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RESEARCH PAPER

Improvement of Dissolution and Bioavailability of Nitrendipine by Inclusion in Hydroxypropyl-β-cyclodextrin

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ABSTRACT

A significant increase in solubility and dissolution rate of nitrendipine, a slightly soluble calcium channel blocker, was achieved by inclusion complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD). The inclusion complex was prepared by solvent evaporation method and characterized by phase solubility method, x-ray diffractometry, infrared spectroscopy, and differential scanning calorimetry. The solubility of nitrendipine increased linearly as a function of HP-β-CD concentration, resulting in A_L-type phase solubility diagram which revealed a formation of inclusion complex in a molar ratio of 1:1, with the apparent association constant of $108.3\,M^{-1}$. The in vitro dissolution rate of nitrendipine in pH 7.4 phosphate buffer was in the order of inclusion complex, physical mixture, and nitrendipine powder. These three different forms of nitrendipine were administered orally to rats with a dose of 10 mg/kg equivalent to nitrendipine. The AUC of inclusion complex was significantly larger than that of nitrendipine powder. T_{max} of inclusion complex was significantly shorter and C_{max} was significantly higher than those of nitrendipine powder. C_{max} of physical mixture was higher than that of nitrendipine powder. T_{max} of physical mixture, however, remained the same. The results indicated that the bioavailability of nitrendipine could be improved markedly by inclusion complexation, possibly due to an increased dissolution rate.

Key Words: Nitrendipine; Inclusion complex; Bioavailability; Hydroxypropyl-β-cyclodextrin; Dissolution rate.

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INTRODUCTION

Natural cyclodextrins (CDs) are cyclic oligosaccharides made up of six (α-CD), seven (β-CD), or eight (γ-CD) D-glucopyranose units linked by α-1,4-glycosidic bonds. They, owing to their configurations, have a cylinder-shaped, electron-rich, internal hydrophobic cavity and a hydrophilic external surface. The lipophilic cavity enables CDs to form noncovalent inclusion complexes with a wide variety of poorly water-soluble compounds in aqueous solutions by the spatial entrapment of a whole molecule, or at least some part of it, into the cavity[1] or in a channel formed by several molecules of CD, whereas the hydrophilic outer surface renders these inclusion complexes water soluble. In addition, inclusion of molecules within the cavity of CDs may protect the guest from the external environment, and hence, CDs may be used to optimize the chemical stability of molecules susceptible to degradation.[2–6] Such molecular encapsulation has been shown to improve a variety of drug properties, such as chemical stability, solubility, dissolution rate,[1,7–11] local irritation,[12] bioavailability,[13,14] and clinical activity.[2,15–17]

Among the CDs, β-CD is of most practical use. However, it has some limitations such as low water solubility (about 1.8% w/v, at 25°C). Therefore, chemically modified β-CDs, such as HP-β-CD, have been developed and more widely used in pharmaceutical formulations due to its amorphous nature, high water solubility (>1 g/mL), high inclusion affinity, low toxicity, and ability to alter the phase solubility behavior in favor of isotherms of the A-type.[18–21]

Nitrendipine(3-ethyl-5methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)3,5-pyridine dicarboxylate) is a potent peripheral vasodilator with antihypertensive activity[22,23] and prolonged duration of action.[24,25] It has been extensively used in the management of angina pectoris and systemic hypertension either as a single agent or in combination with other drug classes because of its greater selectivity for vascular smooth muscle and little effect on cardiac conduction and contractility.[26] However, if administered orally, nitrendipine exhibits large differences in bioavailability due to its low water solubility (solubility = 1.9–2.1 mg/L),[27] slow dissolution rate, and high first- pass effect.[28,29]

Dissolution rates of poorly water-soluble drugs may be improved by the use of solid dispersion systems with water-soluble polymers or inclusion complexes.[7,30–32] Previously, an attempt was made to improve the dissolution behavior of nitrendipine with polyvinylpyrrolidone solid dispersion system.[33]

The objective of the present study was to improve the solubility and dissolution rate by preparing an inclusion complex with HP-β-CD and to obtain higher bioavailability of the drug. The formation of such complex was confirmed by phase solubility study, infrared (IR) spectroscopy, differential scanning colorimetry (DSC), and x-ray diffraction studies. For in vivo study, the complex was administered orally to rats to evaluate the influence of complexation of the drug with HP-β-CD on the bioavailability and to find any correlations between dissolution characteristics and bioavailability parameters.

MATERIALS AND METHODS

Materials

Nitrendipine (Mw = 360.4) powder was provided by Boryung Pharmaceutical Co. (Seoul, Korea). Hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The average molecular weight and molar substitution (number of hydroxyl groups per unit of anhydroglucose) of HP-β-CD were 1500 and 0.8, respectively. Double-distilled water was obtained through a MilliQ system (Waters, USA), and ethanol was purchased from Duksan Pharmaceutical Co. (Seoul, Korea). All other chemicals were of reagent grade and used without further purification.

Preparation of Solid Inclusion Complex and Physical Mixture

An inclusion complex of nitrendipine with HP-β-CD was prepared by the solvent evaporation method, previously described by Chiou and Riegelman.[34] Nitrendipine (0.18 g) and HP-β-CD (0.75 g) were dissolved thoroughly in 500 mL of 50% (v/v) ethanol. The solvent was removed in vacuo in a rotary evaporator (Buchi 461, Switzerland) at about 40°C. The residue was collected and dried in vacuo at room temperature for about 24 hr. The powder obtained was stored in a desiccator over silica gel at room temperature until used. For physical mixture, 0.18 g of nitrendipine and 0.75 g of HP-β-CD were weighed out and powdered in a mortar and thoroughly mixed with a spatula.

Characterization of Products

Raw materials, physical mixture, and inclusion complex were subjected to a series of physicochemical analyses. Differential scanning calorimetry (Perkin-Elmer DSC7, USA) measurements were conducted out on 2-mg samples at a heating rate of 20°C/min over a 60–200°C temperature range. A nitrogen purge was maintained throughout runs, and baseline optimization was performed before each run.

Powder x-ray diffraction patterns were obtained with an x-ray diffractometer (Rigaku Denki, Geigerflex model, Japan) using a Ni-filtered CuK(α) radiation at scanning rate of 1°/min under a voltage of 40 kV and a current of 20 mA for the generator. The investigation was performed in the 2θ range of 6–34°.

Infrared spectra were obtained on a Perkin-Elmer 1310 IR spectrophotometer by KBr semi-microdisk technique from 4000 to 625 cm^{-1}. Scanning rate was 5.278 cm^{-1}/s and scanning time was 12 min.

Phase-Solubility Study

One of the major interests in preparing inclusion complexes of nitrendipine is to increase its water solubility. Phase solubility studies were performed according to the method of Higuchi and Conners.[35] Briefly, each excess amount of nitrendipine (10 mg) was weighed into 30-mL sealed glass vials, containing various concentrations of HP-β-CD ranging from 0.0 to 0.01 M. The resulting suspensions were sonicated for 2 hr and then set in a water bath incubator (KMC 1205SW1, Vision Co., Seoul, Korea) and shaken at 100 cycles/min at 25°C ± 1°C for 10 days to achieve complete equilibration. After equilibrium, the content of each vial was filtered through a 0.45-μm cellulose acetate filter (Millipore, Bedford, USA), appropriately diluted with distilled water and analyzed for nitrendipine concentration by UV/visible spectrophotometer (Shimadzu, UV-1201, Tokyo, Japan) at the wavelength of 236 nm with reference to a suitably constructed standard curve. HP-β-CD was found not to interfere with the assay. The intraday and interday coefficients of variation for the assay were less than 2.4% in all cases.

When working on inclusion complexes, the apparent association constant (K_c) often plays an important role in explaining the various results obtained. The experiment was conducted in triplicate, and K_c for the complex formed was calculated from the slope of the phase-solubility diagram and the solubility of nitrendipine at 25°C in water for S_0, according to the following equation[36]:

$$K_c = \text{Slope}/S_0(1 - \text{Slope}).$$

Dissolution Study

The dissolution studies of pure nitrendipine, nitrendipine/HP-β-CD complex, and nitrendipine/HP-β-CD physical mixture were performed in pH 1.2 and 7.4 phosphate buffer by the USP XXIV rotating paddle method at 37°C ± 0.5°C. A sample equivalent to 10 mg of nitrendipine was gently dispersed in the dissolution medium (500 mL) with stirring at 50 rpm. At appropriate time intervals, 5-mL aliquots of solution were withdrawn, filtered immediately through a membrane filter (pore size 0.45 μm), and analyzed directly or after dilution if needed. A correction was applied for the cumulative dilution caused by the replacement of the samples by equal volumes of the fresh medium. Each experiment was done in triplicate and the standard deviation of the mean value was below ±5%.

Pharmacokinetic Study and Statistical Assessment

Male Sprague-Dawley rats weighing 180–230 g were used for the in vivo bioavailability study. They were kept on a standard diet and fasted for 12 hr prior to oral administration of the drug with free access to water. Nitrendipine, the corresponding amount of 1:1 inclusion complex of nitrendipine/HP-β-CD, or physical mixture of nitrendipine/HP-β-CD was suspended in distilled water (10 mg/kg) and immediately administered orally through ball-tipped needle to each group of eight rats.

The blood samples (about 50 μL) were collected through previously implanted polyethylene cannulae (PE-50, Clay Adams, Parsippany, NJ) in the left femoral artery as described by Upton.[37] The blood samples were withdrawn at 0 (blank plasma) and 10, 20, 30, 40, 60, 120, 240, and 360 min after administration of the drug. Plasma was obtained by centrifugation at 2800 rpm for 15 min. The plasma samples were stored at −20°C until analysis.

The total area under plasma concentration time curve ($AUC_{0-\infty}$), the maximum plasma concentration (C_{max}), and the time to reach the maximum

plasma concentration (T_{max}) were chosen as parameters for pharmacokinetic evaluation. The C_{max} and T_{max} were obtained directly from the experimental data of plasma concentrations vs. time.[38] $AUC_{0-\infty}$ was obtained by adding the $AUC_{0-360\,min}$, which was calculated by the linear trapezoidal method and the area from the last experimental time point to infinite time ($AUC_{360\,min-\infty}$) using the method of Shargel and Yu.[39] Differences in pharmacokinetic parameters were tested statistically by using one-way analysis of variance. In all tests, a probability value of $P < 0.05$ was considered statistically significant.

Determination of Nitrenedipine Plasma Levels

The plasma levels of nitrendipine were measured by high-performance liquid chromatography (HPLC) with slight modifications of the methods reported previously.[40,41] Blood samples collected were centrifuged at 3000 rpm for 10 min to obtain plasma (20 μL), to which 10 μL of nifedipine (100 μg/mL, internal standard) and 10 μL of 1N-NaOH were added. Plasma was extracted with 100 μL of hexane-ethylether (1/1 v/v). After vortexing vigorously for 15 min, the mixture was centrifuged at 3000 rpm for 10 min at 4°C to separate organic phase. After evaporation of the organic phase under a nitrogen stream, the residue was reconstituted in 60 μL of the mobile phase and a 50 μL aliquot was injected onto the HPLC column.

HPLC Conditions

The chromatographic system consisted of Hitach D-7000 system manager software (Hitachi, Ibaraki, Japan), a Model 7725 injector (Rheodyne, Cotati, USA) fitted with a 50-μL sample loop, a Model L-7100 pump (Hitachi, Ibaraki, Japan), a D-7000 interface module (Hitachi), and a Model L-7450 diode array detector (Hitachi). The HPLC separation was performed on reversed phase C18 column (Inertsil ODS-2, 4.6 × 150 mm, 5-μm, GL-Sciences, Tokyo, Japan). The mobile phase consisted of a mixture of water and acetonitrile (30:70 v/v). The mobile phase was filtered through a 0.45-μm HV filter (Millipore, Bedford, USA) and deaerated ultrasonically prior to use. The detector was set at 236 nm (0.005 AUFS), and the eluent was pumped through the column at a flow rate of 1 mL/min. All chromatographic operations were conducted out at ambient temperature.

HPLC Method Validation

Validation of HPLC method was evaluated by linearity, precision (repeatability and intermediate precision), accuracy, and limit of detection. The calibration curve of nitrendipine was linear over the concentration range of 0.01–1.0 μg/mL ($r > 0.9998$). The repeatability was evaluated by six replicate determinations of solutions with two different concentrations of nitrendipine. The relative standard deviation (RSD) obtained for 0.1 and 0.5 μg/mL nitrendipine solutions were 3.27% and 1.93%, respectively. The intermediate precision determined by analyzing these solutions in 5 consecutive days was found to be reproducible, with the RSD of 4.12% (0.1 μg/mL) and 2.35% (0.5 μg/mL). The accuracy, expressed as recovery percentage of nitrendipine from plasma, was assessed by spiking plasma samples with nitrendipine in 0.5 μg/mL concentration and found to be 87.3% ± 3.4% ($n = 6$). The detection limit for the assay was estimated to be 0.01 μg/mL.

RESULTS AND DISCUSSION

Characterization of Solid Inclusion Complex

Figure 1 illustrates the DSC thermal curves of nitrendipine, HP-β-CD, nitrendipine/HP-β-CD mixture, and nitrendipine/HP-β-CD inclusion complex. The DSC trace of nitrendine shows one characteristic sharp endothermic peak at around 158°C, indicating the melting point of the drug. Meanwhile, the DSC trace of HP-β-CD shows shallow and broad endothermic peaks at about 105.1°C, which can be extended because of the release of water from the molecule.[31] The endothermic peak at around 158°C was also observed for physical mixture. However, it disappeared or appeared very broad without distinct phase transition around this temperature due to the inclusion complexation with HP-β-CD in DSC curves, suggesting that the drug is monomolecularly dispersed in the HP-β-CD cavity.

The x-ray diffraction patterns of nitrendipine, HP-β-CD, nitrendipine/HP-β-CD mixture and nitrendipine/HP-β-CD inclusion complex are shown

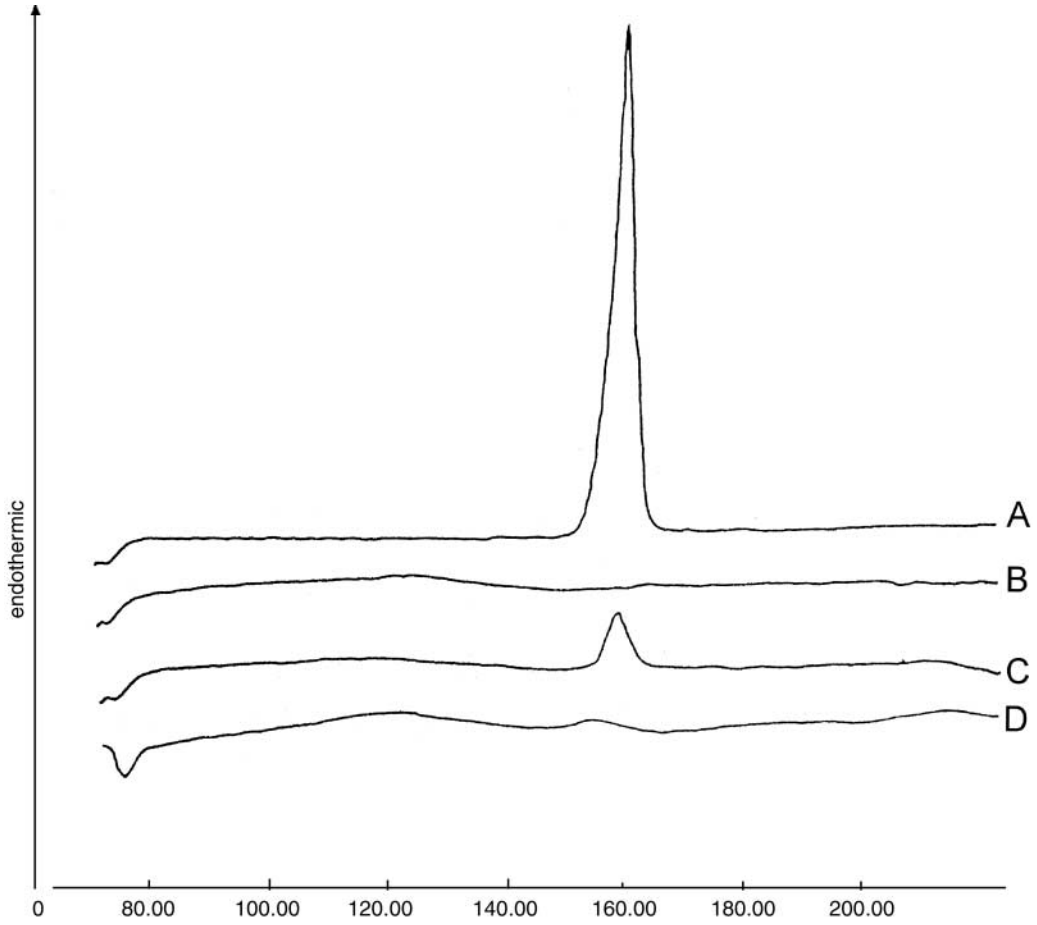

Figure 1. Differential scanning colorimetry curves of nitrendipine/HP-β-CD systems: (A) nitrendipine alone; (B) HP-β-CD; (C) physical mixture; and (D) inclusion complex.

in Fig. 2. Nitrendipine presented well-defined crystalline x-ray pattern, whereas HP-β-CD presented amorphous x-ray pattern. The diffraction pattern of the physical mixture constituted appears to represent the superposition of each component's spectrum although peaks attributable to nitrendipine are remarkably diminished, indicating lower degree of crystallinity. In contrast, inclusion complex showed no diffraction peaks except halo-pattern in x-ray diffractogram, suggesting an amorphous state of the drug in the inclusion complex.

The complex of nitrendipine with HP-β-CD was examined by IR spectroscopy and compared with pure nitrendipine, pure HP-β-CD, and corresponding physical mixture in the same molar ratio as shown in Fig. 3. Pure nitrendipine showed IR absorption band at $1632\,cm^{-1}$ for the ester carbonyl stretching band. The absorption bands at 1413 and $1578\,cm^{-1}$ were denoted for stretching vibration of C=C in the aromatic ring, whereas $1350\,cm^{-1}$ was denoted for NO_2 group and $3316\,cm^{-1}$ for N-H group. The spectrum of pure HP-β-CD showed the vibration of free –OHs between 3000 and $3400\,cm^{-1}$ and those of bound -OHs at $2760\,cm^{-1}$.[42] The spectrum of inclusion complex did not show new peaks, an indication no chemical bonds were created in the formed compound.

In the physical mixture, the spectrum is the superposition of those of the pure products with attenuation of the nitrendipine peaks, showing no significant differences from the respective spectra of the pure components. However, the IR spectrum of the inclusion complex exhibited some significant differences. For the complex, the nitrendipine peak mostly disappeared, and the excess of free HP-β-CD was still visible, suggesting that some interaction, probably hydrogen bonding, occurred between nitrendipine and HP-β-CD in the inclusion complex.

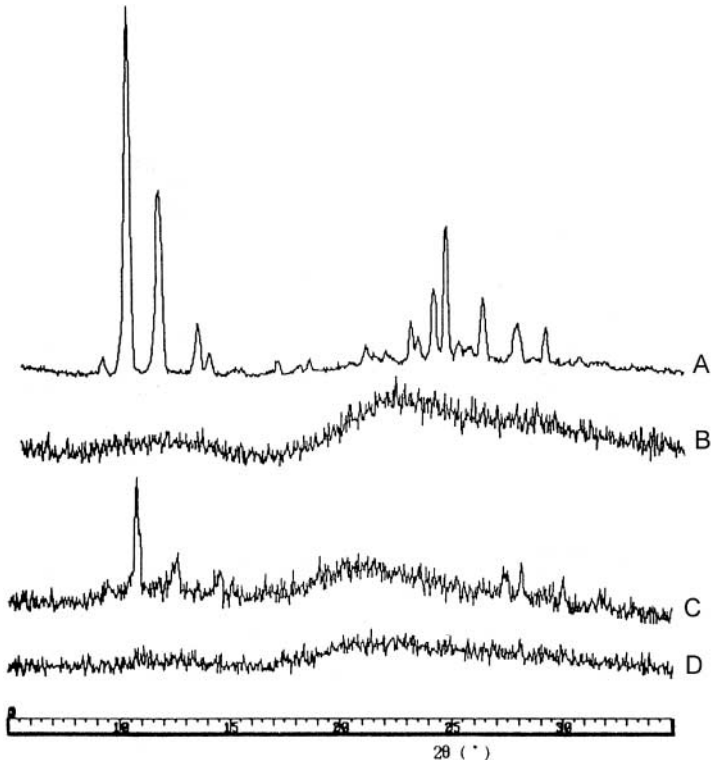

Figure 2. Powder x-ray diffraction patterns of nitrendipine/HP-β-CD systems: (A) nitrendipine alone; (B) HP-β-CD; (C) physical mixture; and (D) inclusion complex.

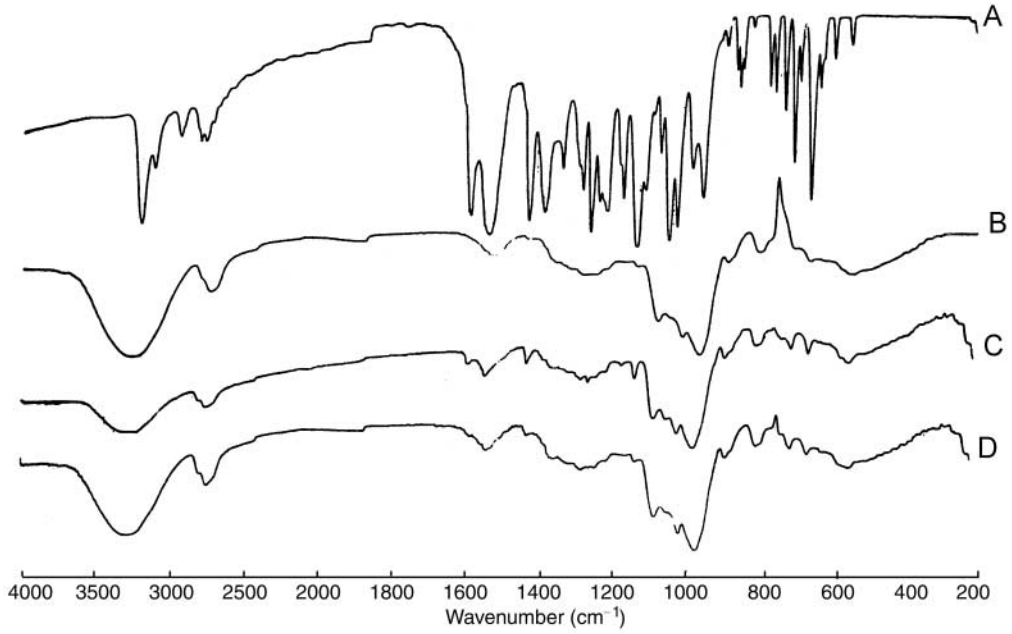

Figure 3. Infrared spectra of nitrendipine/HP-β-CD systems: (A) nitrendipine alone; (B) HP-β-CD; (C) physical mixture; and (D) inclusion complex.

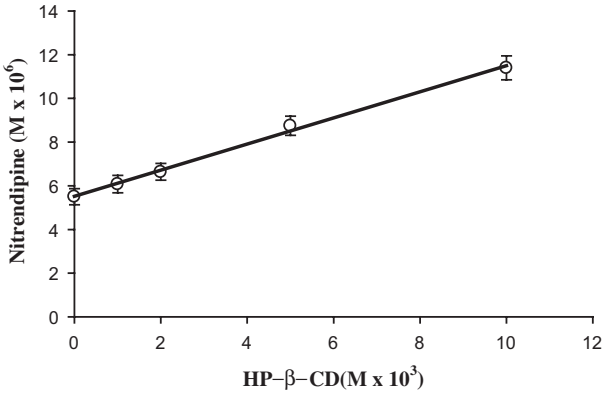

Figure 4. Phase-solubility diagram of nitrendipine-HP-β-CD system in distilled water at 25°C Each data point is the mean ± SD of three determinations.

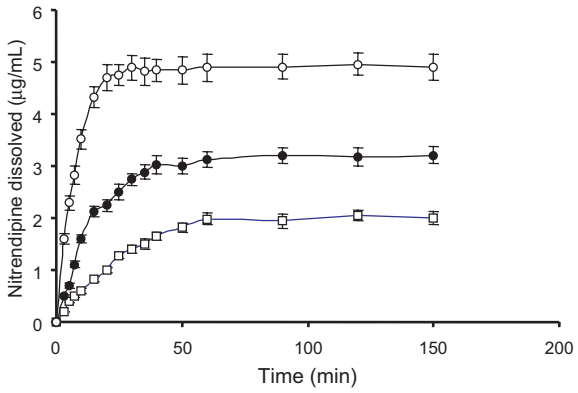

Figure 5. Dissolution profiles of nitrendipine/HP-β-CD systems in pH 7.4 buffer solution at 37°C: (□) nitrendipine alone; (●) physical mixture; and (○) inclusion complex. Each data point is the mean ± SD of three determinations.

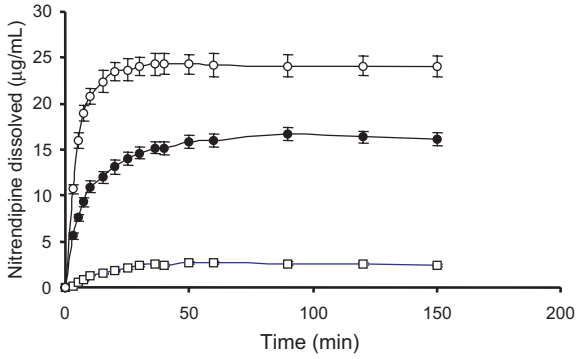

Figure 6. Dissolution profiles of nitrendipine/HP-β-CD systems in pH 1.2 buffer solution at 37°C: (□) nitrendipine alone; (●) physical mixture; and (○) inclusion complex. Each data point is the mean ± SD of three determinations.

Phase-Solubility Study

The aqueous phase solubility profile for the complex formation between nitrendipine and HP-β-CD at 25°C is presented in Fig. 4. This diagram illustrates that the apparent solubility of nitrendipine increased linearly as a function of the HP-β-CD concentration (correlation coefficient: 0.998), resulting in A_L-type phase solubility curve. This finding suggested the formation of the soluble nitrendipine/HP-β-CD inclusion complex with 1:1 stoichiometry according to Highuchi and Connors (35). The apparent association constant of nitrendipine/HP-β-CD complex was calculated to be 108.3 M^{-1}. The aqueous solubility of nitrendipine determined in this study was 1.99 mg/L, which was in good agreement with the result of Kristl et al.[27]

Dissolution Study

The dissolution behaviors of nitrendipine alone, physical mixture, and inclusion complex in pH 1.2 and 7.4 phosphate buffer solution at 37°C are represented in Figs. 5 and 6, respectively. The dissolution rate of inclusion complex was evidently higher than that of drug alone. Corresponding physical mixture also demonstrated higher dissolution rate than pure drug but with significantly slower rate than inclusion complex. The significant enhancement in the dissolution rate of the inclusion complex could be explained from an increase in solubility, a marked reduction in crystallinity as confirmed by x-ray diffraction study and an improved wettability of the drug by the inclusion complexation.[43] Increased dissolution rate of physical mixture might be attributable to the formation of in situ formation of readily soluble complexes in the dissolution media. It should also be attributed to the wetting effect of the HP-β-CD in the initial stage of the dissolution process as suggested by previous authors.[11,44]

Pharmacokinetic Study

The in vivo absorption study was carried out to see whether the enhanced in vitro dissolution rate of nitrendipine from inclusion complex reflected the gastrointestinal absorption of the drug. The profiles of the plasma concentrations of nitrendipine vs. time after oral administration of the pure drug, physical

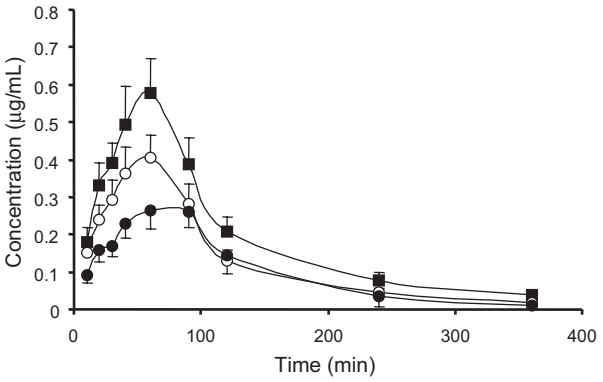

Figure 7. Mean plasma concentration-time curves of nitrendipine after oral administration of 10 mg/kg nitrendipine to rats at a dose of 10 mg/kg nitrendipine equivalent: (●) nitrendipine powder; (○) physical mixture; and (■) inclusion complex Each point represents the mean ± S.D. of eight animals.

Table 1. Bioavailability parameters after oral administration of various forms of nitrendipine at a dose of 10 mg/kg to rats.

	C_{max} (μg/mL)	T_{max} (min)	AUC (μg min/mL)
Inclusion complex	0.581 ± 0.085	63.75 ± 10.61	75.37 ± 12.24
Physical mixture	0.407 ± 0.036	67.5 ± 13.89	49.76 ± 7.94
Nitrendipine powder	0.272 ± 0.051	78.25 ± 22.32	39.88 ± 5.72

Each value represents means ± SD of eight animals.

mixture, and inclusion complex are depicted in Fig. 7. Calculated pharmacokinetic parameters are summarized in Table 1. The time to reach the maximum plasma concentration of inclusion complex (63.7 ± 10.6 min) and physical mixture (67.5 ± 13.9 min) was significantly shorter than that of the free drug (78.3 ± 22.3 min) as shown in Table 1. Nitrendipine/HP-β-CD inclusion complex displayed significantly faster absorption than free drug. T_{max} of physical mixture exhibited a slight delay over that of inclusion complex, although it was not statistically significant. Nitrendipine, physical mixture, and inclusion complex produced C_{max} values of 0.27 ± 0.05, 0.417 ± 0.04, and 0.58 ± 0.09 μg/mL, respectively. C_{max} for inclusion complex was about 2 times greater than that of nitrendipine alone. The AUC of the inclusion complex was about 2 times greater than that of nitrendipine alone. The statistical analysis of the blood level data confirmed that physical mixture and inclusion complex have higher bioavailability than the drug itself, which could be attributed to the increase in solubility and dissolution rate of the drug. These results are in line with Stella and Rajewski's conclusion that the primary use of CDs in oral formulations is to increase bioavailibility through increased rate and extent of drug dissolution.[45] There are numerous reports showing that the aqueous solubility and dissolution rate of poorly soluble compounds are significantly increased in vitro by cyclodextrin complexation.[7,17,46,47]

The present findings demonstrated a significant improvement in bioavailability of nitrendipine by oral administration of its inclusion complex with HP-β-CD in rats, owing to faster T_{max} and higher C_{max}. Inclusion complexation of poorly soluble drugs with HP-β-CD may be expected to provide better bioavailability because of greater dissolution rates of drugs. HP-β-CD may also enable the drug molecules to be kept in a supersaturated state.

CONCLUSION

Nitrendipine was found to form an inclusion complex with HP-β-C by a variety of techniques. Inclusion complex of the drug was remarkably less crystalline and, therefore, more soluble than the nitrendipine itself in aqueous media. Further more, the dissolution rate of inclusion complex was higher than that of pure drug. The appreciable improvement in the physicochemical properties of the drug has resulted in a significant increase in bioavailability of nitrendipine, when administered orally in rats.

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