British Journal of Pharmacology (1997) 120, 399–404

The antihypertensive effect of orally administered nifedipine-loaded nanoparticles in spontaneously hypertensive rats

Young Il Kim, Laurence Fluckiger, Maurice Hoffman, *Isabelle Lartaud-Idjouadiene, *Jeffrey Atkinson & [1]Philippe Maincent

Laboratoire de Pharmacie Galénique et Biopharmacie and *Laboratoire de Pharmacologie Cardio-Vasculaire, Faculté de Pharmacie, Université Henri Poincaré Nancy I, rue Albert Lebrun, 54001 Nancy Cedex, France

1 The therapeutic use of nifedipine is limited by the rapidity of the onset of its action and its short biological half-life. In order to produce a form devoid of these disadvantages we made nanoparticles of nifedipine from three different polymers, poly-ε-caprolactone (PCL), polylactic and glycolic acid (1 : 1) copolymers (PLAGA), and Eudragit RL/RS (Eudragit). Nifedipine in polyethylene glycol 400 (PEG) solution was used as a control.

2 The average diameters of the nanoparticles ranged from 0.12 to 0.21 μm; the encapsulation ratio was 82% to 88%.

3 In spontaneously hypertensive rats (SHR), the initial rapid fall in systolic arterial blood pressure following oral administration of nifedipine in PEG solution (from 193 ± 3 to 102 ± 2 mmHg) was not seen following administration of the same dose in Eudragit nanoparticles (from 189 ± 2 to 156 ± 2 mmHg); with PCL and PLAGA nanoparticles the initial fall in blood pressure was significantly reduced (nadirs PCL 124 ± 2 and PLAGA 113 ± 2 mmHg). Ten hours following administration, blood pressure in rats administered the nifedipine/PEG preparation had returned to normal (183 ± 3 mmHg) whereas that of animals given nifedipine in nanoparticles (PCL 170 ± 3, PLAGA 168 ± 2, Eudragit 160 ± 3 mmHg) was still significantly reduced.

4 All of the nanoparticle dosage forms decreased C_{max} and increased T_{max} and the mean residence time (MRT) values. Relative bioavailability was significantly increased with Eudragit nanoparticles compared to the nifedipine/PEG solution.

5 There was an inverse linear correlation between the fall in blood pressure and plasma nifedipine concentration with all preparations.

6 The nanoparticle nifedipine preparations represent sustained release forms with increased bioavailability, a less pronounced initial antihypertensive effect and a long-lasting action.

Keywords: Nanoparticles; SHR; blood pressure; nifedipine

Introduction

With the development of the vasodilator, 1,4-dihydropyridine calcium entry blockers, the link between the efficacy and the side effects of antihypertensive drugs became clear. The acceptibility of treatment based on dihydropyridines depends on the relationship between their pharmacokinetic and pharmacodynamic properties. Side effects are associated with the rapidity of onset of action and the magnitude of the antihypertensive effect (Myers, 1994).

The dihydropyridine, nifedipine, is a poorly water-soluble drug with a low bioavailability and a short half-life of 2 h (Bittar, 1989). Thus nifedipine has to be administered 2 or 3 times per day. Absorption is rapid and this, coupled with the short elimination half-life, can result in significant fluctuations in plasma drug concentrations (Raemsch & Sommer, 1983). By controlling drug input with a modified release dosage form, it should be possible to maintain the plasma drug concentration at the desired level with minimal fluctuations. This should have the advantage of providing a prolonged therapeutic effect with a reduced incidence of side effects (Pabst *et al*., 1986).

In a previous study, we showed that poly(isobutylcyanoacrylate) nanocapsules of another dihydropyridine, darodipine, displayed a slower release rate than a darodipine-polyethylene glycol 400 (PEG) solution, both *in vitro* and *in vivo* (Hubert *et al*., 1991). In renovascular hypertensive rats, darodipine nanocapsules lowered blood pressure when given orally and intramuscularly, and the initial fall in blood pressure was less marked than that produced by the darodipine-PEG solution.

One potential drawback of the poly(isobutylcyanoacrylate) polymer stems from cellular toxicity *in vitro* coupled with the lack of information on possible toxicity following chronic administration in man (Grangier *et al*., 1991). At the present time there is no dosage form of this polymer available for man. We turned therefore to poly-ε-caprolactone (PCL) and copolymers of lactic and glycolic acids (PLAGA) nanoparticles which have a low toxicity (Ogawa, 1992). Following administration of PLAGA nanoparticles of the dihydropyridine, isradipine, blood pressure fell gradually and the antihypertensive effect in rats lasted for up to 10 h (Maincent *et al*., 1994).

The aim of this study was to extend this work and investigate changes in the pharmacokinetic and pharmacodynamic properties of nifedipine following encapsulation. Our aim was two fold. Firstly, we wished to produce a nanoparticle form of nifedipine which would not provoke an initial rapid and marked fall in blood pressure but a long-lasting lowering of blood pressure with a gradual onset. Secondly, we wished to study the relationship between the pharmacokinetic and pharmacodynamic properties of different nanoparticle forms. We used the biodegradable polymers we had previously used (PCL and PLAGA) and a third, non biodegradable polymer – Eudragit – widely used for the coating of tablets (Lehmann, 1989). Experiments were performed in spontaneously hypertensive rats and changes in blood pressure and the plasma

[1] Author for correspondence

concentration of nifedipine following a single oral administration were followed. Nifedipine dissolved in PEG was used as a 'rapid release' form for comparison.

Methods

Animals

Male, adult spontaneously hypertensive rats (SHR, Iffa-Credo, L'Arbesle, France) were allowed one week to acclimatize before all experiments. They were 11 to 13 weeks old and weighed 230 ± 20 g when experiments were performed.

Preparation of nanoparticles

PCL or PLAGA nanoparticles were prepared as described by Fessi *et al*. (1989). Briefly, polymer (0.125 g) and nifedipine (6 mg) were dissolved in acetone (20 ml). This organic phase was added to an aqueous solution of Pluronic F68 (0.25 g) as a stabilizer. Nanoparticles of Eudragit were prepared by dissolving nifedipine (0.03 g) and polymer (1.5 g of Eudragit RL and 1.5 g of Eudragit RS) in acetone then adding this solution to distilled water without stabilizer (Bodmeier *et al*., 1991). In all cases, nanoparticles form spontaneously following interfacial precipitation. Acetone was eliminated and the final volume of the suspension reduced to 10 ml (PCL and PLAGA nanoparticles) or to 50 ml (Eudragit nanoparticles) by evaporation under reduced pressure.

Nifedipine dissolved in polyethylene glycol 400 (0.6 mg ml^{-1}) was also prepared.

Physicochemical characterization of the dosage forms

Particle size and zeta potential Particle diameters of nanoparticles were determined by photon correlation spectroscopy and zeta potentials by laser doppler velocimetry (ZetaMaster, Malvern, U.K.). All preparations were diluted in a 10^{-3} M NaCl solution in order to maintain constant ionic strength.

Determination of the incorporation efficiency The non-entrapped drug was separated from the particles by gel filtration (Beck *et al*., 1990). A Sepharose CL4B gel (Sigma, St. Louis, Missouri, U.S.A.) in an Econo column (45 cm × i.d. 2.0 cm, Bio-Rad, California, U.S.A.) was used as the stationary phase. Distilled water was used as the mobile phase and the flow rate was adjusted to 1 ml min^{-1} with a Reglo 100 pump (Ismatec SA, Zürich, Switzerland). The concentration of drug loaded into nanoparticles was calculated by subtracting the drug concentration found in the eluant aqueous phase from the total drug concentration.

In vitro *dialysis*

A nanoparticle volume corresponding to 0.9 mg of nifedipine was placed in dialysis bags (i.d. 22 mm, mol.wt. cut off 50,000, Spectrum Medical Industries, Houston, Texas, U.S.A.) which were hermetically sealed and placed in 500 ml of phosphate buffer (pH 7.4) under sink conditions. The system was thermostated at 37°C and stirred at 200 r.p.m. One milliliter of the receptor medium (replaced by fresh buffer) was taken at predetermined time intervals and assayed for nifedipine concentration.

In vivo *studies*

Nifedipine was administered at a dose of 3 mg kg^{-1} in one of the 4 forms (PEG, PCL, PLAGA or Eudragit) described above. The dosing volume was 5 ml kg^{-1} and there were 8 animals per group. Water and non-loaded nanoparticles made out of the three polymers were administered as control dosage forms ($n \geqslant 6$ animals per group).

Measurement of systolic arterial blood pressure SHR were fasted for 15 h before and during the experiments; water was given *ad libitum*. Systolic arterial blood pressure was measured by tail cuff plethysmography (Chillon *et al*., 1992) 0, 0.25, 0.5, 1, 2, 4, 7 and 10 h following oral administration.

Blood sampling The common carotid artery of separate groups of SHR was cannulated under halothane anaesthesia using the technique previously described (Makki *et al*., 1994). The cannula was filled with a solution containing polyvinylpyrrolidone (0.5 mg ml^{-1}), heparine (200 i.u ml^{-1}), sodium chloride (90 mg ml^{-1}) and methylene blue. A minute quantity of methylene blue (< 1 mg l^{-1}) was added in order to give a blue colour to the solution used to fill the cannula. In this way any leak from the cannula could easily be detected. The cannula was sealed and rats were housed in individual cages. Experiments were performed 15 h later. Blood samples were withdrawn at 0, 0.25, 0.5, 1, 2, 4, 7, 10, 13 and 24 h after oral administration of each dosage form (PEG, PLC, PLAGA or Eudragit). Blood samples were centrifuged at 1500 *g* for 10 min at 4°C; plasma samples were analysed immediately by h.p.l.c.

Analytical methods The plasma concentration of nifedipine was determined by a modification of the Miyazaki *et al*. (1984) method. Plasma samples (100 μl), 4 ml of a dichloromethane: n-hexane mixture (3 : 7, v/v) and 1 ml of distilled water were mixed in a light-proof test tube. The mixture was shaken for 10 min with a rotary agitator and centrifuged for 5 min at 1500 *g*. Three millilitres of the supernatant (the organic layer) were transferred to a light-proof reaction vial (Pierce Reacti-Vial, Pierce, Rockford, IL, U.S.A.). The organic phase was evaporated under nitrogen in a dry block sample incubation system (Reacti-Therm III, Rockford, IL, U.S.A.) at 40°C for 15 min. The residue was dissolved in 200 μl of the mobile phase containing n-butyl p-aminobenzoate (butamben) as internal standard (500 μg ml^{-1}). One hundred microlitres of the solution were injected into the high performance liquid chromatography (h.p.l.c.) system. The chromatographic system was equipped with a pump (Spectra-physics, SP 8700, California, U.S.A.), an autosampler (Specta-physics, AS 1000), a u.v. detector (Spectra-physics, UV 100) and an integrator (Spectra-physics, SP 4270). A reverse plase column (Zorbax ODS, 4–6 μm, 25 cm × 4.6 mm i.d.; Dupont de Nemours, Wilmington, DE, U.S.A.) was used. The column was warmed to 55°C. The mobile phase consisted of 0.01 M disodium hydrogen phosphate buffer (pH 6.1)-methanol (50 : 50). The flow rate was 1.0 ml min^{-1} and the detection wavelength of nifedipine was 237 nm.

Statistical analysis

Results are given as means ± s.e.mean or ± s.e. Analysis of variance (ANOVA) and linear regression analysis (a = intercept, b = slope) were performed on the blood pressure and plasma nifedipine data. Means were compared with the Bonferroni test. Differences were considered significant at $P < 0.05$.

Drugs and chemicals

Poly-ε-caprolactone (PCL, mol.wt. 42,000), polylactic and glycolic acid copolymer (PLAGA, Medisorb, D/L, 50/50, mol.wt. 40,000) and Eudragit RL and RS (copolymers synthesized from acrylic and methacrylic acid esters) were supplied by Aldrich Chemie (Steinheim, Germany), Dupont de Nemours (Wilmington, DE, U.S.A.) and Röhm Pharma (Darmstadt, Germany), respectively. Polyvinylpyrrolidone, methylene blue and polyethylene glycol 400 were purchased from Merck AG, Darmstadt, Germany. Nifedipine and aminobutyl p-benzoate were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Pluronic F68 (copolymer of polypropylene oxide and polyethylene oxide), a gift from

BASF (Ludwigshafen, Germany) was used as a nonionic surface active agent. All other reagents and solvents were of analytical grade. All experiments were carried out under light-proof conditions to prevent the photodecomposition of nifedipine (Al-Turk *et al*., 1988).

Results

Physicochemical characteristics

The average diameters, zeta potentials and encapsulation percentages are listed in Table 1. The average diameters of the different preparations of nanoparticles ranged from 0.12 to 0.21 μm. Each dosage form showed a good encapsulation ratio with a maximum of 88% in the case of Eudragit.

In vitro *release of nifedipine*

The release of nifedipine from the PEG solution was rapid, and reached a plateau (about 95% release) after 5 h (Figure 1). With nanoparticles there was a characteristic biphasic release with fast release up to 7 h (maximum 45%, 42% and 35% for PCL, PLAGA and Eudragit nanoparticles, respectively) and slower release from 7 h onwards. The total drug liberation was 57%, 54% and 46% after 24 h for PCL, PLAGA and Eudragit nanoparticles, respectively.

Systolic arterial blood pressure

Administration of empty PLAGA and Eudragit nanoparticles (or distilled water) had no significant effect on systolic arterial blood pressure (Figure 2a). After oral administration of the nifedipine-PEG solution, systolic arterial blood pressure fell very rapidly from 193 ± 3 mmHg to 102 ± 2 mmHg at 0.25 h, then returned rapidly to a value not significantly different from the pre-injection value (183 ± 3 mmHg) at 10 h (Figure 2b). The initial fall in systolic arterial blood pressure following PCL (124 ± 2 mmHg at 0.25 h) and PLAGA nanoparticles (113 ± 2 mmHg at 0.25 h) was significantly less than that obtained with the nifedipine/PEG solution (Figure 2); the hypotensive effect was maintained up to 10 h (170 ± 3 mmHg, 168 ± 2 mmHg, for PCL and PLAGA respectively). After administration of Eudragit nanoparticles, systolic arterial blood pressure fell gradually and the maximal antihypertensive effect (156 ± 2 mmHg) was obtained at 2 h (Figure 2). No initial abrupt fall was observed ($t = 0.25$ h, 166 ± 3 mmHg, $t = 0.5$ h, 168 ± 2 mmHg). At 10 h the systolic arterial blood pressure in the Eudragit group (160 ± 3 mmHg) was no different from that at 2 h (170 ± 2 mmHg).

Plasma concentrations of nifedipine

The plasma concentration-time profiles after oral administration of each dosage form are presented in Figure 3. Calculated pharmacokinetic parameters for each preparation are shown in Table 2. Peak plasma concentrations (C_{max}) were significantly lower (857 ± 34 and 915 ± 111 ng ml^{-1}, following PCL and PLAGA, respectively) than that obtained with the PEG/nifedipine solution (1480 ± 385 ng ml^{-1}). With Eudragit nanoparticles there was a statistically significant difference in peak plasma concentration (C_{max}), 664 ± 69 ng ml^{-1}) and in relative bioavailability (156%) compared to the nifedipine/PEG solu-

Table 1 Mean diameters (nm), zeta potentials (mV) and incorporation ratio (%) of nifedipine-loaded nanoparticles

Nanoparticles	*Diameter* (nm)	*Zeta potentials* (mV)	*Nifedipine incorporation ratio* (%)
PCL	211 ± 5	-21 ± 1	82 ± 3
PLAGA	118 ± 7	-24 ± 2	82 ± 5
Eudragit	172 ± 5	$+29 \pm 1$	88 ± 4

Data shown are means $\pm$ s.e., $n = 3$.

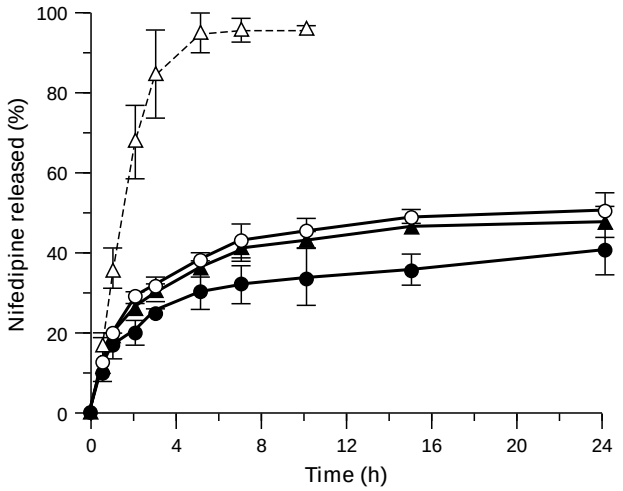

Figure 1 Cumulative percentage of nifedipine released from polyethylene glycol 400 solution and nanoparticles ($n = 3$). (△) PEG solution; (○) PCL nanoparticles; (▲) PLAGA nanoparticles and (●) Eudragit nanoparticles. Each point represents the mean and vertical lines show s.e.mean.

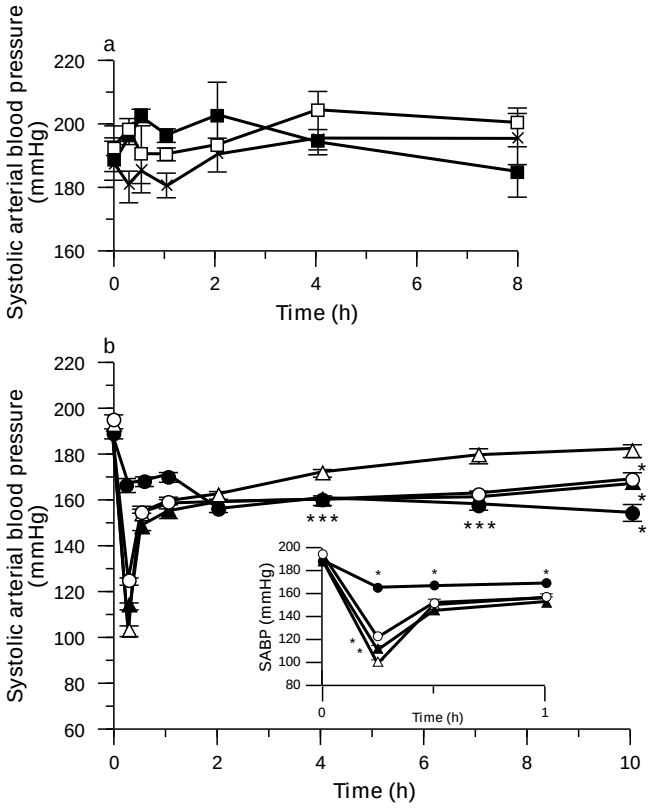

Figure 2 Systolic arterial blood pressure (SABP) in awake spontaneously hypertensive rats following oral administration of empty nanoparticles or distilled water (a) or of different dosage forms of nifedipine (3 mg kg^{-1}) (b). (a) (×) Water; (□) PLAGA empty nanoparticles; (■) Eudragit empty nanoparticles. (b) Nifedipine preparations: (△) PEG solution; (○) PCL nanoparticles; (▲) PLAGA nanoparticles and (●) Eudragit nanoparticles. *$P < 0.05$ versus PEG solution. Each point represents the mean ($n = 8$) and vertical lines show s.e.mean. Insert: data on changes in systolic arterial blood pressure during the first hour following administration of the nifedipine preparations.

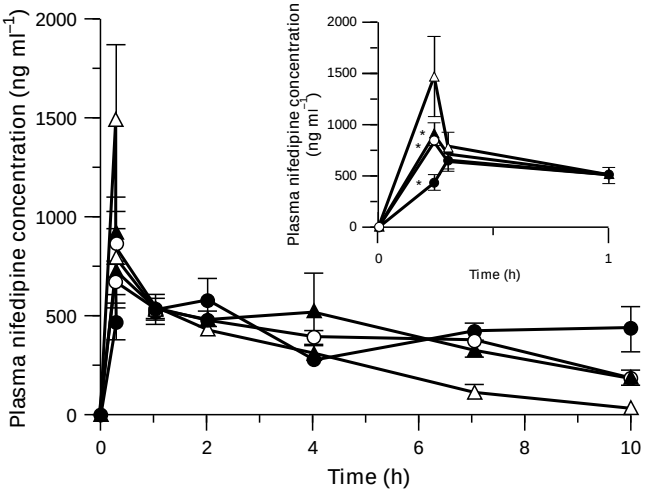

Figure 3 Plasma nifedipine concentration (ng ml^{-1}) in awake spontaneously hypertensive rats following oral administration of different dosage forms of nifedipine (3 mg kg^{-1}). (△) PEG solution; (○) PCL nanoparticles; (▲) PLAGA nanoparticles and (●) Eudragit nanoparticles. *$P<0.05$ versus PEG solution. Each point represents the mean ($n=3$) and vertical lines show s.e.mean. Insert: data on changes in plasma nifedipine concentrations during the first hour following administration of the different forms of nifedipine.

tion. The time to reach the plasma peak (1 h) was greater than with the nifedipine/PEG solution (0.25 h).

Correlation between plasma nifedipine concentration and systolic arterial blood pressure

The linear regression ANOVAs for systolic arterial blood pressure versus plasma nifedipine concentration were similar for all dosage forms (Figure 4 and Table 3).

Discussion

Although the dosage forms had similar physicochemical properties (mean diameter, incorporation ratio, Table 1), PCL and PLAGA nanoparticles had a negative zeta potential (around −23 mV) whereas Eudragit nanoparticles, due to the presence on the polymer backbone of quaternery ammonium groups, had a positive zeta potential (+29 mV). This difference could have an influence on the *in vivo* behaviour of nifedipine nanoparticles due to the overall negative charge of the mucous. *In vitro*, it is obvious that the three preparations of nanoparticles prolong the release of nifedipine when compared with nifedipine dissolved in PEG (Figure 1). The main problem with the dialysis bag technique is that it is not a rapid sink method (Washington, 1989; 1990). A more elegant method to study the release of drugs from colloidal carriers is the cen-

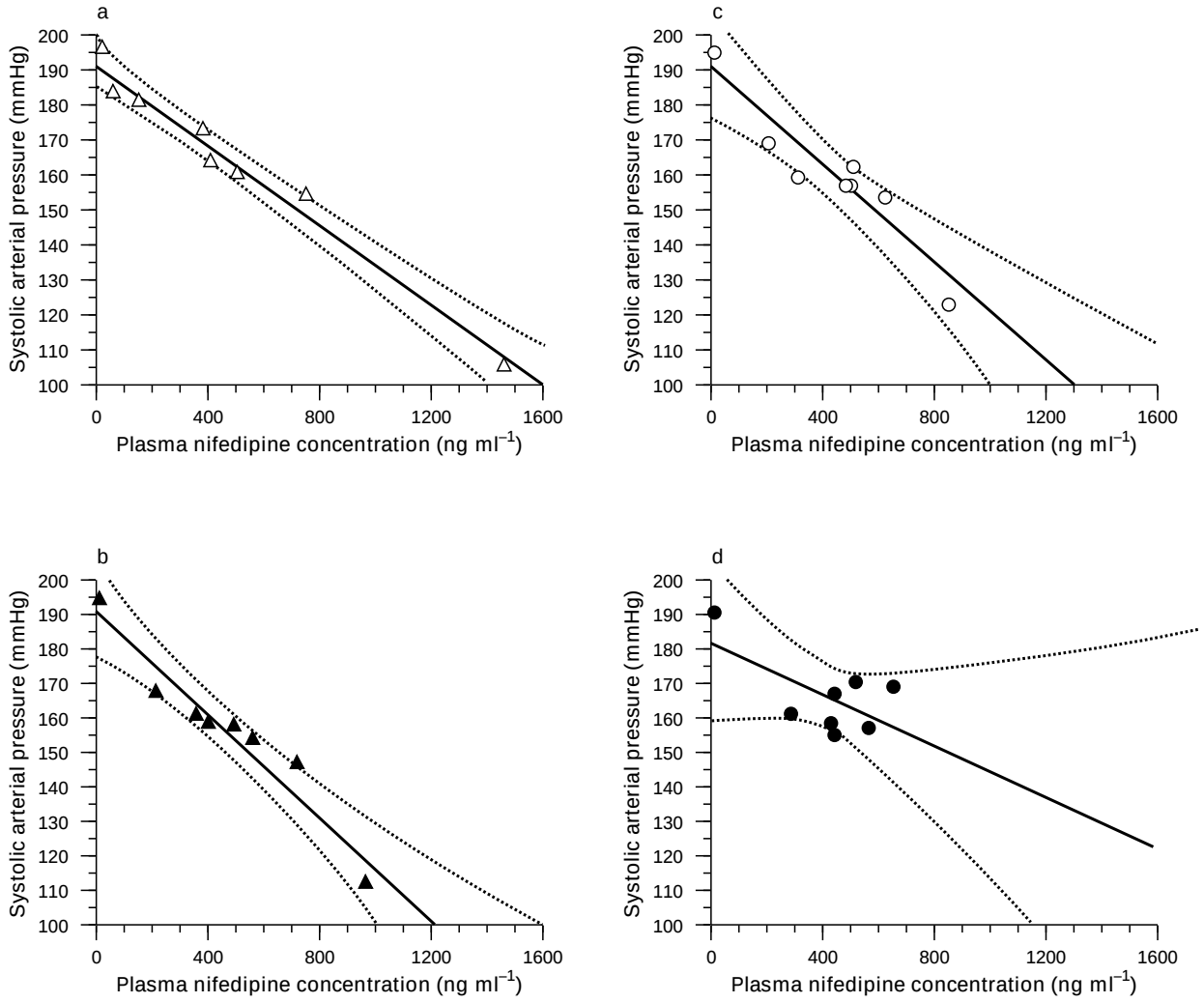

Figure 4 Correlation between nifedipine plasma concentration and systolic arterial blood pressure following oral administration of (a) PEG solution, (b) PLAGA nanoparticles, (c) PCL nanoparticles and (d) Eudragit nanoparticles.

Table 2 Pharmacokinetic parameters of the different dosage forms of nifedipine following oral administration (3 mg kg^{-1})

Parameters	*Nifedipine/PEG*	*PCL NP*	*PLAGA NP*	*Eudragit NP*
C_{max} (ng ml^{-1})	1480±385	857±34*	915±111*	664±69*
T_{max} (h)	0.25	0.25	0.25	1.0±0.5
AUC (ng h ml^{-1})	2835±192	3861±72	3638±786	4420±240*
MRT (h)	2.7±0.2	4.1±3*	3.9±0.3	4.8±0.1*
Relative Bioavailability (%)	100 (reference)	136	128	156*

Data shown are means±s.e.mean, $n=3$. C_{max} = maximal plasma concentration, T_{max} = time to reach peak concentration, AUC = area under concentration-time curve 0 to 10h, MRT = mean residence time. *$P<0.05$ versus nifedipine/PEG solution

Table 3 Linear regression ANOVA of systolic arterial blood pressure (dependent variable) versus plasma nifedipine concentration (independent variable)

	Nifedipine/PEG	*PCL NP*	*PLAGA NP*	*Eudragit NP*
Intercept (mmHg)	191±2	192±4	189±6	181±6
Slope (mmHg ng^{-1} ml^{-1})	−0.058±0.003	−0.07±0.001	−0.069±0.011	−0.034±0.014

trifugal-ultrafiltration technique. With such a technique nifedipine-PEG gave a much faster release time than with the dialysis method (10 min for release of 80% of nifedipine versus about 3 h for the same percentage release with dialysis). Furthermore besides adsorption of nifedipine onto filter membranes, nanoparticles suspensions clogged the filters, invalidating this technique in our case. Albeit, it should be borne in mind that the objective of our *in vitro* study was simply to show differences between a 'fast release' dosage form (PEG) and the nanoparticles formulations. Thus when the dialysis method was used, the time resolution for PEG was much longer than could be expected *in vivo*, yet the dialysis technique allows one to demonstrate clearly a major difference between fast and slow release dosage forms. The general pattern of the release *in vitro* was confirmed *in vivo* since the antihypertensive effect of the nifedipine/PEG solution was of short duration, lasting less than 7 h. Blood pressure initially fell to a value less than the lower limit of cerebral blood flow autoregulation (110 mmHg mean arterial blood pressure in awake, chronically instrumented SHRs of the same sex, age and origin, Bray *et al*., 1991) for approximately 10 min. Extrapolating these data to man, daily repetition of such periods of iatrogenic cerebral ischaemia over several years could have a very detrimental effect on brain functions (Sorkin *et al*., 1985). With the Eudragit preparation the fall in blood pressure was delayed and the nadir reached (at 2 h) −156±2 mmHg - was well above the value for the lower limit of cerebral blood flow autoregulation.

The troughpeak ratio, defined as the ratio between the antihypertensive effect at the end of the interval between doses (trough) and at the maximum effect (peak) (Zanchetti, 1994), was 7% for nifedipine/PEG solution and 30, 25, 88% for nanoparticles of PCL, PLAGA and Eudragit, respectively. The Food and Drug Administration has suggested that the troughpeak ratio should be at least 50–66% (Van Zwieten, 1994). Thus the Eudragit preparation easily meets this criterion.

The change in the pharmacodynamic effect of nifedipine when incorporated into Eudragit nanoparticles cannot be explained by an effect of the polymer on the plasma concentration-effect relationship of nifedipine or an effect of the Eudragit polymer *per se* on blood pressure. Indeed the administration of the empty dosage form did not significantly change blood pressure. Thus an explanation has to be sought for the effect of the polymer on nifedipine pharmacokinetics.

All of the polymer carrier forms decreased C_{max} and increased mean residence time (MTR). The Eudragit form increased the AUC. The decrease in C_{max} can be explained by the slow diffusion of the drug from the nanoparticles, as is generally observed with nanoparticles (Hubert *et al*., 1991). This may explain the decrease in the initial antihypertensive effect with the polymer forms, especially Eudragit which showed a plasma level between 2 and 3 times less than that of the PEG solution at peak (664±69 versus 1480±385 ng ml^{-1}). The AUC values (a measure of the relative bioavailability of Eudragit nanoparticles) were significantly higher than that for the nifedipine/PEG solution. The greater bioavailability observed with the Eudragit nanoparticle form can be explained on the basis of its physicochemical structure. The Eudragit polymer present a low level of quaternary ammonium groups in the backbone. Such groups would increase the frequency of electrostatic interaction with the gut mucus which is composed primarily of negatively charged mucopolysaccharides. This could increase the absorption of the drug and/or the carrier (Lehr *et al*., 1990).

Three possible uptake mechanisms have been suggested for nanoparticles: (1) uptake *via* a paracellular pathway (Volkheimer, 1977), (2) intracellular uptake and transport via the epithelial cells of the intestinal mucosa (Kreuter *et al*., 1989), and (3) lymphatic uptake via the M-cells and the Peyer's patches (Gilley *et al*., 1988). It is not possible to discriminate between the three mechanisms in our study. Albeit were nanoparticles to be taken up by the Peyer patches, nifedipine would diffuse slowly into the lymph. As lymph flow is much slower than blood flow (Tilney, 1971) this mechanism may explain the slow appearance in blood of nifedipine released from nanoparticles. In addition, lymphatic absorption of nifedipine would bypass the liver and so lower the initial metabolism of the drug. Such an absorption mechanism would explain the greater bioaviailability observed with nanoparticles especially Eudragit. Research is in progress to determine the influence of lymphatic absorption on the absorption of nifedipine in different dosage forms. Another possibility is related to the fact that it has also beeen shown that nanoparticles are able to coat the gastrointestinal tract (Grislain *et al*., 1983). Since free nifedipine is mostly absorbed from the jejunum (Raemsch & Sommer, 1983), coating the gut would increase the surface area of intestine in contact with the drug and so increase the drug gradient concentration towards the blood.

In summary, the results obtained *in vitro* and *in vivo* show that colloidal polymer dosage forms of nifedipine are more efficient sustained release forms than a PEG solution. These sustained-released formulations of nifedipine may reduce the initial hypotensive effect and the risk of periodic, iatrogenic cerebral ischaemia. This would also increase patient compliance as the frequency of administration could presumably be diminished.

The authors acknowledge a grant from the French Ministry of Education and Research (JE 250).

References

AL-TURK, W.A., MAJEED, I.A., MURRAY, W.J., NEWTON, D.W. & OTHMAN, S. (1988). Some factors affecting the photodecomposition of nifedipine. *Int. J. Pharm.*, **41,** 227–230.

BECK, P., SCHEREN, D. & KREUTER, J. (1990). Separation of drug-loaded nanoparticles from free drug by gel filtration. *J. Microencapsulation*, **7,** 491–496.

BITTAR, N. (1989). Usefulness of nifedipine for myocardial ischemia and the nifedipine gastrointestinal therapeutic system. *Am. J. Cardiol.*, **64,** 31F–34F.

BODMEIER, R., CHEN, H., TYLE, P. & JAROSZ, P. (1991). Spontaneous formation of drug-containing acrylic nanoparticles. *J. Microencapsulation*, **8,** 161–170.

BRAY, L., LARTAUD, I., MULLER, F., ATKINSON, J. & CAPDEVILLE, C. (1991). Effect of angiotensin I converting enzyme inhibitor perindopril on cerebral blood flow in awake hypertensive rats. *Am. J. Hypertens.*, **4,** 246S–252S.

CHILLON, J.M., CAPDEVILLE-ATKINSON, C., LARTAUD, I., GUILLOU, J., MERTES, P.M. & ATKINSON, J. (1992). Chronic antihypertensive treatment with captopril plus hydrochlorothiazide improves aortic distensibility in the spontaneously hypertensive rat. *Br. J. Pharmacol.*, **107,** 710–714.

FESSI, H., PUISIEUX, F., DEVISSAGUET, J.P., AMMOURY, N. & BENITA, S. (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.*, **55,** R1–R4.

GILLEY, R.M., ELDRIDGE, J.H., OPITZ, J.L., HANNA, L.K., STAAS, J.K. & TICE, T.R. (1988). Development of secretory and systemic immunity following oral administration of microencapsulated antigens. *Proc. Int. Sym. Control. Rel. Bioac. Mat.*, **15,** 123–124.

GRANGIER, J.L., PUIGRENIER, M., GAUTIER, J.C. & COUVREUR, P. (1991). Nanoparticles as carriers for growth hormone releasing factor. *J. Control. Rel.*, **15,** 3–13.

GRISLAIN, L., COUVREUR, P., LENAERTS, V., ROLAND, M., DEPREZ-DECAMPENEERE, D. & SPEISER, P. (1983). Pharmacokinetics and distribution of a biodegradable drug carrier. *Int. J. Pharm.*, **15,** 333–345.

HUBERT, B., ATKINSON, J., GUERRET, M., HOFFMAN, M., DEVISSAGUET, J.P. & MAINCENT, P. (1991). The preparation and acute antihypertensive effects of a nanocapsular form of darodipine, a dihydropyridine calcium entry blocker. *Pharm. Res.*, **8,** 734–738.

KREUTER, J., MULLER, U. & MUNZ, K. (1989). Quantitative and microautoradiographic study on mouse intestinal distribution of polyalkylcyanoacrylate nanoparticles. *Int. J. Pharm.*, **55,** 39–45.

LEHR, C.M., BOUWSTRA, J.A., TUKKER, J.J. & JUNGINGER, H.E. (1990). Intestinal transit of bioadhesive microspheres in an *in situ* loop in the rats - a comparative study with copolymers and blends based on poly(acrylic acid). *J. Control. Rel.*, **13,** 51–62.

LEHMANN, K.O.R. (1989). Chemistry and application properties of polymethacrylate coating systems. In *Aqueous Polymeric Coatings for Pharmaceutical Dosage Forms*. ed. McGinity, J.W. pp. 153–245. New York: Marcel Dekker.

MAINCENT, P., FLUCKIGER, L., LEROUEIL, M., HOFFMAN, M. & ATKINSON, J. (1994). Interest of colloidal carriers as vectors of antihypertensive dihydropyridine calcium-entry blockers to decrease blood pressure in hypertensive rats. *Proc. Int. Sym. Control. Rel. Bioact. Mat.*, **21,** 33–34.

MAKKI, T., TALOM, R.T., NIEDERHOFFER, N., AMIN, F., TANKOSIC, P., MERTES, P.M. & ATKINSON, J. (1994). Increased arterial distensibility induced by the angiotensin-converting enzyme inhibitor, lisinopril, in normotensive rats. *Br. J. Pharmacol.*, **111,** 555–560.

MIYAZAKI, K., KOHRI, N. & ARITA, T. (1984). High-performance liquid chromatographic determination of nifedipine in plasma. *J. Chromatogr.*, **310,** 219–222.

MYERS, M.G. (1994). Dihydropyridine calcium antagonists and the trough: peak ratio: focus on adverse effects. *J. Hypertension*, **12** (suppl 8), S73–S77.

OGAWA, Y. (1992). Monthly microcapsule-depot form of LHRH agonist, leuprorelin acetate (Enantone® Depot): Formulation and pharmacokinetics in animals. *Eur. J. Hosp. Pharm.*, **2,** 120–127.

PABST, G., LUTZ, D., MOLTZ, K.H., DAHMEN, W. & JAEGER, H. (1986). Pharmakinetics and bioavailability of three different galenic nifedipine preparations. *Arzneim-Forsch/Drug Res.*, **36,** 256–260.

RAEMSCH, K.D. & SOMMER, J. (1983). Pharmacokinetics and metabolism of nifedipine. *Hypertension*, **5,** 1118–1124.

SORKIN, E.M., CLISSOLD, S.P. & BROGDEN, R.N. (1985). Nifedipine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in ischaemic heart disease, hypertension and related cardiovascular disorders. *Drugs*, **30,** 182–274.

TILNEY, N.L. (1971). Patterns of lymphatic drainage in the adult laboratory rat. *J. Anat.*, **109,** 369–383.

VAN ZWIETEN, P.A. (1994). Trough: peak ratio: measurement limitation and relevance to treatment of hypertension. Closing remarks. *J. Hypertension*, **12** (suppl 8), S117–S118.

VOLKHEIMER, G. (1977). Persorption of particles: physiology and pharmacology. *Adv. Pharmacol. Chemother.*, **14,** 163–187.

WASHINGTON, C. (1989). Evaluation of the non-sink dialysis method for the measurement of drug release from colloids: effect of drug partition. *Int. J. Pharm.*, **56,** 71–74.

WASHINGTON, C. (1990). Drug release from monodisperse systems. A critical review. *Int. J. Pharm.*, **58,** 1–12.

ZANCHETTI, A. (1994). Trough: peak ratio of blood pressure response to dihydropyridine calcium antagnoists. *J. Hypertension*, **12** (suppl 8), S97–S106.

(Received August 9, 1996
Revised October 14, 1996
Accepted October 17, 1996)