The Preparation and Acute Antihypertensive Effects of a Nanocapsular Form of Darodipine, a Dihydropyridine Calcium Entry Blocker[1]

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We have addressed two problems associated with the use of dihydropyridine calcium entry blockers in antihypertensive therapy, namely, potent vasodilation and short half-lives, by incorporating the representative blocker, darodipine, into a nanocapsular vehicle. In awake, renovascular hypertensive rats, darodipine nanocapsules lowered blood pressure when given orally or intramuscularly, and the initial fall in blood pressure was less marked than that observed with the same dose of darodipine dissolved in polyethylene glycol 400 (PEG). Intramuscular administration of the nanocapsular form of darodipine had an antihypertensive effect which lasted for at least 24 hr.

KEY WORDS: hypertension; rat; dihydropyridine; nanocapsules.

INTRODUCTION

Established hypertension in elderly patients is characterized by (i) blunted counterregulation of the cardiovascular system and (ii) elevated peripheral vascular resistance, partly as a result of calcium influx. Therefore, the use of vasodilating calcium entry blockers has been proposed (2). There is, however, little evidence that age is an independent variable in the hypotensive effect of calcium antagonists (3). Further, potent vasodilators such as dihydropyridine calcium entry blockers, especially in the early phases of treatment, may cause a rapid and pronounced fall in blood pressure to levels at which iatrogenic cerebral ischemia could occur (4). In elderly hypertensive patients the lower limit of cerebral blood flow autoregulation is shifted to a higher pressure level such that resting cerebral blood flow is nearer the threshold for cerebral ischemia (5). The incorporation of a dihydropyridine calcium entry blocker into a colloidal carrier such as a polyisobutylcyanoacrylate nanocapsule, should yield a slow-release preparation producing a more gradual decrease in blood pressure. Nanocapsules are spheric vesicles consisting of an oil droplet surrounded by a polymeric wall; their average size is between 0.2 and 1 μm. Polyisobutylcyanoacrylate is a rapidly biodegradable polymer (6). Insulin nanocapsules have a hypoglycaemic effect in diabetic rats which lasts for more than 2 weeks after a single oral administration (7). Thus nanoencapsulation can dramatically change the *in vivo* behavior of drugs.

Dihydropyridines are generally eliminated rapidly from the circulation (8). This property also causes problems with patient compliance because of frequent dosing schedules (9). In order to investigate changes in the pharmacokinetic and pharmacodynamic properties of dihydropyridines following their nanoencapsulation, we studied the antihypertensive properties of a polyisobutylcyanoacrylate nanocapsular form of darodipine in the renovascular hypertensive rat. As lipophilic substances are better candidates for nanoencapsulation, we used darodipine, an extremely lipophilic dihydropyridine.

MATERIAL AND METHODS

Materials

Polyvinylpyrrolidone, methylene blue, and polyethylene glycol 400 (PEG) were purchased from Merck AG, Darmstadt, West Germany. Darodipine (PY 108-068) was a gift of Sandoz AG, Basel, Switzerland. The monomer isobutylcyanoacrylate was purchased from Sigma, St Louis, Missouri. Miglyol 812 was a gift of Dynamit Nobel, Paris, France.

Animals

Male, outbred Wistar rats of 130 to 160 g body weight were purchased from Iffa-Credo, L'Arbresle, France. They were given food and water ad libitum and allowed 1 week to recover before undergoing the renal artery clip operation. There were eight rats per cage. Body weight was measured every day. Certain rats were housed individually for a few days at a time in order to measure their food (g/kg/day) and water (ml/kg/day) intakes.

Measurement of the Concentration of Darodipine

Following alkalinization of the various plasma samples (100 μl) with 0.5 ml 1 *N* NaOH, darodipine was extracted by shaking with 5 ml toluene followed by centrifugation (2500*g*, 10 min, 4°C). The toluene was evaporated under nitrogen at 45°C for 20 min. The residue was redissolved in toluene (30 μl), then 2 μl was directly injected into a gas chromatographic system (Hewlett Packard 5890, Ni 63 electron capture detector, capillary column OV 1701 Chrompack). The limit of detection was 2 ng/100 μl and the standard curve was linear up to 100 ng/100 μl.

[1] Part of this work was presented at the London meeting of the British Pharmacological Society in December 1988 (1).

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Preparation of the Nanocapsular Form of Darodipine

Darodipine (300 mg) was dissolved in 3 ml chloroform. Miglyol 812 (6 ml), a mixture of saturated fatty acid triglycerides of C8 to C18 chain length, was added to the darodipine–chloroform solution. The chloroform was evaporated, and 4 ml of the darodipine plus Miglyol 812 solution was added to 50.5 ml of a solution of isobutylcyanoacrylate (0.5 ml) in absolute ethanol (50 ml). This solution was then emulsified in an aqueous solution of the nonionic surfactant polyoxyethylene-oxypropylene (Pluronic F 68; 0.5 g/100 ml distilled water). The isobutylcyanoacrylate monomer polymerized instantaneously (interfacial polymerization) in the presence of water at neutral pH (10). The ethanol was evaporated under vacuum, producing a colloidal suspension of nanocapsules of darodipine (2 mg/ml).

Blank nanocapsules were similarly prepared without darodipine. The average size of empty nanocapsules (Coulter Counter Model N4, Coultronics, France) was 139 nm (n = 3, SE = 46), and that of darodipine nanocapsules 144 nm (n = 5, SE = 59).

Two procedures were used to determine the degree of incorporation of darodipine into the nanocapsules. In the first, 5 ml of the nanocapsular suspension of darodipine was centrifuged at 40,000g for 4 hr at 4°C. The density of Miglyol 812 is less than 1, thus nanocapsules are found floating on the top of the preparation. The concentration of darodipine in the aqueous phase was 19 ± 3 μg/ml (n = 12), thus 99% of the darodipine in the colloidal suspension was in a nanocapsular form. In the second procedure, dimethylformamide (4.5 ml) was added to the nanocapsular suspension (0.5 ml). Dimethylformamide destroys the polymer shell of the nanocapsules. Following centrifugation the concentration of darodipine in the supernatant was 199 ± 10 μg/ml (n = 12), thus confirming that 99% of the darodipine in the colloidal suspension was in a nanocapsular form. Nanocapsules of darodipine were stable for up to 6 months. As the solubility of darodipine in an aqueous solution is less than 0.001% (at all pH values tested), a darodipine solution in polyethylene glycol 400 (2 mg/ml) (11) was used as a control.

In Vitro Dialysis of Darodipine from Nanocapsular and Polyethylene Glycol Suspensions

To fulfill the theoretical requirements of *in vitro* dialysis studies, i.e., a dialysis volume ratio of 1:10, and a maximum theoretical concentration in the dialysate equivalent to 20% of the aqueous solubility of the substance dialysed (darodipine) (12), a preparation containing less drug (0.02 mg/ml) than those used for the *in vivo* studies was analyzed because of darodipine's low solubility (<0.001%) in water. Each preparation (10 ml) was placed in a cellulose dialysis bag (molecular weight cutoff, 6000). The dialysis bag was then placed in 100 ml of one of three solutions: (i) bovine serum albumin (15 mg/ml), NaCl (9 mg/ml), amoxicillin (40 mg/ml); (ii) human plasma plus amoxicillin (40 mg/ml); or (iii) human gastric juice. The system was maintained at 37°C, with constant stirring (100 rpm). Samples of the dialysate solution (1 ml) were taken at 0.5, 1, 2, 4, 6, 8, 12, and 24 hr, and the concentration of darodipine was determined.

Induction of Renovascular Hypertension

A solid-silver clip (0.2 mm gap) was placed on the left renal artery via a dorsoventral incision in the left flank, 0.5 cm in a caudal direction from the last rib of the thorax (13). Sham-operated animals underwent a similar operation except that the clip was momentarily placed on the left renal artery and then removed. All operations were performed under ether anesthesia. The abdominal wall was closed using absorbable sutures and the skin incision was closed with wound clips. Rats were allowed to recover for 2 weeks. Renovascular hypertensive rats were selected on the basis of two criteria: (i) a systolic arterial pressure greater than 150 mm Hg (14) and (ii) a marked increase in water consumption (renovascular hypertensive rats, 219 ± 9 ml/kg per day, n = 10; sham-clipped normotensive rats, 83 ± 1 ml/kg per day, n = 10; $P < 0.05$). Using these criteria, 10% of the renal artery clipped rats were eliminated from the study. Autopsy showed that either the clip was no longer on the renal artery or the reduction in renal perfusion pressure was such that unilateral nephrectomy of the clipped kidney had been produced.

Cannulation of the Dorsal Aorta

The abdominal aorta of 36 renovascular hypertensive rats was cannulated under ether anesthesia (14). The cannula was filled with a solution containing polyvinylpyrrolidone (0.5 mg/ml), heparin (200 IU/ml), sodium chloride (90 mg/ml), and methylene blue to produce a light blue color. The cannula was heat-sealed. Rats were housed in individual cages for a 1-week recovery period during which body weight and food and water intake were measured.

Effect of a Suspension of Darodipine on the Mean Arterial Blood Pressure of Awake, Renovascular Hypertensive Rats

During the first 2 or 3 days following cannulation of the abdominal aorta, body weight and food and water intake decreased and then returned to normal 3 to 4 days later. Following recovery, the aortic cannula was connected to a blood pressure recording system consisting of a low-volume strain gauge transducer (Statham, Porto Rico) connected to a polygraph record (Beckman, Palo Alto, California). Blood pressure was continuously recorded until a stable value was obtained (30 to 60 min). Mean arterial pressure was calculated as follows: mean arterial pressure (mm Hg) = diastolic arterial pressure + (0.33 × pulse pressure). Rats (n = 6 per group) received an oral or intramuscular injection (10 ml/kg) of (i) empty nanocapsules, (ii) the nanocapsular suspension of darodipine (20 mg/kg), or (iii) the polyethylene glycol 400 suspension of darodipine (20 mg/kg). Mean arterial pressure was recorded 0.5, 1, 2, 4, 6, 8, and 24 hr later.

Plasma Darodipine Concentrations Following Oral or Intramuscular Administration of Suspensions of Darodipine in Awake, Renovascular Hypertensive Rats

Following each recording of blood pressure the aortic cannula was disconnected and blood was allowed to flow freely into a collecting tube containing heparin. The first 150 μl was discarded. Each blood sample (200 μl) was centrifuged at 2000g for 5 min. Plasma samples were stored at −20°C before measurement of darodipine concentration.

The plasma samples of the rats injected with empty nanocapsules served as the analytical blank control.

The relative bioavailability (15) of darodipine was calculated as the ratio of the areas under the curve of plasma darodipine concentrations, from 0 to 24 hr, following administration of the various darodipine dosage forms.

Statistical Analysis

Results are given as means ± standard error of the mean (SEM) or ± standard error (SE). Means were compared with paired or unpaired *t* test. Analysis of variance (ANOVA) and linear regression analysis were performed on the blood pressure and plasma darodipine data.

RESULTS

In Vitro Dialysis of Darodipine from the Nanocapsular Dosage Form and the Polyethylene Glycol Solution

After 24 hr, 93% of the darodipine in the polyethylene glycol 400 solution had diffused into the albumin–NaCl solution or the human plasma (Fig. 1). Somewhat less (70%) had diffused into the gastric juice dialysate. In contrast, the darodipine diffusion from the nanocapsular suspension was below 10% (Fig. 2). Diffusion of darodipine from the polyethylene glycol suspension reached a plateau after 6 to 12 hr, whereas diffusion from the nanocapsular suspension did not reach equilibrium within 24 hr (Fig. 2). These results demonstrate slow release of darodipine from the nanocapsular suspension.

Change in the Mean Arterial Pressure of Awake Renovascular Hypertensive Rats Following Administration of Darodipine

After oral or intramuscular administration of a suspension of empty nanocapsules, there was a slight but not statistically significant fall in mean arterial pressure at +30 min (Figs. 3 and 4). However, oral administration of a polyethylene glycol 400 solution of darodipine (20 mg/kg) (Fig. 3) produced an abrupt fall in mean arterial pressure to 96 ± 4 mm Hg at 30 min (starting value, 150 ± 5 mm Hg). The corresponding value for the same oral dose of darodipine in a nanocapsular form was 120 ± 5 mm Hg (starting value, 156 ± 5 mm Hg; $P < 0.05$ with darodipine-PEG). Two hours following oral administration, values for the two forms were similar (nanocapsules, 123 ± 10 mm Hg; polyethylene glycol 400, 119 ± 8 mm Hg) and remained similar to the end of the observation period (+24 hr). At this time there was no significant difference between the mean arterial pressure of rats which had received darodipine and the mean of rats which had received empty nanocapsules.

Intramuscular administration of a polyethylene glycol 400 solution of darodipine (20 mg/kg) also produced an abrupt fall in mean arterial pressure to a value of 95 ± 3 mm Hg at +30 min (starting value, 158 ± 4 mm Hg) (Fig. 4). A similar drop was observed following intramuscular administration of the same dose of darodipine in a nanocapsular form (from 166 ± 9 to 103 ± 2 mm Hg), however, recovery of mean arterial pressure was more rapid in nanocapsule-treated rats. At +2 hr their mean arterial pressure was 107 ± 4 mm Hg, whereas rats which had received an intramuscular injection of darodipine in polyethylene glycol 400 had a mean arterial pressure of 93 ± 4 mm Hg ($P < 0.05$). Results at +24 hr were not significantly different (polyethylene glycol 400, 118 ± 5 mm Hg; nanocapsules, 129 ± 12 mm Hg). These values were 40 mm Hg less than the value for rats which had received empty nanocapsules ($P < 0.05$).

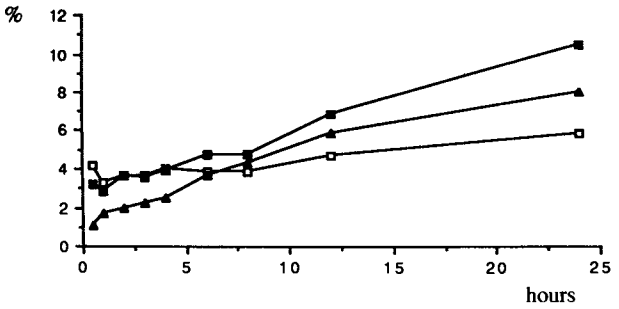

Fig. 2. *In vitro* dialysis of darodipine nanocapsules. Concentrations in the various dialysates are expressed as percentages of the concentration (20 μg/ml) of darodipine in the dialysis solution. Dialysates are represented as follows: gastric juice, open squares; plasma, filled squares; and albumin plus NaCl solution, filled triangles.

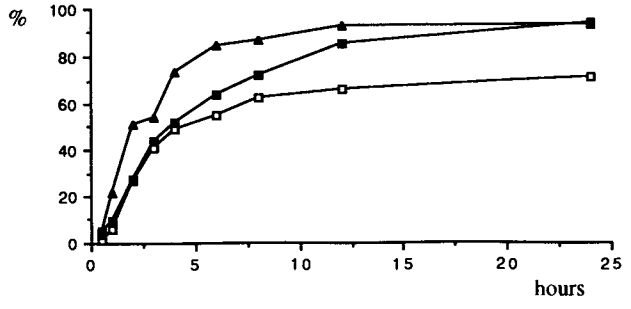

Fig. 1. *In vitro* dialysis of darodipine-PEG solution. Concentrations in the various dialysates are expressed as percentages of the concentration (20 μg/ml) of darodipine in the dialysis solution. Dialysates are represented as follows: gastric juice, open squares; plasma, filled squares; and albumin plus NaCl solution, filled triangles.

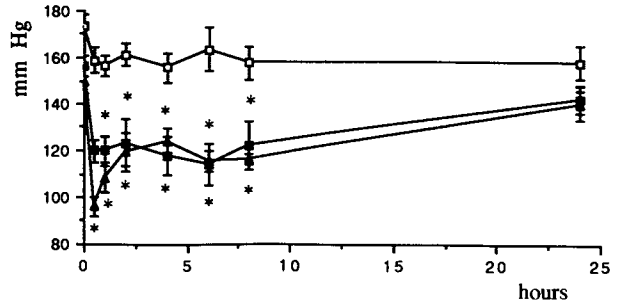

Fig. 3. Fall in mean arterial pressure in awake renovascular hypertensive rats following oral administration of darodipine in PEG (filled triangles) or nanocapsules (filled squares); controls received empty nanocapsules (open squares). The dose of darodipine was 20 mg/kg. (*) $P < 0.05$ versus empty nanocapsules ($n = 6$ per group).

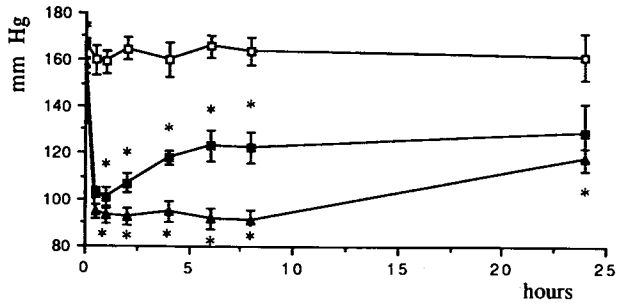

Fig. 4. Fall in mean arterial pressure in awake renovascular hypertensive rats following intramuscular administration of darodipine in PEG (filled triangles) or nanocapsules (filled squares); controls received empty nanocapsules (open squares). The dose of darodipine was 20 mg/kg. (*) $P < 0.05$ versus empty nanocapsules ($n = 6$ per group).

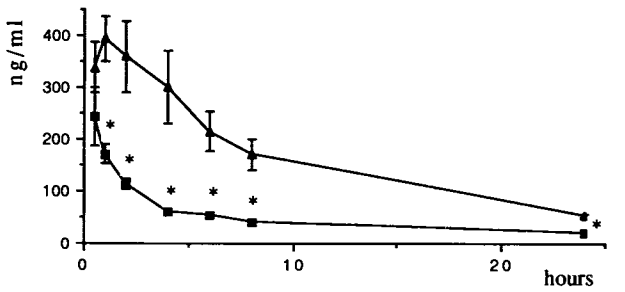

Fig. 6. Plasma darodipine concentrations (ng/ml) in awake renovascular hypertensive rats following intramuscular administration of darodipine in PEG (filled triangles) or nanocapsules (filled squares). The dose of darodipine was 20 mg/kg. (*) $P < 0.05$ versus empty nanocapsules ($n = 6$ per group).

Plasma Concentrations After Administration of the Two Darodipine Pharmaceutical Forms

The plasma concentration–time profiles after oral and intramuscular administration of the two darodipine forms are presented in Figs. 5 and 6. For each route of administration the concentrations were higher for the PEG solution. The peak concentrations corresponded, in each case, with the maximum hypotensive effect. Taking the area under the plasma concentration–time curve for the PEG solution as equal to 1, the relative bioavailability of darodipine in a nanocapsular form is 1.05 (±0.11) and 0.27 (±0.01) for the oral and intramuscular route, respectively.

Correlation Between Plasma Darodipine Concentrations and Mean Arterial Pressure

Linear regression analysis of all results for both preparations of darodipine and both administration routes gave a significant value for the regression of maximal fall in mean arterial pressure versus plasma darodipine concentration determined simultaneously (Fig. 7). The regression was maximal fall in mean arterial pressure = [(32.5 × log plasma darodipine concentration) − 23.5], $r = 0.70$, df = 22, $P < 0.05$.

DISCUSSION

This report presents data showing that a nanocapsular form of the antihypertensive dihydropyridine, darodipine, lowers the blood pressure of awake renovascular hypertensive rats when administered by either the oral or the intramuscular route. The blood pressure lowering effect of the nanocapsular form lasts at least as long as that of the preparation of darodipine dissolved in PEG. With both forms blood pressure is significantly lowered for 8 hr following oral administration and 24 hr following intramuscular administration. The nanocapsular form of darodipine has the advantage of a less pronounced, initial hypotensive effect compared to the darodipine-PEG form.

The relative bioavailability of the nanocapsular form of darodipine after oral administration was 1.05, suggesting that incorporation of darodipine into a colloidal carrier did not diminish its intestinal absorption. However, the form of the curve of the plasma concentration of darodipine following oral administration of nanocapsules was different from that of the darodipine-PEG preparation: the peak was lower and later in onset. A similar pattern of change of blood pressure following oral administration was observed. The initial hypotensive peak obtained with the nanocapsular form of darodipine was less marked, but from 2 hr onward the drop in blood pressure was similar with the two preparations. The attenuation of the initial hypotensive effect may diminish the risk of the hypotension-induced cerebral ischemia following a rapid fall in blood pressure to a level below that of the lower limit of cerebral blood flow autoregulation. The results obtained *in vitro* and *in vivo* show that nanocapsules of darodipine are a slower release form than darodipine-PEG so-

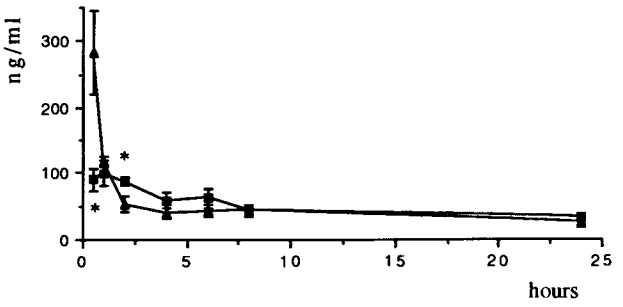

Fig. 5. Plasma darodipine concentrations (ng/ml) in awake renovascular hypertensive rats following oral administration of darodipine in PEG (filled triangles) or nanocapsules (filled squares). The dose of darodipine was 20 mg/kg. (*) $P < 0.05$ versus empty nanocapsules ($n = 6$ per group).

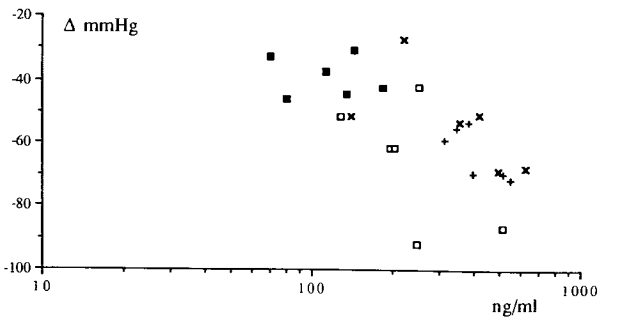

Fig. 7. Maximal fall in mean arterial pressure (mm Hg) as a function of plasma darodipine concentration (ng/ml). Administration form and route for darodipine are PEG/oral (+), PEG/intramuscular (×), nanocapsules/oral (■), and nanocapsules/intramuscular (□).

lutions. However, while *in vitro* dialysis was still increasing after 24 hr, *in vivo* plasma concentrations reached a peak 1 hr after oral administration. This disparity may be explained by gastrointestinal absorption of intact nanocapsules as has previously been demonstrated by Aprahamian *et al.* (16). Darodipine then diffuses from the nanocapsules across their polymer wall. As nanocapsules are concentrated mainly in the Kuppfer cells of the liver (12), it is probable that the latter organ is the site of liberation of darodipine from the nanocapsules following rapid enzymatic hydrolysis of the polymer of the nanocapsule wall.

The darodipine concentrations measured in the plasma samples are probably those of free darodipine, as there was a relatively good correlation between the increase in plasma darodipine concentrations measured in plasma and the decrease in blood pressure (see Fig. 7). The points outside the 95% fiducial limits of this regression did not represent the members of any specific group.

Following intramuscular administration, the relative bioavailability of darodipine administered in nanocapsular form was far less (0.27) than that of the darodipine-PEG. Peak plasma concentration was approximately half that following administration of darodipine-PEG, and plasma levels fell more rapidly. The timing of the relative changes in plasma darodipine concentration parallelled those in blood pressure, which began to rise following darodipine-nanocapsules after 1 hr (whereas for darodipine-PEG such a rise in blood pressure was not observed until 8 hr after administration). The fall in blood pressure was, however, still observable 24 hr after intramuscular administration of the nanocapsular form of darodipine, at a time when the effects of darodipine-PEG started to wane. This result suggests that the liberation of darodipine from the muscle mass was limited by slow dialysis across the polymer wall, a phenomenon similar to that observed in our *in vitro* studies. Under these conditions it is possible that the intramuscular administration of a nanocapsular form of darodipine could produce satisfactory control of blood pressure for periods much longer than 24 hr. A note of caution should be added, however, regarding intramuscular injection of the two dosage forms, as injection of darodipine-PEG produced edema, myositis, and tissue necrosis at the injection site (unpublished results). These effects were far less pronounced following intramuscular injection of the nanocapsular form darodipine, but although minor, they were still present. As the toxicity of PEG 400 is reported to be low (17), we cannot ascribe these complications to PEG 400 alone but must consider darodipine toxicity as well.

Our results obtained in young, renovascular hypertensive rats cannot presently be extrapolated to the use of dihydropyridine vasodilators in the elderly, but they suggest that darodipine nanocapsules may serve as a slow-release formulation with reduced side effects.

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