we have occasionally experienced that a compound showing biological activity in vitro does not exhibit the same activity when administered orally because of limited bioavailability, and we have given up the development for that reason. It is well known that the bioavailability of poorly water-soluble drugs is influenced by dissolution rate. Accordingly, it is very important to enhance the absorption of such a compound in order to succeed in the development of new drugs. To date, many efforts have been made to increase the dissolution rate of poorly water-soluble drugs and many techniques, including particle size reduction, solvent deposition, lyophilization, solvate formulation and solid dispersion etc., have been reported as methods for an increased dissolution rate.1

In general, to improve such disadvantageous pharmaceutical properties as poor water solubility and low bioavailability, synthetic polymers have been most commonly used as drug carriers,2–4 but some researchers have recently focused on natural polymers such as egg albumin or gelatin, being safe to human, as drug carriers.5–11 On the other hand, it has been reported that several fatty acids and glycerides enhance the intestinal absorption of some drugs.12–16 Previously, we developed a new technique, pulverizing oily substances such as tocopherol, lamprey oil and various vegetable oils to powder using egg albumin.17 On the basis of these findings, we modified the new technique to make it applicable to the improvement of disadvantageous pharmaceutical and pharmaceutical properties of drugs, and the modified method, forming a ternary formulation of drug using egg albumin and olive oil or fatty acids, was applied to indomethacin (IND), a very poorly water-soluble drug, which has widely been used clinically as an anti-inflammatory drug, to increase its solubility in water.18,19 As a result, the IND formulation showed an increase in the dissolution rate, in the AUC value and, furthermore, in the anti-inflammatory action of IND, compared with IND alone or a freeze-dried albumin–IND complex. Also, this formulation exhibited a lower adverse effect, ulcerogenicity, than that of IND alone or the freeze-dried albumin–IND complex.

In this work, to investigate whether the modified method is useful for improvement of the bioavailability of other poorly water-soluble drugs, meclizine (MZ, an anti-motion sickness drug), prednisolone (PRED, an anti-inflammatory drug) and norfloxacin (NFLX, an anti-microbial drug) were selected as test drugs because these drugs are very poorly soluble in water and show lower bioavailability in vivo. The ternary formulations of these drugs were prepared using egg albumin and olive oil, and then, to confirm the improvement in their pharmaceutical characteristics by preparing the ternary formulations, we examined the powder X-ray diffractometry, the dissolution profiles and the plasma drug concentrations of these formulations compared with each drug alone.

MATERIALS AND METHODS

Materials Egg albumin, olive oil, dexamethasone, MZ dihydrochloride monohydrate, triethylenamine and pyrene were obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Pyrene was recrystallized from ethanol. NFLX was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. p-Nitrophenyl acetate acid was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. PRED and sodium carboxymethylcellulose were purchased from Nacalai Tesque, Inc., Kyoto, Japan. All other reagents used were of analytical grade.

Preparation of Formulations Freeze-dried ternary formulations were prepared according to the method

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described elsewhere. Individual drugs and olive oil were dissolved in 25 mL of acetone. Then, each solution was added to 180 mL of 5% (w/v) aqueous egg albumin solution and stirred vigorously with a high-speed homogenizer (BM-1, Nihonseiki Kaisha Ltd., Tokyo, Japan) at 25,000 rpm for 2 h under cooling in ice water. Acetone was removed from the emulsion under reduced pressure, and the remaining substance was poured into a dish in the freeze-dryer (FD-80, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and freeze-dried. After that, solid freeze-dried formulations were sieved using a sieve of 100 mesh, and white porous powders were finally obtained. The ternary formulations prepared by this method were as follows: albumin–MZ–olive oil (9:1:4.3), albumin–PRED–olive oil (9:1:4.3) and albumin–NFLX–olive oil (9:1:4.3).

**Physical Mixtures**

The physical mixtures were prepared by the simple mixing of the albumin–olive oil complex (9:4:3), prepared as described previously, and each drug in a ceramic mortar.

**High Performance Liquid Chromatography (HPLC) Conditions**

The concentrations of the drugs in the formulations and in the plasma of rats were determined by HPLC. The chromatographic system consisted of a system controller (801-SC, Japan Spectroscopic, Tokyo, Japan), an HPLC pump (880-PU, Japan Spectroscopic, Tokyo, Japan), a sampler (850-AS, Japan Spectroscopic, Tokyo, Japan), a UV detector (870-UV, Japan Spectroscopic, Tokyo, Japan) and a data processor (C-R 3A, Shimadzu, Kyoto, Japan). The reversed-phase columns used were as follows: (a) MZ and NFLX, Cosmosil 5C18 column (4.6 mm i.d. × 150 mm, Nacalai Tesque, Inc., Kyoto, Japan); (b) PRED, Cosmosil 5C8-p column (4.6 mm i.d. × 150 mm, Nacalai Tesque, Inc., Kyoto, Japan). All the columns were maintained at a temperature of 40 °C with a water-jacket. The analytical conditions were as follows: (a) MZ, internal standard (IS) solution—pyrene dissolved in n-hexane (5 mg/2000 μL), mobile phase (MP)—methanol:0.1 M acetate buffer, pH 6.2, (9:1) at a flow rate of 1.5 mL/min, detection wavelength (WL)—232 nm; (b) PRED, IS solution—dexamethasone dissolved in methanol (50 mg/100 μL), MP—methanol:water (6:4) at a flow rate of 1.5 mL/min, detection WL—254 nm; (c) NFLX, IS solution—p-nitrophenyl acetic acid dissolved in methylene chloride (50 mg/2000 μL), MP—the solution containing 1 g of sodium acetate trihydrate, 2 g of citric acid monohydrate and 1 mL of triethylamine–acetonitrile (6.5:1) at a flow rate of 1.5 mL/min, detection WL—277 nm. All mobile phases were prepared daily, and filtered through a 0.5 μm membrane filter before use.

**Determination of Drug Content in the Formulations**

Fifty mg of each formulation was sonicated in 20 mL of methanol to dissolve each drug completely. After the insoluble material was filtered, the volume of filtrate was adjusted to 50 mL with methanol. Then, the contents of drugs dissolved in the solution were determined by HPLC. The recovery of drugs in this extraction procedure was more than 98%.

**Dissolution Studies**

The equivalent of 10 mg of drug as a 100 mesh powder was weighed and put in a jacket beaker which contained 20 mL of the dissolution medium No. 1 (pH 1.2) or No. 2 (pH 6.8) described in the dissolution test in JP XII. The dissolution rates of the drugs from the formulations and of the each drug alone were measured by stirring at 150 rpm at 37 °C. Aliquots (0.2 mL) of the solution were withdrawn, at appropriate intervals through a pipette attached to a cotton wool filter, and were diluted to the required concentration with water. The sample was taken from the same position in the container and the dissolution medium, 0.2 mL, was added to the dissolution container after sampling. The amounts of drugs dissolved were determined by HPLC.

**Powder X-ray Diffraction Studies**

Powder X-ray diffraction analysis was performed with a diffractometer (RAD-IIA Rigaku Denki Company, Ltd., Tokyo, Japan). Operating conditions were as follows: X-ray source radiation; voltage, 40 kV; current, 30 mA; receiving slit, 0.30 mm; scanning speed, 1°/min; angle, 2θ.

**Animals**

Male Sprague Dawley rats (200–250 g) purchased from Charles River, Japan, were used. They were housed in raised mesh-bottom cages under conditions of 22 ± 2 °C temperature, 55 ± 5% relative humidity and 12 h light (from 7 a.m. to 7 p.m.), and were given a commercial pellet diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and allowed tap water ad libitum.

**Bioavailability Studies**

Groups of 4–5 rats were used. The rats were treated with single oral administration of the formulations and each drug alone as a suspension in 0.5% sodium carboxymethylcellulose. After that, about 0.2 mL of blood was taken, before and at 1, 2, 5 or 6, 8 and 24 h after administration as the formulations or each drug, from the tail artery with a heparinized syringe. The plasma was separated immediately by centrifugation at 3000 rpm for 10 min. To determine the drug concentrations in plasma, 100 μL of the plasma was submitted to HPLC as described above.

**RESULTS AND DISCUSSION**

First, we prepared the ternary formulations of poorly water-soluble drugs, MZ, PRED and NFLX chosen as test samples, showing their chemical structures in Chart 1, and examined their dissolution profiles in JP XII.
disintegration medium No. 1 (pH 1.2) or No. 2 (pH 6.8), at 37°C in comparison with each drug alone and their physical mixtures. The results are shown in Fig. 1 (data of physical mixtures not shown). The dissolution rates of the physical mixtures were almost the same as those of the drugs alone in both the disintegration media, No. 1 and 2, respectively. However, the ternary formulations of PRED and NFLX exhibited significantly greater dissolution rates than those of the drugs alone in both the disintegration media. The dissolution rate of the MZ formulation was greater than that of MZ alone in medium No. 1. In contrast, both the MZ formulation and MZ alone exhibited very slow dissolution rates in medium No. 2, but the former showed a slightly increased dissolution

![Graphs showing dissolution profiles of different formulations](image)

Fig. 1. Dissolution Profiles of MZ, PRED, NFLX and Their Ternary Formulations in JP XII Disintegration Media (No. 1 and 2) at 37°C

A) ○, MZ; ●, egg albumin-MZ-olive oil (9:1:4.3) formulation; B) ○, PRED; ●, egg albumin-PRED-olive oil (9:1:4.3) formulation; C) ○, NFLX; ●, egg albumin-NFLX-olive oil (9:1:4.3) formulation.

![Graphs showing powder X-ray diffraction patterns](image)

Fig. 2. Powder X-Ray Diffraction Patterns of Ternary Formulation Systems

A) MZ alone; B) egg albumin-MZ-olive oil (9:1:4.3) formulation; C) PRED alone; D) egg albumin-PRED-olive oil (9:1:4.3) formulation; E) NFLX alone; F) egg albumin-NFLX-olive oil (9:1:4.3) formulation.
rate compared with the latter. Such an increase in the dissolution rate of a drug by preparing the ternary formulation was also observed in the IND ternary formulation reported previously. It is thought that the increased dissolution rates of these drugs can be attributed to forming the ternary formulation because, in the case of physical mixtures, their dissolution rates did not improve.

Figure 2 depicts the powder X-ray diffractograms of the three ternary formulations (egg albumin-MZ–olive oil, egg albumin-PRED–olive oil and egg albumin-NFLX–olive oil) and of each drug alone. Albumin, the drugs alone and the physical mixtures showed peaks for crystalline forms (data of the physical mixtures not shown), but no peaks for crystalline drugs were detected in the MZ and NFLX ternary formulations (curves B and F), and the peaks in the PRED ternary formulation (curve D) disappeared considerably compared with its physical mixture. Previously, we have reported that the dissolution rates and the bioavailability of IND were greatly enhanced by forming a ternary formulation of IND with egg albumin and olive oil or various fatty acids. After various considerations on the basis of experimental results, we estimated that the improvement in these pharmaceutical characteristics was attributed to a change in the crystalline form of IND to an amorphous form by preparing the ternary formulation. It is thought that this idea would also be applicable to the increased dissolution rates of MZ, PRED and NFLX in the same formulation. In the formulations, in which the molar ratio of albumin and these drugs is albumin: PRED = 1:14 and albumin: NFLX = 1:16, respectively, these drugs are presumed not to bind to a specific site on the albumin, but to disperse into the albumin–olive oil complex in an amorphous form as did IND, as reported previously. Thus, the increased dissolution rates of each drug in the formulations in the disintegration media No. 1 and 2 might be explained by the results obtained from the measurements of powder X-ray diffraction.

To confirm the improvement in the bioavailability of MZ, PRED and NFLX, we examined the plasma concentrations of the drugs after oral administration in the formulations and as the drugs alone. Figure 3 indicates the time courses of the plasma concentrations of drugs after oral administration as drugs alone and in formulations at the doses shown in Table 1. The pharmacokinetic parameters calculated from the data obtained are also summarized in Table 1. The plasma concentrations of these drugs formulated increased significantly compared with each drug alone. As Table 1 shows, the pharmacokinetic parameters, the values of the area under the concentration–time curve (AUC) and the maximum concentrations (Cmax) of MZ and PRED formulations also increased. The values of AUC and Cmax of the ternary formulations of MZ, PRED and NFLX were 2.1 and 3.1 times, 1.6 and 1.3 times, and 1.3 and 1.1 times those of the drugs alone, respectively. The Tmax value of the MZ formulation (1 h) was smaller than that of MZ alone (2 h). However, there was no difference in Tmax values between the PRED and NFLX formulations and the corresponding drugs alone. From these results, it was found that the values of the AUC of MZ and PRED were enhanced by their oral administration in the formulations, but not that of the NFLX formulation. It is well known that the

<table>
<thead>
<tr>
<th>Product administered</th>
<th>Dose (mg/kg)</th>
<th>AUC (ng h/ml)</th>
<th>Cmax (ng/ml)</th>
<th>Tmax (h)</th>
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</thead>
<tbody>
<tr>
<td>MZ alone</td>
<td>50</td>
<td>6910.9 ± 1224.0</td>
<td>559.6 ± 85.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Albumin–MZ–olive oil</td>
<td>50*</td>
<td>14508.3 ± 1304.4</td>
<td>1729.3 ± 714.5</td>
<td>1.0</td>
</tr>
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<td>(9:1:4.3) formulation</td>
<td></td>
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</tr>
<tr>
<td>PRED alone</td>
<td>3</td>
<td>975.6 ± 152.9</td>
<td>458.6 ± 36.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Albumin–PRED–olive oil</td>
<td>3*</td>
<td>1590.0 ± 171.1</td>
<td>575.3 ± 118.9</td>
<td>1.0</td>
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<tr>
<td>(9:1:4.3) formulation</td>
<td></td>
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<tr>
<td>NFLX alone</td>
<td>20</td>
<td>1344.4 ± 200.0</td>
<td>409.0 ± 90.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Albumin–NFLX–olive oil</td>
<td>20*</td>
<td>1756.2 ± 107.9</td>
<td>459.0 ± 35.3</td>
<td>1.0</td>
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<td>(9:1:4.3) formulation</td>
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Results are expressed as the means ± S.E.M. of 4 rats. a) Substantial dose of the drugs in the formulations. b) Significantly different from drug alone, p < 0.05. c) Significantly different from drug alone, p < 0.01.
amorphous form of a drug has a higher solubility than that of its crystalline form.\textsuperscript{20} Furthermore, it has been reported that the intestinal absorption of drug is enhanced by fatty acids and glycerides\textsuperscript{13-16}. Accordingly, in case of the MZ and PRED ternary formulations, it was considered that two factors, namely the change from a crystalline form to an amorphous form by preparing the formulation and also the action of olive oil as an absorbefaciency agent, participated in the increased AUC value.

On the other hand, in the case of NFLX, the AUC value of the formulation was not so large compared with NFLX alone, in spite of the increase in the dissolution rate (Fig. 1C) and the conversion of a crystalline form into an amorphous form in the formulation (Fig. 2). The lack of enhancement in the AUC value in this experiment seems to be attributed to a difference in the absorption mechanism of NFLX from those of other drugs because NFLX, possessing a carboxyl group at the 3 position and a piperazinyl group at the 7 position, has a zwitterionic structure in the solution in the intestinal tract, around pH 7. However, further investigations should be made to clarify the reason.

In conclusion, considering the results obtained in the present study and reported in previous papers,\textsuperscript{18,19} the new modified method, forming the ternary formulation, seems considerable to be useful in improving the bioavailability of a number of drugs with poor water-solubility.

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REFERENCES