Utility of 2-Hydroxypropyl-β-cyclodextrin in an Intramuscular Injectable Preparation of Nimodipine

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Possible utility of hydroxyalkylated β-cyclodextrin (β-CyD) derivatives as parenteral drug carriers was investigated, using nimodipine, a dihydropyridine derivative with calcium antagonistic action, as a model drug. The aqueous solubility of nimodipine increased linearly with increase in the concentration of hydroxyalkylated β-CyDs, showing an A_L-type phase solubility diagram. The stability constant of nimodipine–hydroxyalkylated β-CyD complexes was in the order of 2,3-dihydroxypropyl-β-CyD < β-CyD < 2-hydroxyethyl-β-CyD < 3-hydroxypropyl-β-CyD < 2-hydroxypropyl-β-CyD, and the solubilizing ability of the β-CyDs was also in that order. The results of powder X-ray diffractometry and thermal analysis suggested 1 : 3 (guest : host) complex formation of nimodipine with 2-hydroxypropyl-β-CyD in the solid state. The dissolution rate of nimodipine–2-hydroxypropyl-β-CyD complex was much faster than that of the drug alone. Nimodipine–2-hydroxypropyl-β-CyD complex gave higher plasma levels of the drug after intramuscular administration to rabbits, *i.e.*, the area under the plasma concentration–time curve and the maximum plasma concentration of the complex were about 2.5 times higher than those of the drug alone. The muscular damage after the injection of nimodipine was reduced by the administration of the complexed form.

Keywords hydroxyalkylated β-cyclodextrin; nimodipine; complexation; solubilization; intramuscular administration; bioavailability; muscular damage

Introduction

Nimodipine, isopropyl 2-methoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (Fig. 1), is a dihydropyridine derivative with calcium antagonistic action, and can be used in the treatment of cerebrovascular spasms because of its preferential effect on cerebral vessels.[1,2] However, the clinical usefulness of nimodipine is severely restricted by its insufficient intestinal absorption and first-pass metabolism.[3] From the viewpoint of minimizing such loss of the therapeutic efficacy of nimodipine, parenteral dosage forms of the drug may need to be used.

Molecular encapsulation of drugs with cyclodextrins (CyDs) has received increasing attention because of its potential to improve the solubility and other pharmaceutical properties of drugs.[4] The usefulness of natural, crystalline CyDs can be improved by chemical modification into amorphous mixtures of their derivatives.[5] Among these types of derivatives, hydroxyalkylated CyDs have gained acceptance in pharmaceutical applications because of their high solubility in water and low toxicity.[6,7] We have recently reported some physico- and bio-pharmaceutical properties of various hydroxyalkylated β-CyDs.[8] Thus, the present study was undertaken to survey the possible utility of hydroxyalkylated β-CyDs as solubilizers to prepare water-soluble dosage forms of nimodipine suitable for parenteral use. 2-Hydroxypropyl-β-CyD, as an example, was applied to the intramuscular injectable preparation, anticipating an improvement in the bioavailability of nimodipine.

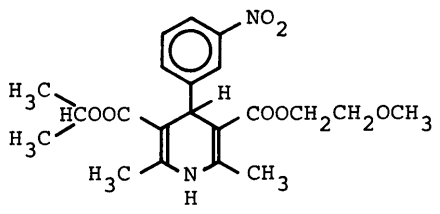


Fig. 1. Chemical Structure of Nimodipine

Methods

Materials Nimodipine was supplied by Bayer Yakuhin Ltd. (Osaka, Japan). 2-Hydroxyethyl- (2-HE-), 3-hydroxypropyl- (3-HP-) and 2,3-dihydroxypropyl- (DHP-) β-CyDs were donated by the Tokyo Research Division of Wako Pure Chemical Ind., Ltd. (Saitama, Japan) and β-CyD and 2-hydroxypropyl- (2-HP-) β-CyD were from Nipon Shokuhin Kako Co. Ltd. (Tokyo, Japan). The degrees of substitution (D.S.) for the hydroxyalkylated β-CyDs were 5.8, 5.8, 6.1 and 5.9 for 2-HE-, 2-HP-, 3-HP- and DHP-β-CyDs, respectively, as determined by secondary ionization mass spectrometry.[8,9] Other chemicals were from commercial sources, and deionized, double-distilled water was used.

Solubility Studies Solubility measurements were carried out according to Higuchi and Connors.[10] An excess amount (10 mg) of nimodipine was added to aqueous solutions containing CyDs at various concentrations, and the solutions were shaken for about 10 d at 25 °C. After equilibrium was attained, filtered aliquots were analyzed by spectrophotometry at 358 nm. The experiment was carried out in the dark to avoid the photodegradation of nimodipine.

Preparation of Solid Complex The solid complex was prepared according to the kneading method,[11] *i.e.*, nimodipine (40 mg) and 2-HP-β-CyD in various molar ratios were triturated with a small amount of water (about 1 ml) and the slurry was kneaded thoroughly for about 45 min in the dark. The resulting paste was dried under reduced pressure at room temperature for 2 d. Differential thermograms and powder X-ray diffractograms were taken under conditions similar to those reported previously.[12]

Dissolution Studies The dissolution rate was measured according to the dispersed amount method.[13] An excess amount of nimodipine (10 mg, < 100 mesh) or its 2-HP-β-CyD complex (equivalent to 10 mg of the drug) was put into 200 ml of water at 37 °C, and the dissolution medium was stirred at 91 rpm. At appropriate intervals, 0.5 ml of solution was sampled by using a pipet with a cotton plug, and the drug in the aliquot was extracted with 6 ml of ethyl acetate containing nifedipine as an internal standard for high-performance liquid chromatography (HPLC). A 5 ml aliquot of the organic phase was evaporated under reduced pressure, the residue was redissolved in 100 μl of acetonitrile–methanol–water (2 : 2 : 1), and the drugs were assayed by HPLC under the following conditions: pump and detector, a Hitachi 635A liquid chromatograph (Tokyo, Japan) with a Hitachi 638-41 UV monitor (Tokyo, Japan); column, Erma ERC-ODS-1282 (6 mm diameter × 150 mm, Tokyo, Japan); mobile phase, acetonitrile–methanol–water (2 : 2 : 1); flow rate, 1.0 ml/min; detection, 358 nm.

Intramuscular Administration Five male albino rabbits, weighing 2.5—3.0 kg, were fasted for 24 h prior to drug administration. Intervals of at least 2 weeks were used in a cross-over matrix to minimize the cumulative effect of the preceding dose. A test powder (5 mg/kg of body weight as nimodipine, < 100 mesh) was injected as a suspension in 1 ml of normal sterile saline into *M. vastus lateralis* of rabbits using a 23-gauge 0.5 inch needle. At predetermined intervals, 2.0 ml of blood was taken from the

marginal ear vein using a citrated syringe and centrifuged (1400 × *g*) for 10 min. Nimodipine in the plasma was determined by gas chromatography (GC), after oxidation of the dihydropyridine ring of nimodipine to the pyridine, *i.e.* 1 ml of the plasma was added to isopropanol (0.5 ml) containing nisoldipine as an internal standard for GC. Nimodipine was oxidized by adding 0.1 N HCl (0.5 ml) and 1% sodium nitrite (0.3 ml) and agitating for 1 h at 45 °C. After addition of 2 N NaOH (0.5 ml), the oxidized drugs were extracted with benzene (5 ml), the organic phase (4 ml) was evaporated under reduced pressure, the residue was redissolved in ethyl acetate, and the drugs were analyzed by GC under the following conditions: a Shimadzu GC-7A gas chromatograph with an electron capture detector (Kyoto, Japan); column, 3% OV-1 on Chromosorb WAW DMCS (80—100 mesh) in a glass column (3 mm diameter × 3 m); column and injection temperatures, 250 and 280 °C, respectively.

The intramuscular irritation study was carried out by the method of Shintani *et al.*[14] Nimodipine or its complex was injected into the *M. vastus lateralis* of rabbits, in the same manner as described above. Two days after the injection, the rabbits were killed. The muscle was exposed and cut longitudinally, and the lesions were scored as described.[14]

Results and Discussion

Complex Formation of Nimodipine with Hydroxyalkylated β-CyDs Figure 2 shows the phase solubility diagrams in water of nimodipine with various hydroxyalkylated β-CyDs having a similar degree of substitution (D.S. about 6). The solubility (5.5×10^{-6} M) of nimodipine increased linearly with increase in CyD concentration, showing A_L type solubility diagrams according to Higuchi and Connors.[10] Assuming that a 1 : 1 complex is initially formed, the apparent stability constants (K') of the complexes were calculated, in terms of Eq. 1,[10] using the slope and intercept of the straight lines of the diagram.

$$K' = \frac{\text{slope}}{\text{intercept } (1 - \text{slope})} \quad (1)$$

The K' values of the complexes were in the order of DHP-β-CyD (260 M^{-1}) < β-CyD (480 M^{-1}) < HE-β-CyD (560 M^{-1}) < 3-HP-β-CyD (870 M^{-1}) < 2-HP-β-CyD (950 M^{-1}). The inclusion ability of β-CyD toward nimodipine was enhanced by the introduction of the relatively hydrophobic hydroxypropyl group into the host molecule, but diminished by the highly hydrophilic dihydroxypropyl group. The ultraviolet (UV) spectral change and the induced circular dichroism spectra of nimodipine caused by the binding to 2-HP-β-CyD were the largest among the derivatives.

The solid complex of nimodipine with 2-HP-β-CyD, having the highest solubilizing effect among the CyD derivatives, was prepared by the kneading method[11] and their interaction was investigated by powder X-ray diffractometry and thermal analysis. Figure 3 shows the X-ray diffractograms of nimodipine–2-HP-β-CyD solid complexes at molar ratios of 1 : 1 to 1 : 3 (guest : host). 2-HP-β-CyD gave a diffused diffraction pattern due to its amorphous character, which was in contrast to the case of nimodipine. The diffraction patterns of the 1 : 1 and 1 : 2 complexes were similar to those of the simple physical mixture of each component, although the intensity was decreased slightly. In the case of the 1 : 3 complex, on the

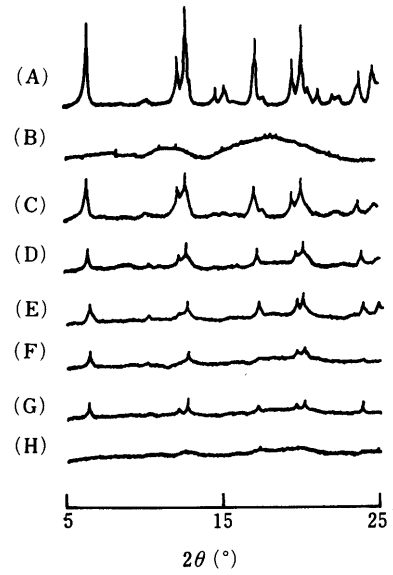


Fig. 3. Powder X-Ray Diffraction Patterns of Nimodipine–2-HP-β-CyD Systems at Various Molar Ratios of the Guest and Host Molecules

(A), nimodipine; (B), 2-HP-β-CyD; (C), 1 : 1 (guest : host) physical mixture; (D), 1 : 1 complex; (E), 1 : 2 physical mixture; (F), 1 : 2 complex; (G), 1 : 3 physical mixture; (H), 1 : 3 complex.

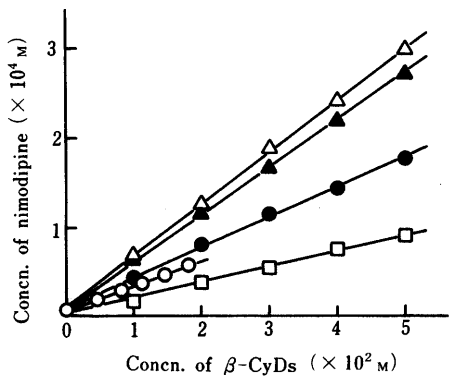


Fig. 2. Phase Solubility Diagrams of Nimodipine–Hydroxyalkylated β-CyD Systems in Water at 25 °C

—○—, β-CyD; —●—, HE-β-CyD; —△—, 2-HP-β-CyD; —▲—, 3-HP-β-CyD; —□—, DHP-β-CyD.

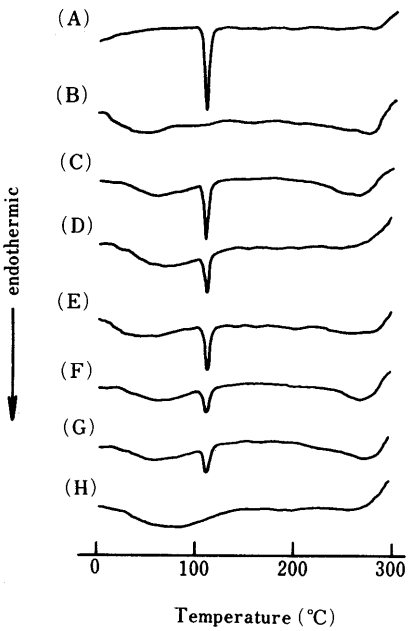


Fig. 4. DTA Thermograms of Nimodipine–2-HP-β-CyD Systems at Various Molar Ratios of the Guest and Host Molecules

(A), nimodipine; (B), 2-HP-β-CyD; (C), 1 : 1 (guest : host) physical mixture; (D), 1 : 1 complex; (E), 1 : 2 physical mixture; (F), 1 : 2 complex; (G), 1 : 3 physical mixture; (H), 1 : 3 complex.

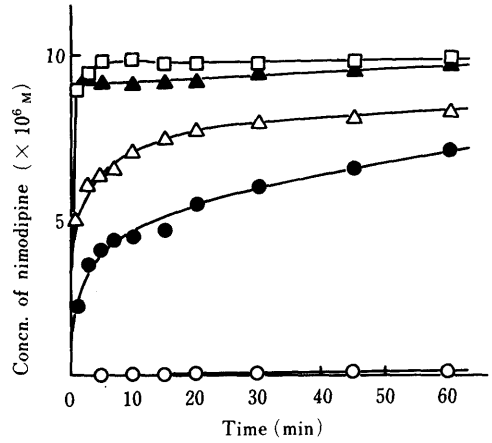


Fig. 5. Dissolution Profiles of Nimodipine–2-HP-β-CyD Complexes with Various Molar Ratios in Water at 37 °C

—○—, nimodipine alone; —●—, 1 : 1 (guest : host) complex, —△—, 1 : 2 complex; —▲—, 1 : 3 complex; —□—, 1 : 5 complex.

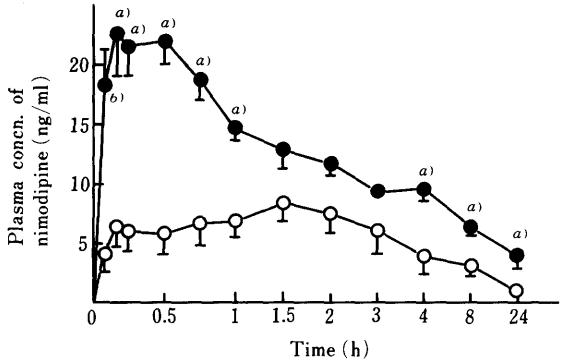


Fig. 6. Plasma Concentrations of Nimodipine Following Intramuscular Administration of Nimodipine or Its 2-HP-β-CyD Complex (Equivalent to 5 mg/kg Nimodipine) Suspensions to 5 Rabbits

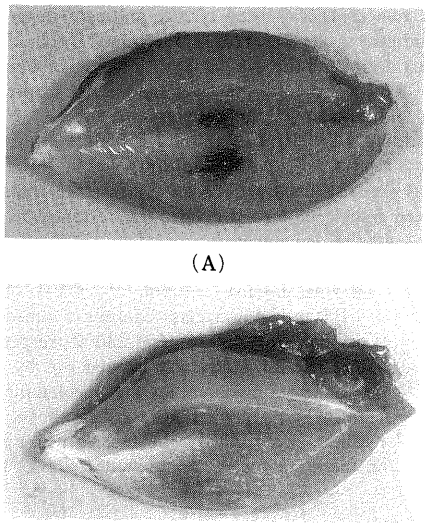
—○—, nimodipine alone; —●—, 1 : 3 complex. *a*) $p<0.05$ in complex *versus* nimodipine alone. *b*) $p<0.01$ in complex *versus* nimodipine alone.

Table I. Pharmacokinetic Parameters Following Intramuscular Administration of Suspensions of Nimodipine or Its 2-HP-β-CyD Complex (Equivalent to 5 mg/kg Nimodipine) to 5 Rabbits

System	C_{max} (ng/ml)	t_{max} (h)	$AUC^{a)}$ (h·ng/ml)
Nimodipine alone	9.13±1.78	0.78±0.21	31.90±8.78
2-HP-β-CyD complex	23.18±2.94	0.35±0.06[b)]	82.55±8.92[b)]

a) Up to 24 h post-administration. *b*) $p<0.01$ *versus* nimodipine alone.

other hand, the diffraction peaks of nimodipine disappeared and the pattern was apparently different from that of the physical mixture. Figure 4 shows differential thermal analysis (DTA) thermograms of the complexes with molar ratios of 1 : 1 to 1 : 3. Nimodipine gave a sharp endothermic peak at 110 °C due to melting, whereas 2-HP-β-CyD had no peaks around that temperature. The intensity of the endothermic peak of the drug decreased with increase in the molar ratio of the host molecule, and the peak completely disappeared at the 1 : 3 ratio, in good agreement with the results of X-ray diffractometry. These results suggest that nimodipine interacts with three 2-HP-β-CyD molecules, *i.e.* partial inclusion occurs, giving an amorphous solid



(A)

(B)

Fig. 7. Macrographs of *M. vastus lateralis* at 2 d after the Injection of Nimodipine or Its 2-HP-β-CyD Complex (Equivalent to 5 mg/kg Nimodipine) Suspensions

(A), nimodipine alone; (B), 1 : 3 complex.

Table II. Intramuscular Irritation Scores of *M. vastus lateralis* at 48 h after Intramuscular Administration of Suspensions of Nimodipine or Its 2-HP-β-CyD Complex (Equivalent to 5 mg/kg Nimodipine) to 8 Rabbits

System	Irritation score
Nimodipine alone	1.88±0.48
2-HP-β-CyD complex	0.85±0.44

complex.

Dissolution of Nimodipine–2-HP-β-CyD Complex Figure 5 shows the dissolution profiles in water of nimodipine–2-HP-β-CyD complexes with various molar ratios. The dissolution rate of nimodipine was extremely slow, whereas the complexes dissolved much more rapidly, which may be due to the enhanced solubility in water (Fig. 2) and amorphous character (Fig. 3) of the complexes. The dissolution rate of the complexes increased with increase in the molar ratio of the host molecule, and leveled off above the 1 : 3 ratio where a burst of dissolution followed by a slower dissolution phase was observed. This leveling phenomenon may support the occurrence of 1 : 3 complex formation.

Plasma Level after Intramuscular Administration Figure 6 shows the plasma levels of nimodipine after intramuscular administration of nimodipine or its 2-HP-β-CyD complex (molar ratio of 1 : 3) in the form of suspension to the *M. vastus lateralis* of rabbits, and Table I summarizes the pharmacokinetic parameters obtained by moment analysis.[15] The plasma levels of nimodipine were much higher when the drug was administered as the complexed form. The maximum plasma level (C_{max}) and the area under the plasma concentration–time curve (AUC, up to 24 h) of the complex were about 2.5 times higher than those of the drug alone, while the t_{max}, *i.e.*, the time to reach the C_{max}, was about half of that of the drug alone. The higher plasma level may be attained mainly due to the enhanced solubility in water of the drug as a result of the complexation and the rapid release of the drug from the dispersed solid

complex into mucus around the injection site. The pharmacokinetic parameters obtained from the muscular administration were almost equal to those obtained from the oral administration of a 2-fold larger amount (10 mg/kg) of nimodipine or the complex to rabbits.[16]

The muscular damage after the injection of the 2-HP-β-CyD complex was compared with that in the case of the drug alone. Figure 7 shows macrographs of *M. vastus lateralis* of rabbit dissected at 2 d after the single injection of nimodipine or its complex, and Table II lists the irritation score estimated according to Shintani *et al.*[14] In the case of the drug alone, a discoloration of the muscle with hemorrhage was observed, whereas the complex gave only a slight hyperemia. The irritation score for the complex was about half of that for the drug alone.

The present data suggest that 2-HP-β-CyD is of considerable pharmaceutical use as a drug-carrier for injectable preparations of nimodipine, because it provides high aqueous solubility and low muscular irritation.

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