J. Pharm. Pharmacol. 1994, 46: 505–507
Received July 27, 1993
Accepted December 5, 1993

In-vivo and in-vitro evaluations of a modified-release oral dosage form of nifedipine by hybridization of hydroxypropyl-β-cyclodextrin and hydroxypropylcelluloses in dogs

ZHENG WANG, FUMITOSHI HIRAYAMA, KANETO UEKAMA, *Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan*

Abstract—To maintain a suitable blood level of nifedipine for a long period of time, double-layer tablets consisting of 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) and 3% nonionic surfactant (HCO-60) as a fast-release portion and hydroxypropylcelluloses (HPCs) with different viscosity grades (low, medium and high) as a slow-release portion were prepared, and their in-vitro and in-vivo release behaviours were investigated. Among the seven formulations, the tablet having the mean dissolution time of 0·8–1·3 h gave prolonged plasma nifedipine levels without decrease of AUC after oral administration to dogs. Consequently, the double-layer tablet consisting of HP-β-CyD with 3% HCO-60/(HPC-low:HPC-medium) in a weight ratio 1/(1·5:1·5) was selected as an appropriate modified-release formulation because it elicited almost comparable retarding effects with superior oral bioavailability compared with those of a commercially available slow-release nifedipine product.

Conventional formulations of nifedipine, a calcium-channel antagonist, must be dosed either twice or three times daily, because of the rapid absorption and short elimination half-life, which often results in significant fluctuations in plasma drug concentrations (Foster et al 1983; Kleinbloesem et al 1984). To attain a prolonged therapeutic effect and a reduced incidence of side-effects, many attempts have been made to maintain a suitable plasma level of nifedipine for a long period of time with minimal frequency of administration (Sugimoto et al 1982; Pabst et al 1986; Kleinbloesem et al 1987). For this purpose, use of rationally-designed drug carrier materials is of potential in controlling the drug release at the desired level. In previous papers (Wang et al 1993a, b), we reported that the release rate of nifedipine can be modified by the hybridization of hydroxypropyl-β-cyclodextrin (HP-β-CyD) and hydrophilic polymers such as hydroxypropylcelluloses (HPCs) and polyvinylpyrrolidone (PVP) with different viscosity grades. In particular, the double-layer tablets consisting of nifedipine/HP-β-CyD/HCO-60 as a fast-release portion and nifedipine/HPCs (low (L), medium (M) and high (H)) as a slow-release portion were found to be useful in the control of the release rate of nifedipine depending on the composition ratio. In the present study, therefore, the rationale for the double-layer tablet was further investigated by in-vivo absorption studies in a dog model, to seek the optimal formulation of nifedipine.

Materials and methods

Materials. HP-β-CyD with an average degree of substitution of 5·8 was donated by Nihon Shokuhin Kako Co. Ltd (Tokyo, Japan). Nifedipine and its commercially available slow-release preparations (Adalat L-10 and L-20) were supplied from Bayer Yakuhin Ltd (Osaka, Japan). Three HPCs, HPC-L, HPC-M and HPC-H with different viscosity grades (viscosity ranges of 2% aqueous solution at 20°C of 6–10, 150–400 and 1000–4000 cPs, respectively), were supplied by Nippon Soda Co. (Tokyo, Japan). Nonionic surfactant, HCO-60, was purchased from Nikko Chemicals Co. (Tokyo, Japan). Tetragastrin was purchased from Mekto Co. (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study. All experiments were carried out under light-protected conditions to prevent the photodecomposition of nifedipine.

Correspondence: K. Uekama, Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan.

Preparation of tablets. A spray-drying method was employed to prepare the nifedipine powders, as reported previously (Wang et al 1993a, b). Formulations of plain (F_0) and double-layer tablets (F_1–F_7) with various composition ratios are listed in Table 1. Tablets of 4 mm (content of nifedipine, 5 mg) and 7 mm (content of nifedipine, 20 mg) diameters were prepared for in-vitro and in-vivo studies, respectively, by compressing sample powder under a pressure of 98 MPa for 1 min in a hydraulic press (Model R-303, Riken Seiki, Tokyo, Japan). In the case of double-layer tablets, the spray-dried product of nifedipine/HP-β-CyD (1:4 weight ratio corresponding to 1:1 molar ratio) containing 3% HCO-60 as the fast-release portion was lightly compressed in the die, and then that of nifedipine/HPCs (1:4 weight ratio) as the slow-release portion was directly added onto the tablet before preparation in the same manner as the plain tablets. The two portions were combined in a 1:3 weight ratio. For example, the F_3 formulation (nifedipine content 20 mg, see Table 1) consisted of 5 mg/20 mg of nifedipine/HP-β-CyD containing 3% HCO-60 and 15 mg/(40+20+0 mg) of nifedipine/(HPC-L+HPC-M+HPC-H).

In-vitro release studies. The release of nifedipine from the compressed tablets (4 mm diam., drug content of 5 mg) was measured, using an automatic dissolution testing apparatus described previously (Uekama et al 1990) at 37°C and stirring speed of 100 rev min^{-1}. The dissolution medium was continuously circulated through a glass filter (G3; porosity 20–30 μm) at a flow rate of 3 mL min^{-1}, using an HPLC pumping unit (Hitachi 655A-11, Tokyo). The amount of nifedipine dissolved in water was automatically measured by an HPLC UV-monitoring unit (Hitachi L-4000, Tokyo) at a wavelength of 254 nm, under which conditions the photodegradation of nifedipine was negligible. The amount of nifedipine in tablets, except for

Table 1. Formulations of plain (F_0) and double-layer tablets (F_1–F_7).

Formulation	Fast-release portion	Slow-release portion		
		HPC-L	HPC-M	HPC-H
F_0	4	0	0	0
F_1	1	3	0	0
F_2	1	2	1	0
F_3	1	1·5	1·5	0
F_4	1	1	2	0
F_5	1	0	3	0
F_6	1	0	1·5	1·5
F_7	1	0	0	3

The fast-release portion contained HP-β-CyD and 3% HCO-60. The slow-release portion contained HPCs of various viscosity grades.

Adalat L-20, was lower than its solubility ($3{\cdot}2 \times 10^{-5}$ M), thus maintaining a sink condition.

In-vivo absorption studies. The in-vivo absorption studies were carried out using gastric acidity-controlled beagle dogs, in order to minimize the individual difference in gastric acidity. Four healthy male beagle dogs, 9–11 kg, were fasted for about 24 h but allowed free access to water. Tetragastrin (4 μg kg^{-1}) was injected intramuscularly twice, 15 min before and 45 min after oral administration of the nifedipine tablet (7 mm diam., drug content of 20 mg) according to the protocol reported previously (Yamada et al 1989). The gastric pH of dogs was confirmed to be about 1·5 for at least 2 h with negligible pH deviation (Uekama et al 1993). Nifedipine tablets were administered orally along with water (50 mL) to gastric pH-controlled dogs. Plasma samples (1·0 mL) were collected at appropriate intervals and agitated with isopropanol (0·5 mL) containing an internal standard (nisoldipine). After addition of 0·1 M HCl (2·0 mL) and 1% sodium nitrite (0·3 mL), the sample was warmed at 45°C for 1 h. The oxidized nifedipine and nisoldipine were extracted with benzene (5·0 mL), 4·0 mL of which was evaporated under reduced pressure. The residue was dissolved in ethyl acetate (200 μL), and assayed for nifedipine by gas-chromatography (Kondo et al 1980; Uekama et al 1992) using a Shimadzu GC-7A gas chromatograph with a GC-7A ECD electron-capture detector (Kyoto, Japan), 3% OV-1 Chromosorb WAW DMCS (80–100 mesh, Gas-Chro Co., Tokyo, Japan), column temperature 230°C and injection temperature 260°C.

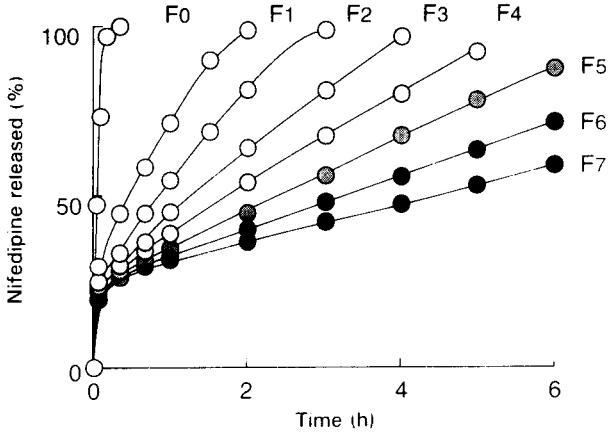

FIG. 1. Release profiles of nifedipine (equivalent to 5 mg nifedipine) from plain tablet (F_0) and seven double-layer tablets (F_1–F_7) in water at 37°C. Formulations of the tablets are shown in Table 1.

Results and discussion

In-vitro release behaviour. Fig. 1 shows the release profiles of nifedipine from the formulations F_0–F_7. The fixed ratio in double-layer tablets of 1:3 was chosen because the amount of the immediately released nifedipine from the fast-release portion was appropriate to attain an effective plasma level and the drug was slowly released from the other portion according to zero-order kinetics, as described previously (Wang et al 1993a). The release of nifedipine from the slow-release portion decelerated with increase in the amount of HPC of high viscosity grade. The in-vitro release of nifedipine from the double-layer tablets was little affected by rotation speed (50–150 rev min^{-1}) of paddle, compression pressure (49–147 MPa) in the tablet preparation or ageing (28 days at 60°C and 75% relative humidity).

In-vivo absorption behaviour. Table 2 summarizes the in-vivo

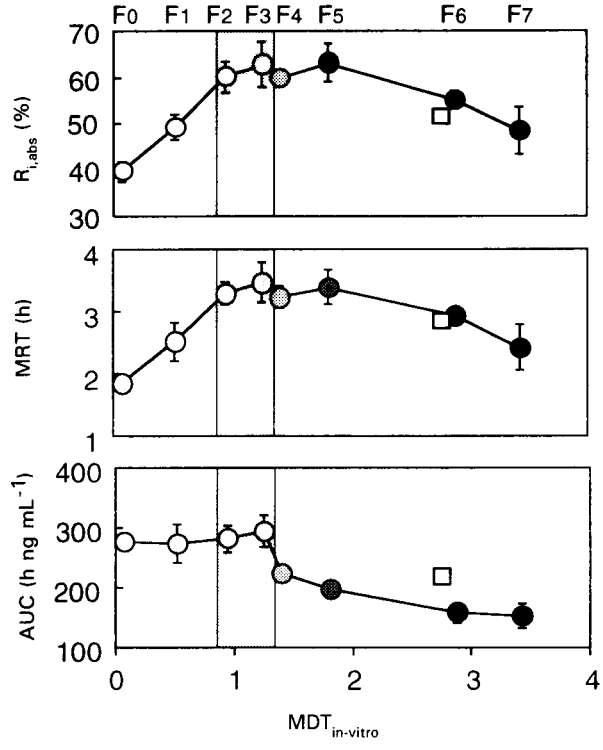

FIG. 2. Relationship between in-vivo absorption parameters and $MDT_{in\text{-}vitro}$ for various nifedipine preparations. □ Commercial slow-release preparation (Adalat L-20).

Table 2. In-vivo absorption and in-vitro release parameters[a] for nifedipine preparations (n = 4).

Tablet	In-vivo					In-vitro
	C_{max} (ng mL^{-1})[b]	t_{max} (h)[c]	AUC (h ng mL^{-1})	MRT (h)	$R_{i,abs}$ (%)	$MDT_{in\text{-}vitro}$ (h)
F_0	132·6 ± 21·17	0·625 ± 0·125	277·5 ± 14·5	1·849 ± 0·051	39·66 ± 2·06	0·080 ± 0·004
F_1	91·00 ± 9·44	0·625 ± 0·125	273·0 ± 32·6	2·519 ± 0·317	49·21 ± 2·96	0·519 ± 0·017
F_2	72·92 ± 4·87	1·500 ± 0·500	281·0 ± 21·0	3·295 ± 0·173	60·10 ± 3·24	0·943 ± 0·020
F_3	82·06 ± 6·18	1·625 ± 0·800	293·1 ± 26·2	3·474 ± 0·325	62·72 ± 4·88	1·252 ± 0·019
F_4	71·40 ± 5·32	1·125 ± 0·625	224·3 ± 6·4	3·238 ± 0·188	59·64 ± 1·88	1·398 ± 0·020
F_5	57·64 ± 14·02	0·750 ± 0·144	196·3 ± 14·0	3·388 ± 0·285	63·09 ± 4·08	1·822 ± 0·029
F_6	50·44 ± 8·50	1·500 ± 0·612	158·1 ± 16·0	2·933 ± 0·106	55·20 ± 2·01	2·885 ± 0·053
F_7	61·93 ± 13·53	0·875 ± 0·375	151·8 ± 20·7	2·423 ± 0·377	48·43 ± 4·94	3·427 ± 0·047
Intact nifedipine[d]	36·73 ± 7·86	0·625 ± 0·125	94·90 ± 6·8	2·933 ± 0·374	56·88 ± 6·54	3·101 ± 0·072
Adalat L-20	66·12 ± 12·13	2·250 ± 0·479	220·1 ± 29·7	2·874 ± 0·177	50·61 ± 1·66	2·787 ± 0·097[e]

[a] The parameters were calculated using data up to 12 h; [b] the maximum plasma level; [c] the time required to reach the maximum plasma drug level; [d] diluent: starch; [e] Adalat L-10 (10 mg of drug content) was used to maintain a sink condition in the in-vitro release study.

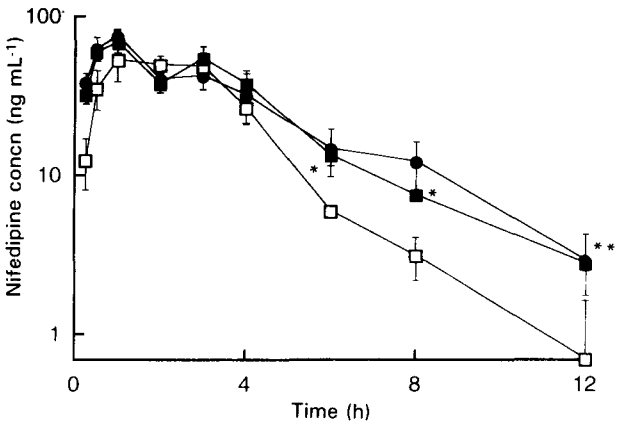

FIG. 3. Typical plasma level profiles of nifedipine after oral administration of nifedipine tablets (equivalent to 20 mg nifedipine dosed) in dogs. ■ Double-layer tablet F_2, ● double-layer tablet F_3, □ Adalat L-20. Each value represents the mean ± s.e. of four dogs. $*P<0.05$, $**P<0.01$ compared with Adalat L-20.

pharmacokinetic parameters and the in-vitro release parameters of nifedipine for all the tablets tested. From the inspection of the data in Table 2, the in-vitro release behaviour of nifedipine was clearly reflected in the plasma levels. For example, the F_0 tablet gave an initial rapid increase in plasma nifedipine levels, but the level decreased rapidly. In the case of double-layer tablets, the plasma level initially increased up to a constant value (about 60 ng mL^{-1}) due to the fast drug release, and decreased with the rates depending on the composition of the slow-release portion (HPC-L, M and H). However, the plasma profile of the tablets containing large amounts of the low viscosity HPC-L (e.g. F_1) was similar to that of the F_0 tablet. On the other hand, the tablets containing large amounts of the high viscosity HPC-H (e.g. F_6 and F_7) gave a fast decrease of plasma levels. This may be due to the incomplete release from the slow-release portion in the gastrointestinal tract, and subsequent considerable first-pass metabolism. Interestingly, the formulations F_2 and F_3 showed prolonged maintenance of nifedipine levels with an increase in area under the plasma concentration-time curve (AUC), which was 2·8 times greater than that of a physical mixture of intact nifedipine and starch (see Table 2).

For the estimation of the optimal formulation of nifedipine, correlations between the in-vitro release and in-vivo absorption parameters were examined. Fig. 2 shows the plots of the AUC, the mean residence time (MRT) in the systemic circulation and the absolute retarding parameter ($R_{i,abs}$) proposed by Nimmerfall & Rosenthaler (1986) vs the in-vitro mean dissolution time ($MDT_{in\text{-}vitro}$). The MRT and $MDT_{in\text{-}vitro}$ values were calculated by moment analysis (Yamaoka et al 1981), using the data of plasma nifedipine level-time profiles and the data of in-vitro release profiles, respectively. It is apparent that MRT and $R_{i,abs}$ values increased with increase of $MDT_{in\text{-}vitro}$, but decreased with further increases in $MDT_{in\text{-}vitro}$. The maximum MRT and $R_{i,abs}$ values were observed between 0·8–1·8 h $MDT_{in\text{-}vitro}$. On the other hand, the AUC value was almost the same up to 1·3 h $MDT_{in\text{-}vitro}$, but decreased with further increases in $MDT_{in\text{-}vitro}$. These results suggest that the tablet having the $MDT_{in\text{-}vitro}$ value of 0·8–1·3 h (shaded portion in Fig. 2) gives prolonged plasma nifedipine levels without decrease of AUC, which may be sufficient to overwhelm the subsequent first-pass metabolism. Therefore, of the double-layer tablets employed, the F_2 or F_3 formulations may be the most promising as retarding preparations of nifedipine. Fig. 3 shows typical plasma level-time curves of nifedipine after oral administration of the double-layer tablets (F_2 and F_3) and commercial slow-release tablet (Adalat L-20) in the gastric pH-controlled dogs. Indeed, the retarding effect of both double-layer tablets was almost comparable with that of Adalat L-20, where the F_3 formulation showed the most superior oral bioavailability among the preparations tested.

These limited data will provide a rational basis for design and evaluation of modified-release dosage forms of nifedipine, which may offer a more balanced bioavailability with prolonged therapeutic effects in oral preparations.

References

Foster, T. S., Hamann, S. R., Richards, V. R., Bryant, P. J., Graves, D. A., McAllister, R. G. (1983) Nifedipine kinetics and bioavailability after single intravenous and oral doses in normal subjects. J. Clin. Pharmacol. 23: 161–170

Kleinbloesem, C. H., Van Brummelen, P., Van de Linde, J. A., Voogd, P. J., Breimer, D. D. (1984) Nifedipine: kinetics and dynamics in healthy subjects. Clin. Pharmacol. Ther. 35: 742–749

Kleinbloesem, C. H., Van Brummelen, P., Danhof, M., Faber, H., Urquhart, J., Breimer, D. D. (1987) Rate of increase in the plasma concentration of nifedipine as a major determinant of its hemodynamic effects in humans. Clin. Pharmacol. Ther. 41: 396–401

Kondo, S., Kuchiki, A., Yamamoto, K., Akimoto, K., Takahashi, K., Awata, N., Sugimoto, I. (1980) Identification of nifedipine metabolites and their determination by gas chromatography. Chem. Pharm. Bull. 28: 1–7

Nimmerfall, F., Rosenthaler, J. (1986) Modified release of drug: a way to its quantification. Int. J. Pharm. 32: 1–6

Pabst, G., Lutz, D., Molz, K. H., Dahmen, W., Jaeger, H. (1986) Pharmacokinetics and bioavailability of three different galenic nifedipine preparations. Arzneim. Forsch. 36: 256–260

Sugimoto, I., Sasaki, K., Kuchiki, A., Ishihara, T., Nakagawa, H. (1982) Stability and bioavailability of nifedipine in fine granules. Chem. Pharm. Bull. 30: 4479–4488

Uekama, K., Matsubara, K., Abe, K., Horiuchi, Y., Hirayama, F., Suzuki, N. (1990) Design and in vitro evaluation of slow-release dosage form of piretanide: utility of β-cyclodextrin:cellulose derivative combination as a modified-release drug carrier. J. Pharm. Sci. 79: 244–248

Uekama, K., Ikegami, K., Wang, Z., Horiuchi, Y., Hirayama, F. (1992) Inhibitory effect of 2-hydroxypropyl-β-cyclodextrin on crystal-growth of nifedipine during storage: superior dissolution and oral bioavailability compared with polyvinylpyrrolidone K-30. J. Pharm. Pharmacol. 44: 73–78

Uekama, K., Horikawa, T., Horiuchi, Y., Hirayama, F. (1993) In vitro and in vivo evaluation of delayed-release behaviour of diltiazem from its *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin complex. J. Contr. Rel. 25: 99–106

Wang, Z., Horikawa, T., Hirayama, F., Uekama, K. (1993a) Design and in-vitro evaluation of a modified-release oral dosage form of nifedipine by hybridization of hydroxypropyl-β-cyclodextrin and hydroxypropylcellulose. J. Pharm. Pharmacol. 45: 942–946

Wang, Z., Hirayama, F., Ikegami, K., Uekama, K. (1993b) Release characteristics of nifedipine from 2-hydroxypropyl-β-cyclodextrin complex during storage and its modification by hybridizing polyvinylpyrrolidone K-30. Chem. Pharm. Bull. 41: 1822–1826

Yamada, I., Mizuta, H., Goda, T., Haga, K., Ogawa, K. (1989) Gastric pH profile and control in fasting beagle dogs. Chem. Pharm. Bull. 37: 2539–2541

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobiodyn. 4: 879–885