Jpn. J. Hosp. Pharm.
一 般 論 文
23(2) 101 — 107 (1997)

Pharmaceutical Properties of Nifedipine from a Ground Mixture with Nifedipine, Casein, Magnesium Silicate and Cellulose Acetate Phthalate

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(Received January 18, 1996
Accepted November 12, 1996)

The dissolution step of practically insoluble drugs plays an important role in drug absorption. In this paper, the pharmaceutical properties of a ground mixture of nifedipine are described. A ground mixture of nifedipine was prepared by grinding with casein, magnesium silicate, and cellulose acetate phthalate in a vibrational ball mill. The X-ray powder diffraction patterns and differential scanning calorimetry data thereafter suggested that nifedipine was present in its amorphous form in the ground mixture. The wettability of the ground mixture was better than that of the physical mixture. The solubility of amorphous nifedipine in water was also better than that in crystalline. Both the dissolution rate and the bioavailability of amorphous nifedipine in the ground mixture were significantly greater than those of the physical mixture. The above results thus suggest that a mixture grinding method not only improves the solubility of nifedipine and the dissolution profile of its preparation but also enhances its bioavailability after oral administration.

Key words —— nifedipine, ground mixture, amorphous

Introduction

Nifedipine (NP), a crystalline powder, is practically insoluble in water with about 12 μg/ml of solubility[1]. When poorly water-soluble drugs are administered orally, the dissolution rate of the drug in the digestive tract becomes a limiting factor for drug absorption[2]. NP was reported to have a low rate of absorption as 20% when administered orally in a crystalline powder form[1].

The dissolution property of the drug has been recently improved by developments in pharmaceutical technology such as the grinding mixture[3], the roll mixing[4], and the solid dispersion methods[5]. The grinding mixture method is especially superior method since it employs a dry formulation process to eliminate the and therefore residual solvent in the formulation is not a concern.

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In the present study, the grinding mixture method by adding a protein (casein), an antacid (magnesium silicate) and an enteric soluble cellulose derivative (cellulose acetate phthalate, CAP) was adopted to improve dissolution property and bioavailability of NP[6].

Experiments

1. Materials

NP was purchased from Teikoku Chemical Industries Co., Ltd. (Osaka, Japan). Casein from Imanaka Corporation (Osaka, Japan), magnesium silicate from Kyowa Chemical Co., Ltd. (Kagawa, Japan)., and cellulose acetate phthalate was purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). All other materials were employed of guarantee grade.

2. Methods

All experiments were carried out in darkness in view of the high sensitivity of NP to light.

a. Preparation of samples

Ground mixture (sample 1) was prepared in the following manner. NP, casein and magnesium silicate were blended by weight ratio of 2 : 3 : 2 respectively, and the mixture (105 g) was ground using a vibrational ball mill (Model MB-1, Chuo Kakohki Co., Ltd., Aichi, Japan) for 120 minutes at room temperature. Then 35 g of the above ground powder and 70 g of CAP was mixed and ground by the vibrational ball mill for 20 minutes at room temperature. The final ground powder (105 mg, including 10 mg of NP) was encapsulated into JP #3 hard gelatin capsules.

Physical mixture (sample 2) was prepared as follows : NP, casein, and magnesium silicate were separately ground using the vibrational ball mill at room temperature, and the three ground powders were mixed together in the same ratio as the ground mixture. The final mixture (105 mg, including 10 mg of NP) was filled into JP #3 hard capsules.

b. X-ray powder diffraction (powder XRD)

Measurement of X-ray powder diffraction was conducted using a X-ray powder diffraction apparatus (JDX-3530, JEOL) with the Ni-filter set at a diffraction angle of $2\theta = 0\sim50^\circ$.

c. Differential scanning calorimetry (DSC)

Differential scanning calorimetry was conducted using a DSC-200 (Seiko Electronics Co., Ltd.).

d. Measurement of wetting on powder

Penetration rate[7] of water into the powder was measured by the capillary rise method[8]. About 0.30 g of sample was packed into a glass capillary tube (3 mm i. d. × 25 cm) with the bottom covered with a filter paper.

e. Dissolution property

Sample powder (105 mg ; equivalent to 10 mg of NP) was added to 10 ml of purified water or 4% SDS (Sodium Lauryl Sulfate) solution at 37℃. Then the solution stirred vigorously for 2 minutes, and centrifuged at 3000 rpm for 10 minutes. The supernatant of the solution was filtered (with a 0.45 μm membrane filter), and the amount of NP in the filtrate was determined using an UV spectrophotometer (Hitachi 557, 350 nm) and was calculated beforehand from a standard calibration curve.

f. Dissolution test

Dissolution test apparatus (rotating dialytic cell, type PTSW, Pharmatest Co., Germany) was employed to obtained the dissolution profile of NP, and a hydrophilic membrane filter (Durapore 0.45 μm, Millipore) was used as a dialytic membrane. For the dissolution test, 4% of SDS was added to either

the 1 st fluid (pH 1.2) or the 2 nd fluid (pH 6.8) listed in disintegration test of JPXII.

Two capsules from each sample were put in the rotating dialytic cell with 10 ml of the dissolution fluid. The cell was immersed and rotated at a rotation speed of 26 rpm in 900 ml of dissolution fluid maintained at 37℃. Five ml of test solution was withdrawn through a membrane filter (pore size 0.45 μm) at 30 minute intervals for up to 6 hour and immediately replaced with an equal volume of the fluid. Then the amount of NP dissolved was calculated from the absorbance at 350 nm using the standard calibration curve.

g. Bioavailability study in rabbits

Doses were administered by the crossover arrangement after a time interval of one week. After fasting for 24 hours but with free access to water, 11 white male rabbits, weighing approximately 2.5 kg, were orally administered the test sample equivalent to 10 mg of NP with water. Plasma samples were collected before administration and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after administration. Plasma samples were then assayed for NP by high-performance liquid chromatography (HPLC, model LC-9 A, Shimadzu, Co., Ltd.) equipped with a UV detector (model SPD-6 A, Shimadzu, Co., Ltd.) using n-butyl p-hydroxybenzoate as an internal standard. UV absorption was measured at 350 nm. HPLC samples were chromatographed at 50℃ on a Nucleosil 7 C 18 column (4.6 mm i.d. × 250 mm, M. NAGEL, Co., Ltd.). The mobile phase was water-methanol (3 : 7). The flow rate was 1.0 ml/min.

Results and Discussion

1. Crystal form

A ground mixture consisting of NP crystal with several excipients was prepared by grinding and was studied by X-ray powder diffraction and measurement of DSC.

Figure 1 shows the X-ray powder diffraction patterns of NP bulk substance, samples 1 and 2. Many diffractional peaks derived from the crystal form of the NP was observed in NP of sample 2, while it was hardly observed in that of sample 1.

Figure 2 shows results from DSC measurement of the NP bulk substance, samples 1 and 2. The

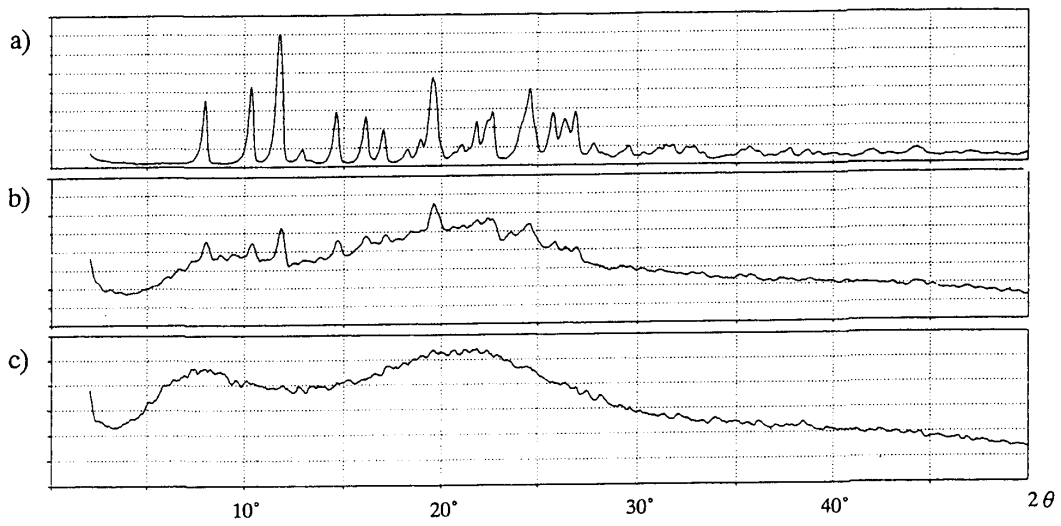

Fig. 1. X-Ray Powder Diffraction Patterns of Nifedipine
a) Alone, b) Physical Mixture, c) Ground Mixture

endothermic peak induced by melting at around 173℃, peculiar to crystal form of NP, was not observed in sample 1.

From the above findings, it was assumed that the NP of sample 1 existed as an amorphous form.

2. Physical properties of amorphous nifedipine

The amorphous NP formulation was applied for further investigation on its physical properties as an oral dosage form. It is known that the bioavailability and the dissolution behavior in an insoluble drug are correlated, and the wetting property of the powder surface is one of factors influencing the dissolution profile.

a. Wetting

The effect of wetting of the sample with water was measured using the powder capillary method and was plotted according to Washburn's equation (1) as shown in **Fig. 3**.

$$L^2 = r\,\gamma\cos\theta \cdot t/2\eta \quad \cdots\cdots(1)$$

L : height of liquid after time t

r : average radius of capillary space of packed area

γ : surface tension of the liquid

θ : contact angle between the liquid and the powder surface

η : viscosity of the liquid

The penetration rate of sample 1 was higher than that of sample 2. Comparison of these samples at 10 minutes showed that L^2 value of sample 1 was 2.3-fold higher than that of sample 2. This result suggests that the ground mixture has a higher wettability than the physical mixture.

b. Dissolution property

The improvement of the dissolution property in water-insoluble drugs by amorphization has been already shown in a study using glyceoflubin[9]. We studied the dissolution property of NP for purified water and 4% SDS solution (**Table 1**). The dissolved percent of NP in sample 1 to 10 ml purified

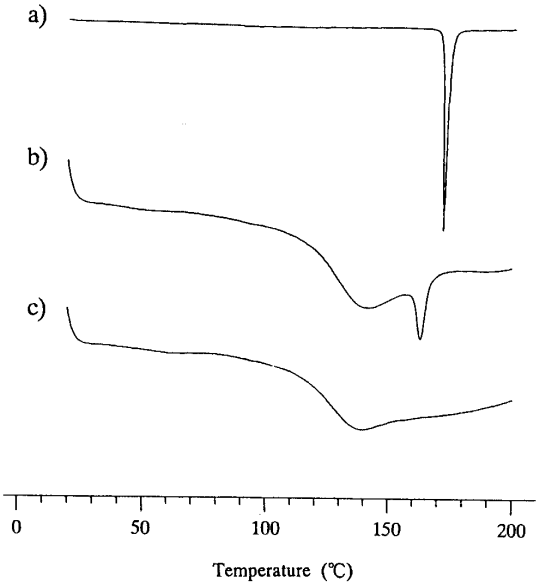

Fig. 2. DSC Curves of Nifedipine
a) Alone, b) Physical Mixture,
c) Gorund Mixture

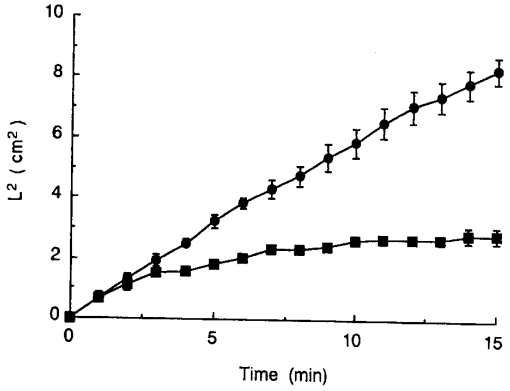

Fig. 3. Penetration of Water into Powder Bed Plotted According to Washburn's Equation
Each value is the mean ± SD (n=5).
(●) Sample 1, (■) Sample 2

Table 1. Dissolution Property of Samples (Nifedipine ; 10 mg) in 10 ml of Water

Solvent	Dissolved percent	
	Sample 1	Sample 2
Water	1.80 ± 0.24	0.51 ± 0.06
4% SDS solution	98.59 ± 4.35	29.57 ± 1.98

Each value is the mean ± SD (n=5).

water was 1.80% to 10 mg of NP, while that of sample 2 was 0.51%. The value in sample 1 was about 3.5-fold higher than that of sample 2. Moreover, NP in sample 1 showed a high dissolution property (98.6%) on 4% SDS solution, whereas in sample 2 under the same condition it was only 30%. These results also suggest that the ground mixture (amorphous NP) was superior in dissolution property to that of the physical mixture (crystalline NP).

c. Dissolution

It is known that the dissolution behavior of water-insoluble drugs influences the bioavailability. The dissolution rate of NP were determined using the rotating dialysis cell method. As shown in **Fig.4**, sample 1 showed a higher dissolution rate than that of sample 2 from the beginning in both fluids. Six hours later, the dissolution rates were 84.2% for sample 1 and 68.6% for sample 2 in the 1 st fluid, while the rates were 87.8% for sample 1 and 72.6% for sample 2 in the 2 nd fluid.

Thus, the ground mixture (amorphous NP ; sample 1) showed a higher dissolution rate than that of the physical mixture (crystalline NP ; sample 2) in both acidic solution (1st fluid) and the neutral solution (2nd fluid). These results demonstrated that amorphous NP by mixture grinding method has shown good dissolution behavior.

It was reported that the dissolution behavior of NP by the rotating dialysis method has a direct and positive relationship with AUC when administered orally[10]. Also, Nakai et al. reported that the dissolution rate of ground mixture of phenytoin and crystalline cellulose exhibited a good correlation to bioavailability[11]. Therefore, the amorphous NP was expected to be a superior formulation in bioavailability.

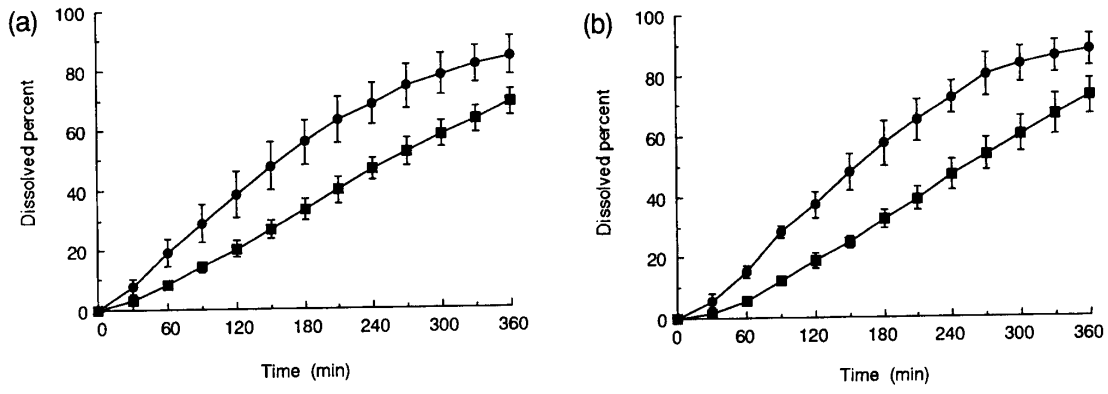

Fig. 4. Dissolution Profiles of Samples in J.P. Disintegration Fluid containing 4% SDS by PTSW Rotating Dialysis Cell Method
(a) 1st Fluid (pH 1.2), (b) 2nd Fluid (pH 6.8)
Each value is the mean ± SD (n=3).
(●) Sample 1, (■) Sample 2

3. Bioavailability

Further experiments were performed to study the bioavailability difference between amorphous NP and crystalline NP. Plasma concentration–time curves of NP after oral administration of test samples (10 mg of NP) to rabbits are shown in **Fig.5.** Three parameters were examined to assess the bioavailability from the plasma concentration data (**Table 2**). The C_{MAX} value of sample 1 was about 7–fold higher and the AUC (the area under the time plasma concentration curve) value about 3–fold larger than those of sample 2. Furthermore, the T_{MAX} of sample 1 was faster than that of sample 2. These results demonstrated that, when administered orally, absorption of amorphous NP was superior to that of the crystalline form, and the AUC was greatly improved by amorphization using grinding mixture method. Therefore, the amorphous NP was expected to be a superior formulation in bioavailability.

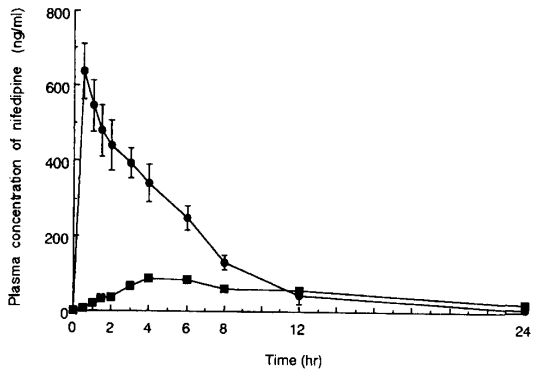

Fig. 5. Plasma Concentration–Time Curves of Nifedipine in Rabbits after Oral Administration of Samples
Each value is the mean ± SE (n=11).
(●) Sample 1, (■) Sample 2

Table 2. Comparison of Bioavailability Parameter of Samples in Rabbits

Sample	AUC (μg·min/ml)	Cmax (ng/ml)	Tmax (hr)
1	3.35 ± 0.40	652.7 ±72.7	1.0 ± 0.2
2	1.15 ± 0.09	94.4 ± 8.6	6.2 ± 0.7

Each value is the mean ± SE (n=11).

This investigation indicated that amorphous NP using the grinding mixture method was easily absorbed and the AUC was greatly improved by amorphization.

From the above results, it is suggested that a grinding mixture method not only improves the dissolution property of nifedipine and the dissolution profile of its preparation but also enhances the bioavailability after oral administration.

So far, many oral solid dosage forms have been manufactured as crystalline form of bulk drugs, and these drugs show relatively lower bioavailability in comparison with that of the amorphous form. Accordingly, it becomes necessary to increase the dose to improve the amount of drug absorption. In the future, the grinding mixture method is expected to be used to reduce the required dose of these crystalline drugs by improvement of bioavailability.

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