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Improvement of dissolution characteristics and bioavailability of poorly water-soluble drugs by novel cogrinding method using water-soluble polymer

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Abstract

A novel cogrinding method for improving dissolution characteristics of poorly water-soluble drugs was developed. A coground mixture of nifedipine (NP)-polyethylene glycol 6000-hydroxypropylmethyl cellulose system prepared in the presence of small amount of water showed remarkable effect with respect to NP dissolution and its apparent solubility. Although the performance of the coground mixture was superior to that of spray-dried powder with the same composition, the X-ray diffraction pattern indicated the mixture did not change to amorphous state. In addition, the transmission electron microscopy revealed that NP seemed to exist in coacervate-like fine particles (50–200 nm) in water. This cogrinding method was also effective for other poorly water-soluble drugs such as griseofluvin and indomethacin. Some drug–polymer interactions through hydroxypropoxyl groups seemed to participate in the mechanism of the improvement of dissolution characteristics, because the content of the functional group affected the extent of solubility-enhancing effect. Furthermore, the addition of small amount of water in cogrinding was especially effective, because it promoted the interactions. The coground mixture showed the same plasma concentration as NP solution of PEG 400 when it was orally administered to beagle dogs. From these results, it is clear that the present cogrinding method is very effective for improvement of bioavailability of poorly water-soluble drugs. © 1998 Elsevier Science B.V.

Keywords: Poorly water-soluble drug; Water-soluble polymer; Coground mixture; Solubility; Dissolution; Bioavailability

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1. Introduction

Poorly water-soluble drugs often show low bioavailability when administered orally, because the absorption of the drugs in the gastrointestinal-tract can usually be a rate-limiting step. Therefore, it is important for such kind of drugs to enhance their dissolution rate. To enhance the dissolution rate, increasing the drug solubility is necessary according to the Noyes–Whitney equation (Abdou, 1989). Various studies have been done in attempt to improve solubilities of poorly water-soluble drugs; they include micronization (Otsuka and Kaneniwa, 1984), solid dispersion (Ford, 1986), solvent deposition (Takeuchi et al., 1987), ordered mixture (Nystrom and Westerberg, 1985), roll-mixing (Nozawa et al., 1984) and complexation (Moyano et al., 1995). Some of the dissolution-enhancing methods have been applied for the production of pharmaceutical preparations.

Among these, a solid dispersion is one of the effective method for enhancing the drug dissolution rates. Solid dispersions are prepared mainly by a melting method or a solvent method, which are usually consisted of two components; a poorly water-soluble drug and a water-soluble polymer like polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), methylcellulose (MC), polyvinylalcohol (PVA) and so on. In addition, a multi-component solid dispersion has been recently reported (Law et al., 1992). In the melting method, water-soluble polymers which can solve drugs are limited, and the drugs often decompose during the dissolving at high temperature. On the other hand, in the solvent method, we often can not avoid using organic solvents, such as chloroform or dichloromethane, which are highly toxic to human bodies. Furthermore, since drugs are usually amorphous in solid dispersions, they should be physically unstable; crystallization of the drugs will occur during the storage in highly humid conditions, resulting in a decrease in the solubility (Sugimoto et al., 1981). Similar physically unstable properties have been also investigated in coground mixtures consisted of a poorly water soluble drug and a polymer (Kawano and Nakai, 1983).

Recently, we found that cogrinding of a poorly water-soluble drug with water-soluble polymers in the presence of small amount of water was remarkably effective to improve its apparent solubility. Although the composition of the coground mixture is similar to that of solid dispersions, the drug crystallinity is maintained to some extent. This finding prompted us to develop a novel technique that can apply for peroral solid preparations of various poorly water-soluble drugs which possess problems in the bioavailability. The purpose of this study is to investigate the properties of the coground mixture in vitro and in vivo. Furthermore, factors affecting the solubilization will be discussed.

2. Materials and methods

2.1. Materials

Nifedipine (NP), griseofulvin (GR), and indomethacin (IM) were purchased from Wako Pure Chemical (Osaka, Japan). HPMC (type:TC-5R, 60SH-50 and 65SH-50; Shin-Etsu, Tokyo, Japan), MC (type: SM-25 and SM-100; Shin-Etsu), HPC (type: SL; Nihon Soda, Tokyo, Japan), PVP (type: k-30; B.A.S.F., Germany), PVA (type: gosenol EG-05; Nihon Gosei, Tokyo, Japan), PEG (type: 6000; Nihon Soda) were used as received. All other chemicals used were of reagent grade.

2.2. Preparation of coground mixture

A poorly water-soluble drug (500 mg) was dispersed in the fused PEG6000 (500 mg) at 70°C. A water-soluble polymer (2.5 g) was then added to the mixture at 70°C. After cooling to room temperature, the solidified mass was ground for 10 min using a vibration ballmill (spex mixer, Spex). According to the experimental purposes, 3 ml of solvent (water, methanol, ethanol, dichlormethane/methanol) was added to the mixture, which was further ground for 10 min. After the grinding, the added solvent was removed by drying at 70°C for 6 h. The resultant mass was lightly pulverized to pass through a 200 μm screen.

2.3. Preparation of spray-drying powder

NP (1 g), PEG 6000 (1 g), and TC-5R (5 g) were dissolved in 360 ml of dichlorometane/methanol mixture (10/3). The solution was spray-dried using Pulvis mini spray (type: GS31, Yamato Scientific, Japan) under the following conditions: inlet temperature, 70°C, outlet temperature, 46°C.

2.4. Solubility measurement

The apparent solubility of a poorly water-soluble drug in the coground mixture, spray-drying powder and physical mixture was determined in water at 37°C. Each preparation equivalent to 60 mg of the drug was added to 50 ml of water in a jacketed beaker which was heated by external water circulation to maintain a constant temperature. The solution was stirred at a constant speed. At appropriate time intervals, aliquots were removed and filtered with a 0.45 μm membrane filter (FR40, Fuji Film, Tokyo) for analysis of the drug concentration with a spectrophotometer (UV2100, Shimadzu, Kyoto, Japan). Apparent drug solubility was determined, when super-saturation was observed.

2.5. Dissolution rate measurement

Dissolution rate of a drug was measured by the JP paddle method in 900 ml of water at 37°C with constant stirring at 100 rpm. Aliquots were automatically withdrawn and filtered with a glass filter at specified times. Drug concentrations in the filtrate were determined with a spectrophotometer (UV-160, Shimadzu, Kyoto, Japan).

2.6. Powder X-ray diffraction studies

The powder X-ray diffraction patterns were measured at room temperature with a Geigerflex X-ray diffractometer (type: 2013, Rigaku Denki, Tokyo). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 35 mA; receiving slit, 0.3 mm; time constant, 10 s; scanning speed, 0.25°/min.

2.7. Transmission electron microscopy (TEM)

A transmission electron microscope (TEM) (JEM-100CX, Jeol, Tokyo) was used to observe the filtrate that was used for solubility measurement. The filtrate was passed through a membrane filter (pore size: 0.45 μm), followed by dying with a 1% phosphorus wolframate solution for the TEM-observations.

2.8. Absorption study

Four male beagle dogs weighing about 10 kg were used. The dogs were fasted for 20 h before the drug administration, while receiving water ad libitum. A physical mixture or a coground mixture (NP:PEG 6000:TC-5R = 1:1:5) equivalent to 10 mg of NP was suspended in a 50 ml of water, and it was administered orally through a sonde. As a reference, NP solution of PEG 400 was also administered in the same manner. After dosing, blood samples (3 ml) were collected at predetermined times and centrifuged ($2000 \times g$, 10 min). The obtained plasma was frozen until analysis.

2.9. Assay of NP by HPLC

One ml of plasma samples, 0.5 ml of 1 N NaOH and 8 ml of diethylether were mixed with a voltex mixer, followed by centrifugation at $2000 \times g$ for 10 min. The 6 ml of dietylethel layer was pippeted and evaporated to dryness under a nitrogen stream. A 0.5ml of mobile phase containing *p*-aminobenzoic acid butyl ester as an internal standard (1 mg/ml) was added to dissolve the residue, and a 200 ml of the aliquot was injected into the HPLC (LC 6A, Shimadzu, Kyoto, Japan). A mixture of acetonitrile/phosphate buffer (pH 6.1) (0.6:1) was used as the mobile phase. A reverse-phase column (Hypersil 5C18 4f $\times$ 150, Chemco Scientific, Osaka, Japan) was used, and UV detection for quantification was performed at 237 nm. A linear detector response was observed over the concentration range of interest.

3. Results and discussion

3.1. Improvement of dissolution characteristics of NP by cogrinding in ternary systems

Law et al. (1992) studied the effect of phosphatidyl choline as an additional ingredient to the NP/PEG solid dispersion, which represented remarkable enhancement of bioavailability. On the other hand, Nozawa et al. (1986) studied roll mixing of (i) NP and PVP with additional solvent like ethanol and water, and (ii) NP/PVP/PEG. We investigated the effect of cogrinding of NP-HPMC-PEG6000 ternary system to improve NP dissolution. Coground mixing process seemed to be more effective than roll mixing, since it can generate higher physical energy in a short time. Furthermore, because NP can be dissolved in PEG 6000 (solubility: about 180 mg/g at 80°C), the addition of PEG 6000 in the coground mixing seemed to bring about some advantages for the improvement of NP dissolution. The effect of addition of water in the coground mixing was also investigated.

Fig. 1 shows change of NP solubility with time for NP-PEG 6000-HPMC (TC-5R) systems (1:1:5) prepared by various techniques. The solubility of physical mixture is also shown as a reference. To compare with the physical mixture, the ground mixture prepared in the absence of water demonstrates only a little increase in its apparent solubility. On the other hand, a remarkable enhancement of solubility was observed in the case of coground mixture with water, even though the other cogrinding conditions were identical. The super-saturation, which is about 20 times greater than that of the physical mixture, is maintained over 60 min. The spray dried powder also showed a large increase in the apparent solubility at the initial stage of dissolution. However, the crystallization of the drug resulted in the rapid decrease in apparent solubility.

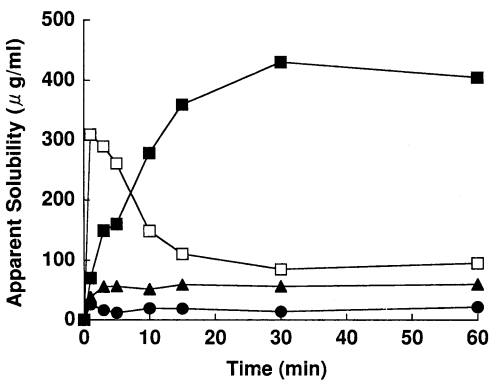

Fig. 1. Apparent solubility of NP in NP-PEG6000-HPMC (TC-5R) (1:1:5) systems. (●), physical mixture; (▲), coground mixture; (■), coground mixture prepared in the presence of water; (□), spray dried powder. The amount of water added for preparing the coground mixture was 40% of total weight.

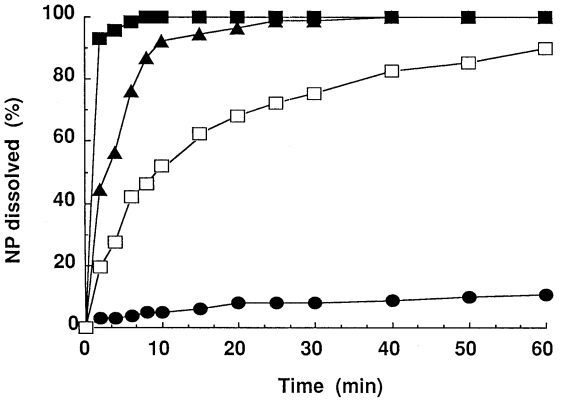

Fig. 2. Dissolution profiles of NP in NP-PEG6000-HPMC (TC-5R) (1:1:5) systems. (●), physical mixture; (▲), coground mixture; (■), coground mixture prepared in the presence of water; (□), spray dried powder. The amount of water added for preparing the coground mixture was 40% of total weight.

Fig. 2 shows the dissolution profiles of NP in NP-PEG 6000-HPMC (TC-5R) systems (1:1:5) prepared by various methods. Reflecting the solubility differences shown in Fig. 1, the coground mixture prepared in the presence of water shows the highest dissolution rate among the four preparations. Unexpectedly, the spray dried powder had low dispersibility, because drug particles aggregate together due to its high energy amorphous state. Its dissolution rate resulted in lower one than anticipated from its solubility shown in Fig. 1. In the case of the coground mixture with water, it seems that NP disperses quickly in water accompanied with water-soluble polymer to dissolve, and that a supersaturation is maintained for a long period without crystallization.

From the results shown in Figs. 1 and 2, it was demonstrated that the cogrinding of NP with HPMC (TC-5R) and PEG can be an effective method to improve the dissolution rate, and that this method is superior to the conventional spray drying product. In particular, cogrinding in the presence of a small amount of water found to be remarkably effective with respect to the improvement of apparent solubility and dissolution of poorly water soluble drug.

3.2. Solid- and liquid-state analysis of the coground mixture

In order to clarify the physicochemical characteristics of the coground mixture, X-ray diffraction measurements were conducted. Fig. 3 shows X-ray diffraction patterns of NP-PEG 6000-HPMC (TC-5R) ternary systems prepared by different methods. The composition of the three preparations was identical except intact NP powder; NP-PEG 6000-HPMC (TC-5R) (1:1:5). Although the characteristic diffraction peaks for NP in the spray dried powder were not observed, the pattern of the coground mixture unexpectedly had some diffraction peaks arised from NP, suggesting that it was not entirely amorphous.

The filtrate through a membrane filter (pore size: 0.45 μm) of the dispersion of the coground mixture prepared looked slightly turbid. The increase in the solubility shown in Fig. 1 was partly ascribed to the turbidity of filtrate. Therefore, to determine the feature of the turbidity, the microscopic observation of the filtrate was carried out. The TEM photographs of the filtrate of NP-PEG 6000-HPMC (TC-5R) coground mixture in the presence or absence of water were shown in Fig. 4. The size of particles coground in the absence of water are 300–400 nm, and the reticulated structure due to the deposition of polymer was observed. On the other hand, the size of particles coground in the presence of water are 50–200 nm, and they were dispersed homogeneously. Moreover, NP in this turbid supersaturated solution showed the same UV absorption as NP solution. Consequently, the pulverized NP is finely dispersed in the coground mixture with partly maintaining its crystallinity, and when it is dispersed in water, NP can be exist in the coacervate-like particles that is observed by TEM as a hypersaturated solution.

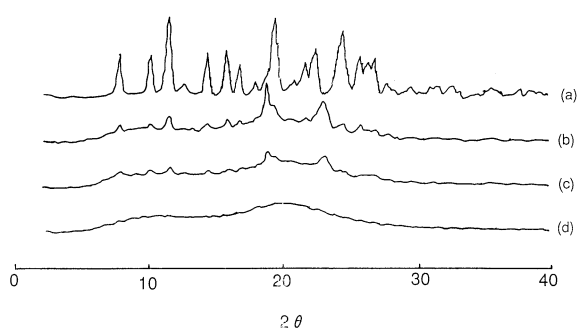

Fig. 3. X-ray diffraction spectra of NP-PEG6000-HPMC (TC-5R) (1:1:5) systems. (a) Intact NP powder; (b) physical mixture; (c) coground mixture prepared in the presence of water; (d) spray-dried powder.

3.3. Effect of polymer species in the coground mixture on NP solubility

Effect of water-soluble polymer species in the coground mixture prepared in the presence of water is listed in Table 1. HPMC, HPC, PVP and PVA were used as water-soluble polymers, and apparent solubility was observed in water. As shown in Table 1, HPMC was most effective, on the other hand PVP showed little effectiveness among these polymers with respect to solubility enhancement. From these results, it was suggested that hydroxyl groups in the water-soluble polymer participate in the solubility enhancement, and that some interactions between NP and the polymer may have occurred during the cogrinding process through the functional groups in a small amount of water added.

Furthermore, in an attempt to validate the reason mentioned above, the effect of hydroxypropoxyl groups on apparent NP solubility of coground mixture was investigated by using HPMC and MC with different hydroxypropoxyl group content. As listed in Table 2, the content of the substituent groups in the water-soluble polymers obviously affected the apparent solubility of NP, suggesting that some interactions between NP and the hydroxypropyl groups of the polymer

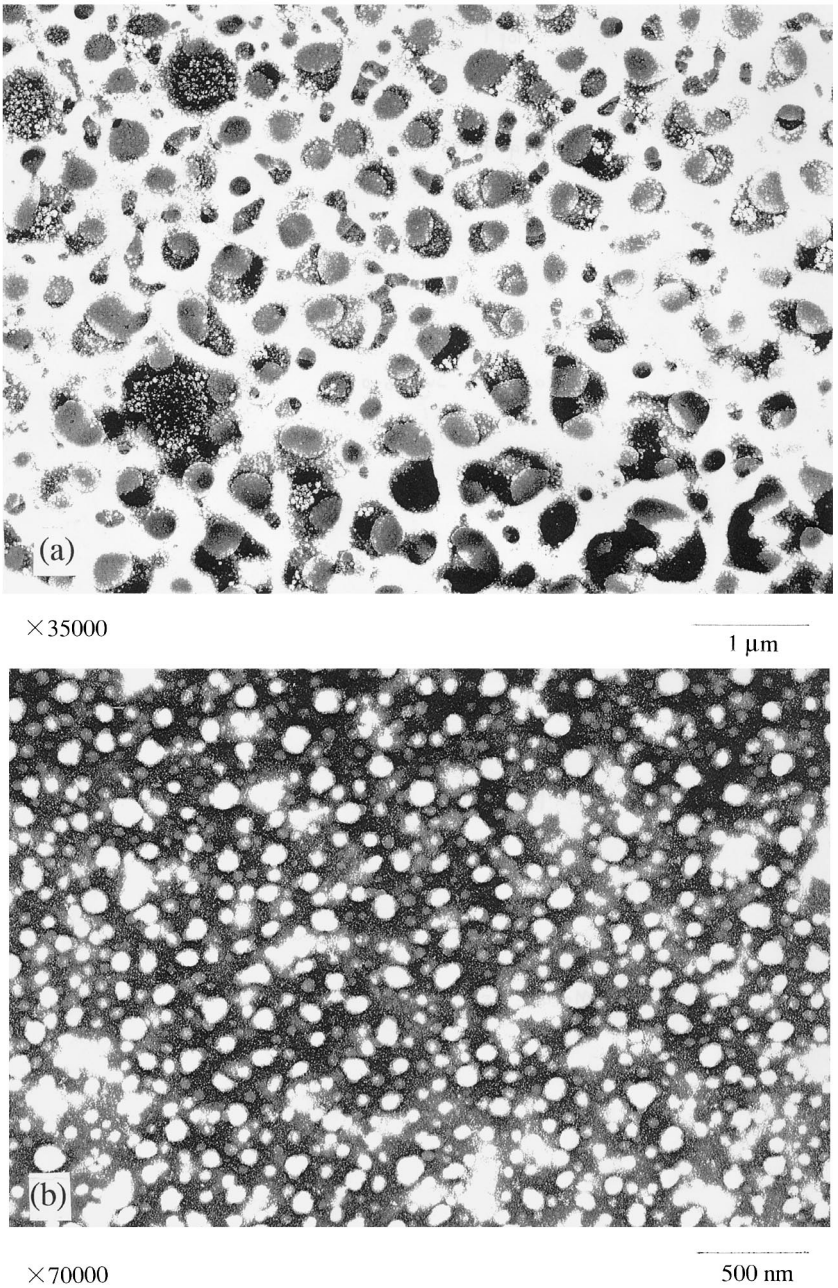

Fig. 4. Transmission electron micrograph of the filtrate of NP-PEG6000-HPMC (TC-5R) (1:1:5) coground mixture. (a) Coground mixture; (b) coground mixture prepared in the presence of water.

play an important role in the enhancement of the apparent NP solubility. Hasegawa et al. (1985) previously reported that the supersaturation of NP solution was maintained effectively by HPMC and MC, slightly by HPC. The supersaturation was brought about by their inhibitory effect on the recrystallization through hydrophobic interactions between NP and the water-soluble polymers.

Table 1
Effect of polymer species in the coground mixture on apparent solubility of NP[a] in water at 37°C

Polymer[b]	Apparent solubility of NP (μg/ml)
HPMC	417
HPC	367
PVA	151
PVP	24

[a] Coground mixture was prepared in the presence of water. The composition of the coground mixture is NP-PEG6000-Polymer (1:1:5).
[b] Polymer grade: HPMC (TC-5R); HPC (SL); PVA (EG-05); PVP(k-30).

Table 3
Effect of solvent added in the cogrinding[a] on solubility of NP in water at 37°C

Solvent	Apparent solubility of NP (μg/ml)
None	56
Water	417
Methanol	108
Ethanol	105
Dichloromethane/methanol (7/3)	145

[a] The coground mixtures were prepared in the presence of each solvent. Composition: NP-PEG6000-HPMC (TC-5R) (1:1:5).

The discrepancy observed in this study therefore can be attributed to differences in the mechanism of supersaturation.

In order to farther clarify the characteristics of the interactions, the effect of solvent added in the cogrinding was investigated. Water, methanol, ethanol and dichloromethane/methanol (7:3) were used in the cogrinding of NP-PEG 6000-HPMC (TC-5R) ternary system. As listed in Table 3, a remarkable enhancement was observed in the case of water, suggesting that the NP and HPMC easily interact in a higher polar microenvironment. However, a slight increase in apparent solubility was also observed when other solvents were used.

Therefore, other mechanisms should be concerned with the solubility enhancement. Since NP is hardly dissolved in the cogrinding in the presence of water, pulverizing effect for NP can also participate in the solubility enhancement. In addition, the pulverizing effect will promote the drug–polymer interactions.

3.4. Applicability of the cogrinding method for other poorly water-soluble drugs

To preliminary see if the present cogrinding method can be effective in the enhancement of other poorly water-soluble drugs, IM and GR were selected as model compounds besides NP. Their solubilities in water are known as same level as NP. The results are listed in Table 4. In the case of coground mixture prepared in the presence of a small amount of water, apparent IM solubility was remarkably enhanced as well as NP, but the enhancement of GR solubility was lower than that of other two drugs. Although NP and IM are soluble in PEG 6000 which is contained compo-

Table 2
Effect of substituent groups of water-soluble polymers on apparent solubility of NP in water at 37°C[a]

Polymer	$CH_3CH(OH)CH_2$-substituent (%)	CH_3-substituent (%)	Apparent solubility of NP (μg/ml)
HPMC	7.0–12.0[b]	28.0–30.0	535
	4.0–7.5[c]	27.0–30.0	347
MC	0[d]	26.0–33.0	112
	0[e]	26.0–33.0	148

[a] NP-PEG6000-Polymer (1:1:5) systems were prepared in the presence of water.
[b] Polymer grade: Metolose 60SH-50.
[c] Polymer grade: Metolose 65SH-50.
[d] Polymer grade: Metolose SM-25.
[e] Polymer grade: Metolose SM-100.

nent in the ternary coground mixture, GR is practically insoluble in PEG 6000. Therefore, this difference in drug solubility in PEG 6000 may be affect the performance of the present coground mixtures. Consequently, it appears that the present method can extensively be applied to enhance poorly water-soluble drugs. However, further detail investigations should be necessary, especially with respect to the relationship between drugs and PEG 6000 in the dissolution-improved coground mixture.

3.5. In vivo absorption in beagle dogs

The in vivo performance of the coground mixture was investigated. The plasma concentration–time profiles following the oral administration was examined in beagle dogs. Fig. 5 shows a comparison of the plasma concentration–time profiles of NP after oral administration of the coground mixture prepared in the presence of water and the physical mixture of NP-PEG 6000-HPMC (TC-5R) ternary systems. Additionally, the profiles of NP solution of PEG 400 is also shown in Fig. 5 as a reference. The pharmacokinetic parameters calculated from the plasma concentration curves are summarized in Table 5. The C_{max} of the coground mixture was about ten times

Table 4
Solubilization of various drugs by cogrinding of drug-PEG6000-HPMC systems[a]

Drug	Preparation	Apparent solubility (μg/ml)
NP	Physical mixture	20
	Coground mixture	56
	Coground mixture (water)[b]	417
IM	Physical mixture	35
	Coground mixture	84
	Coground mixture (water)[b]	351
GR	Physical mixture	34
	Coground mixture	51
	Coground mixture (water)[b]	194

[a] Composition: Drug-PEG-6000-HPMC (TC-5R) (1:1:5).
[b] Prepared in the presence of water.

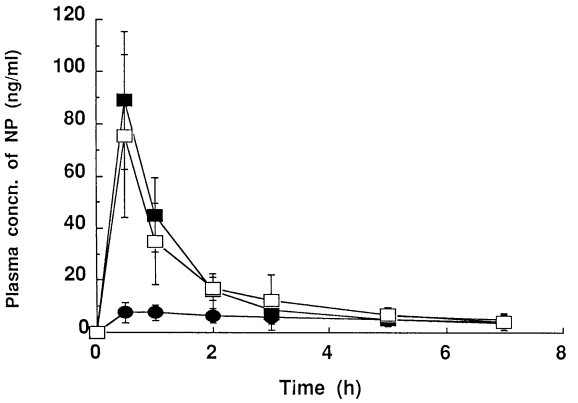

Fig. 5. Mean plasma concentration–time profiles of NP after oral administration of various preparations equivalent to 10 mg of NP to beagle dogs. Each point represents the average ± S.E. of four determinations. (●), physical mixture; (■), coground mixture prepared in the presence of water; (□), NP solution of PEG400.

greater than that of the physical mixture, which was almost same as that of NP solution of PEG 400. The AUC of the coground mixture was also significantly higher than that of the physical mixture. These results indicates that the coground mixture prepared in the presence of a small amount of water disperses quickly in the gastrointestinal tract to produce the emulsion-like hyperfine particles which include NP. Moreover, the supersaturation of NP may be maintained for a long period in vivo, resulting in the remarkable increase in the AUC. According to the results of the in vivo absorption study, the NP dispersed in

Table 5
Comparison of pharmacokinetic parameters after oral administration of varius preparations of NP

Preparation	C_{max} (ng/ml)	AUCi (ng·h/ml)	t_{max} (h)
Physical mixture	9.1 ± 4.1	47.0 ± 29.5	1.4 ± 1.1
Coground mixture (water)[a]	89.0 ± 26.6*	122.3 ± 32.9*	0.5 ± 0.0
PEG400 solution	75.4 ± 3.4*	121.0 ± 54.1*	0.5 ± 0.0

Each value represents the mean ± S.D. ($n = 4$).
[a] Prepared in the presence of water.
* $p < 0.05$ versus physical mixture.

the hyperfine particles may exist in a solution-like state that should be equivalent to the NP solution of PEG 400.

4. Conclusions

The cogrinding of NP with HPMC (TC-5R) and PEG in the presence of water was found to be effective with respect to the improvement of apparent solubility and dissolution. The pulverized NP by the cogrinding is finely dispersed in the coground mixture with partly maintaining its crystallinity; NP can exist in the coacervate like particles sized 50–200 nm in water. Drug–polymer interactions seem to be involved in the mechanism of the solubility enhancement of the cogrinding method presented. When NP-PEG 6000-HPMC (TC-5R) coground mixture was administered orally to beagle dogs, its bioavailability was almost equal to NP solution of PEG400. The novel cogrinding method presented can be an effective method for poorly water-soluble drugs to improve their bioavailability.

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