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Relationship between Plasma Concentration and Antihypertensive Effect of the Dihydropyridine Calcium Antagonist, Benidipine, in Rats

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Abstract

Although benidipine hydrochloride, a hydrophobic and weakly cationic dihydropyridine calcium antagonist, has slow onset but long-lasting anti-hypertensive effect in experimental animals and man, after administration it rapidly disappears from the plasma. Correlation of the plasma concentration with reduction in blood pressure has been investigated in spontaneously hypertensive rats, and concentrations in the mesenteric artery were compared with those in plasma after oral and intravenous administration of benidipine to rats.

After oral administration of benidipine to spontaneously hypertensive rats, the fall in blood pressure showed an anti-clock hysteresis phenomenon. Using concomitant analysis, plasma concentrations and anti-hypertensive activity could both be closely fitted to a one-compartment open model and the effect compartment for the pharmacological activity of benidipine could be described by a Hill equation. Concentrations in the mesenteric artery increased rapidly and then declined more slowly than in plasma. The mean residence time of benidipine in the mesenteric artery corresponded closely to the reciprocal of the rate constant for elimination of benidipine from the effect compartment.

From these results it seems that benidipine is retained longer in the plasma membrane, an effective site, because of its physicochemical properties, and thus shows a more sustained anti-hypertensive effect than would be predicted from its disposition in plasma.

Benidipine hydrochloride (Fig. 1) is a dihydropyridine calcium antagonist, synthesized in the Pharmaceutical Research Laboratories of Kyowa Hakko Kogyo. After oral and intravenous administration to rats and dogs its onset of action is slow but its anti-hypertensive effect is long-lasting compared with other dihydropyridines such as nifedipine, nicardipine and nitrendipine (Karasawa et al 1988a, b, c). Because of its long-lasting effect, this drug can be used clinically once daily for hypertension or twice daily for angina pectoris. However, the plasma concentration of this compound increases rapidly and falls quickly after oral administration to experimental animals and to man (Kobayashi et al 1990; Noda et al 1990). Recently, it was reported that the relationship between the plasma concentration and anti-hypertensive effect of dihydropyridine calcium antagonists such as benidipine in Japanese patients with essential hypertension could be explained by the effect-compartment model (Shimada et al 1996). In this study, we measured the plasma concentration and blood pressure after oral administration of benidipine to spontaneously hypertensive rats (SHR) to clarify the correlation between plasma concentration and its anti-hypertensive effect. In addition, the concentration–time profile of benidipine in the mesenteric artery was investigated and compared with that in plasma to study the relationship between the concentration of benidipine at the target site and in the plasma concentration.

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Materials and Methods

Chemicals

Benidipine hydrochloride ((±)-(*R**)-3-[(*R**)-1-benzyl-3-piperidyl] methyl 1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridine dicarboxylate hydrochloride) and (±)-3-(1-phenylethyl-4-piperidinyl)methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride, the internal standard used for quantitation by gas chromatography (GC), were synthesized in Sakai Laboratories or in the Pharmaceutical Research Laboratories of Kyowa Hakko Kogyo. Other reagents were analytical grade and purchased from Kanto Chemicals (Tokyo, Japan).

Experimental animals and sample collection

Investigation of the relationship between anti-hypertensive effect and plasma concentration was conducted on 22-week-old male spontaneously hypertensive rats (SHR, 290–355 g; Hoshino Experimental Animals, Saitama, Japan). After training by measurement of blood pressure at least once, benidipine suspended in 0·3% aqueous sodium carmellose was administered to rats, after an overnight fast, by gastric intubation at a dose of 1 mg kg^{-1}; this dose previously produced anti-hypertensive effects in SHR (Karasawa et al 1988a). Water was freely available to the animals, and they were fed 8 h after drug administration. At a predetermined time after administration the systolic blood pressure was measured by the plethysmographic tail method (VSM-105-R, Ueda Seisakusho, Tokyo, Japan). The animals were then killed under mild anaesthesia with diethyl ether and blood was collected in heparinized test-tubes. Plasma was separated and stored at −20°C until analysis. Each group consisted of four rats.

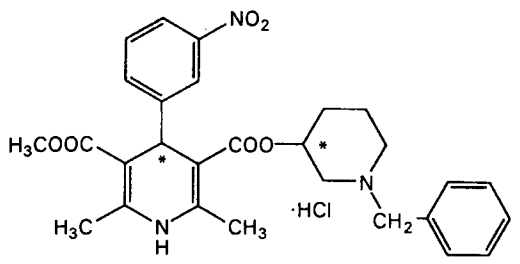

FIG. 1. The chemical structure of benidipine hydrochloride.

Male Wistar rats, 192–260 g (Japan SLC, Shizuoka, Japan) were used for measurement of plasma and mesenteric artery concentrations. After oral administration of benidipine suspended in 0·3% aqueous sodium carmellose by gastric intubation (after overnight fast), or after intravenous administration of benidipine dissolved in 0·5% Tween 80-saline to non-fasting rats, the animals were killed under mild anaesthesia with diethyl ether and blood was collected in heparinized test-tubes. Immediately, the mesenteric artery and vein with the mesenteric membrane were excized. The artery was separated from fatty tissue, membrane and vein, and rinsed with saline. Total wet weight was measured. Plasma and artery were stored at −20°C until analysis. Each group given the drug orally or by intravenous administration consisted of 8 and 4–5 rats, respectively.

Measurement of plasma and arterial concentrations of benidipine

Plasma and arterial concentrations of the unchanged drug were measured by a specific GC-ECD method. After addition of internal standard (10 ng) and saturated aqueous $NaHCO_3$ solution (0·25 mL) to plasma (0·25 mL), benidipine was extracted with diethyl ether (3 × 2 mL). After back-extraction into H_2SO_4 (0·33 M; 1·5 mL) the organic layer was removed and NaOH was added to make the aqueous phase alkaline. Benidipine was re-extracted with diethyl ether (5 mL) and the organic layer was evaporated to dryness under nitrogen and redissolved in 20 μL ethyl acetate for GC injection. For measurement of benidipine in the mesenteric artery the artery was cut into small pieces with scissors. After addition of internal standard, benidipine was extracted three times with ethanol by use of a glass homogenizer. After separation of arterial tissue, ethanol was evaporated to dryness under nitrogen and the residue was re-dissolved by the addition of saturated $NaHCO_3$ solution. Benidipine was extracted twice with diethyl ether and back-extracted in the same way as for the plasma assay.

Chromatography was performed with an HP5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a ^{63}Ni electron-capture detector, a split-splitless injector and a 5 m × 0·25 mm i.d. × 0·25 μM film-thickness DB5 fused-silica capillary column (J&W, Folsom, CA). Helium was used as carrier gas at a flow rate of 2 mL min^{-1}. Nitrogen was used as a make-up gas at a flow rate of 60 mL min^{-1}. Injection port and detector temperatures were maintained at 250°C and 320°C, respectively. The initial oven temperature was maintained at 220°C for 1 min then increased to 300°C at 10°C min^{-1}. Split injection was performed at a split ratio of 1 : 10. Peak heights were measured by means of a Shimadzu Model C-R4A Chromatopac (Kyoto, Japan). The lower limits of quantitation were 0·2 ng mL^{-1} and 1 ng g^{-1} for plasma and artery, respectively.

Pharmacokinetic analysis

Plasma and mesenteric arterial concentrations of benidipine were logarithmically transformed, and plotted against time. The maximum concentration (C_{max}) and the time (T_{max}) to reach C_{max} in plasma and mesenteric artery were determined by visual inspection. The slope of the linear part of the elimination phase (elimination rate constant k) was calculated by the linear least-squares method. The elimination half-life ($t_{1/2}$) was calculated from 0·693/k, and the area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity using k. Mean residence time (MRT) was calculated from the area under the first-moment curve (AUMC) and AUC. The plasma concentration–time profile after intravenous administration was analysed by the non-linear least-squares program MULTI (Yamaoka et al 1981) using a two-compartment open model (weight = 1/C).

Pharmacokinetic-pharmacodynamic model analysis

Plasma concentrations and the reduction in blood pressure after oral administration of benidipine at a dose of 1 mg kg^{-1} to SHR were concomitantly fitted by 'MULTI' using the model mentioned below to analyse the relationship between the pharmacokinetics and pharmacodynamics of benidipine. In this analysis, the mean plasma concentration of benidipine (C_p) was supposed to be described by a one-compartment open model with first-order absorption which is attached to the effect compartment (Sheiner et al 1979). The mean reduction in systolic blood pressure (E) was supposed to be described using the Hill equation as a function of concentration in the effect compartment (C_e). The absorption rate constant (k_a), the elimination rate constant (k_e), the elimination rate constant from the effect compartment (k_2), the maximum effect of the reduction in blood pressure (E_{max}), a constant that relates the changes in response to the change in concentration (s) and a constant related to the affinity of benidipine for its binding site (1/Q) were obtained from this analysis.

Results

Relationships between plasma concentrations and the reduction in blood pressure after oral administration of benidipine to SHR

The plasma concentrations and the reduction in blood pressure after oral administration of benidipine at a dose of 1 mg kg^{-1} to fasted SHR are shown in Fig. 2. The plasma concentrations increased rapidly, reached a maximum of 1·17 ± 1·09 ng mL^{-1} (mean ± s.d., n = 4) at 0·5 h and then decreased mono-exponentially. The reduction in blood pressure, which was measured immediately before blood sampling, was more gradual compared with the increase in the plasma concentration of benidipine and the peak hypotensive effect was observed 4 h after administration. Supposing that a 10% reduction in blood pressure is effective, then its hypotensive effect lasted for 8 h and the blood pressure returned to baseline within 24 h of administration. Because anti-clockwise hysteresis was observed in the plot of mean plasma concentrations against the absolute values of the reduction in blood pressure,

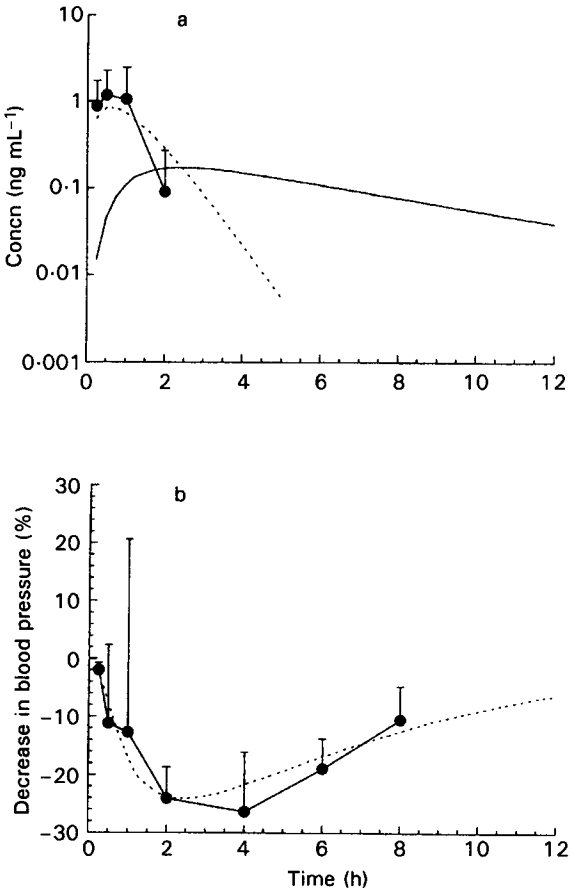

FIG. 2. a. Observed (●) and calculated (dotted line) benidipine concentrations in plasma and calculated concentrations in the effect compartment (solid line). b. Observed (●) and calculated (dotted line) percentage reduction in blood pressure after oral administration of benidipine hydrochloride (1 mg kg^{-1}) to SHR. Mean ± s.d. (n = 8).

as shown in Fig. 3, plasma concentrations and the reduction in blood pressure were analysed concomitantly using the effect compartment. The calculated values of k_e, k_a, k_2, E_{max}, s and 1/Q were 1·51 h^{-1}, 1·80 h^{-1}, 0·17 h^{-1}, 54·2% and 1·22 and 0·21 ng mL^{-1}, respectively. Simulations of plasma concentrations, concentrations in the effect compartment and the reduction in blood concentrations are shown in Fig. 2, together with the observed values. Concomitant analysis of plasma concentrations and the reduction in blood pressure using the effect compartment described closely the relationship between the plasma concentrations and the pharmacological effect of benidipine in SHR.

Concentration–time profiles in mesenteric artery after intravenous and oral administration of benidipine to rats

Plasma and mesenteric arterial concentration–time profiles after oral administration of benidipine at a dose of 1 mg kg^{-1} to fasted male Wistar rats are shown in Fig. 4; the pharmacokinetic parameters are listed in Table 1. Benidipine appeared in plasma rapidly after administration, as in SHR, reached a maximum concentration and disappeared quickly. Mesenteric arterial concentrations are higher than those in plasma at all sampling times and reached a maximum 0·75 h later than T_{max} for plasma concentrations. The MRTs of benidipine in plasma and mesenteric artery were calculated as 1·70 and 9·73 h,

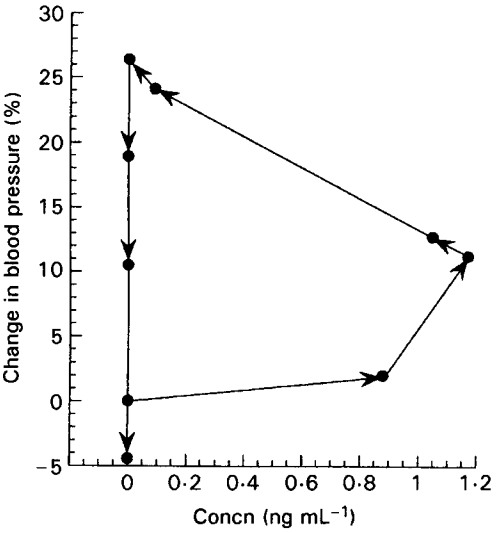

FIG. 3. Correlation of mean plasma concentrations and mean percentage reduction in blood pressure in SHR after oral administration of benidipine hydrochloride (1 mg kg^{-1}). Arrows indicate time-course.

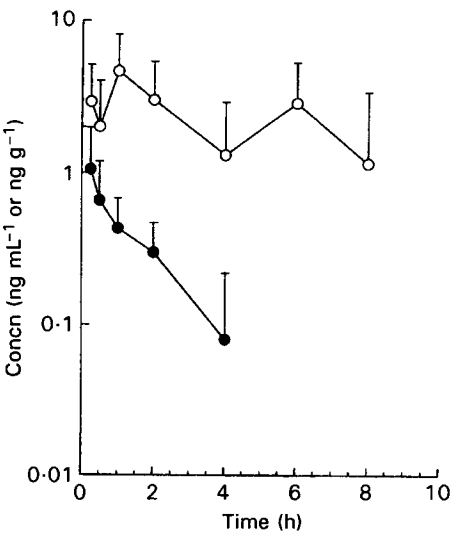

FIG. 4. Plasma (●) and mesenteric arterial (○) concentrations after oral administration of benidipine hydrochloride (1 mg kg^{-1}) to Wistar rats. Mean ± s.d. (n = 8).

respectively, the difference between these values being 8·03 h. The AUC for the mesenteric artery was almost 20 times larger than that for plasma.

Plasma and mesenteric arterial concentration-time profiles after intravenous administration of benidipine at a dose of 0·1 mg kg^{-1} to fasted male Wistar rats are shown in Fig. 5; the pharmacokinetic parameters are listed in Table 2. Benidipine was eliminated from plasma bi-exponentially and its MRT in plasma was 1·27 h. The MRT of benidipine in mesenteric artery was 5·60 h, 4·33 h longer than in plasma.

Discussion

The relationship between the anti-hypertensive effect and plasma concentration of benidipine has been investigated in patients with essential hypertension after oral administration of

Table 1. Pharmacokinetic parameters of benidipine after oral administration of 1 mg kg^{-1} to male Wistar rats.

Parameter	Plasma	Mesenteric artery
Time to maximum concentration (h)	0·25	1
Maximum concentration (ng mL^{-1} or ng g^{-1})	1·06 ± 0·92	4·65 ± 3·49
Half-life (h)	1·21	6·94
Area under the plasma concentration–time curve (ng h mL^{-1} or ng h g^{-1})	1·51	33·13
Mean residence time (h)	1·70	9·73
Mean residence time in the artery – mean residence time in the plasma (h)	–	8·03

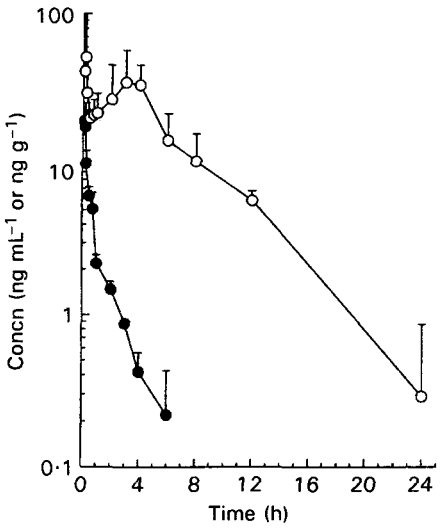

FIG. 5. Plasma (●) and mesenteric arterial (○) concentrations after intravenous administration of benidipine hydrochloride (1 mg kg^{-1}) to Wistar rats. Mean ± s.d. (n = 4–5).

benidipine at a dose of 8 mg (Noda et al 1990). The plasma concentrations of benidipine reached a maximum 2·5 h after administration and then decreased relatively rapidly with a biological half-life of approximately 2 h; however, the anti-hypertensive effect reached a maximum 4 to 7 h after administration and was even present 24 h after administration. The authors reported that there was no direct relationship between plasma concentrations and hypotensive activity of benidipine in man. In addition, Karasawa et al reported that the fall in blood pressure after oral or intravenous administration to rats and dogs appeared gradually, behaviour similar to that in man (Karasawa et al 1988a, b, c). However, benidipine appeared rapidly in plasma and then fell comparatively quickly after oral administration to rats and dogs (Kobayashi et al 1990). Recently, Shimada et al (1996) reported that the long-lasting anti-hypertensive effects of eight calcium antagonists (nicardipine, nifedipine, nilvadipine, benidipine, manidipine, barnidipine, nitrendipine and efonidine) relative to their short plasma elimination half-lives in Japanese patients with essential hypertension could be explained by an effect-compartment model or an ion-channel binding model based on the slow rates of association-dissociation of the drug at the calcium channel.

To investigate the relationship between plasma concentration and anti-hypertensive activity more precisely, we measured the plasma concentrations and blood pressure concomitantly after oral administration of benidipine at a dose of 1 mg kg^{-1} to SHR. The hypotensive effect and plasma concentration time-profiles agreed well with results already reported (Karasawa et al 1988a, b; Kobayashi et al 1990). The plasma concentrations and anti-hypertensive effect showed an anti-clock hysteresis. Although the formation of active metabolite(s) is one possible explanation of the anti-clock hysteresis, no active metabolite has been found in plasma after oral administration of benidipine to rats (Ishii et al 1988a; Kobayashi et al 1988). The long-lasting anti-hypertensive effect in SHR compared with its elimination half-life in plasma could be closely fitted by the effect-compartment model as in the analysis of data from man (Shimada et al 1996). In man, the elimination rate constant from the effect compartment (k_2) was reported as 0·12 h^{-1}, very consistent with that of rats (0·17 h^{-1}). From this we concluded that the rat would be a suitable model animal for study of the relationships between the pharmacodynamics and pharmacokinetics of benidipine and, perhaps, other dihydropyridines.

The target site of benidipine is the calcium channels (Ishii et al 1988b). As for its anti-hypertensive activity, it was expected that the extent of binding of benidipine to calcium channels in arterial smooth muscles is an index of its pharmacological activity .

Concentrations of benidipine in mesenteric artery were higher than those in plasma after oral and intravenous administration to rats. The MRT of benidipine in the mesenteric artery was 4·33–8·03 h longer than that in plasma. This showed that benidipine was retained in the artery compared with plasma and the slow elimination from the resistant vessels

Table 2. Pharmacokinetic parameters of benidipine after intravenous administration of 0·1 mg kg^{-1} to male Wistar rats.

Parameter	Plasma	Mesenteric artery
Time to maximum concentration (h)	0·083	0·167
Maximum concentration (ng mL^{-1} or ng g^{-1})	19·44 ± 3·17	51·15 ± 53·42
Half-life (h)	1·46	3·07
V_1 (L kg^{-1})	3·83	
Area under the plasma concentration–time curve (ngh h mL^{-1} or ng h g^{-1})	13·37	261·16
Mean residence time (h)	1·27	5·60
Mean residence time in the artery – mean residence time in the plasma (h)	–	4·33

would contribute to the duration of the anti-hypertensive effect of this compound. In addition, the elimination rate constant from the effect compartment (k_2) was 0·17 h^{-1} from analysis using the Hill equation. Its reciprocal ($1/k_2$) was calculated as 5·88 h, in good agreement with the in-vivo MRT of benidipine in the mesenteric artery. This could be interpreted by postulating that the elimination of benidipine from the effect compartment was rate-limiting for the disposition of this compound in the artery.

After oral and intravenous administration the C_{max} of benidipine in the mesenteric artery was observed at 1 h. Thus, the translocation of benidipine to target organ or tissue is rapid and inconsistent with the time to maximum effect in SHR. To explain this discrepancy, the following hypothesis is proposed. For binding of hydrophobic dihydropyridine derivatives to the calcium channels, the dissolution of these compounds in the biological membrane is the first step; this is followed by side-diffusion and attachment to the binding site (Affolter & Coronado 1986; Rhodes et al 1985; Chester et al 1987; Herbette et al 1989, 1991; Young et al 1992). Gotoh et al (1989) have also reported that the vasodilating activity of 3 nM benidipine on the spontaneous contracting activity of rat excized portal vein and K^+-induced contraction of rat aorta was diminished by removal of benidipine and addition of the calcium channel agonist BayK8644; however, removal of BayK8644 restored the vasodilating activity of this compound. This showed that non-specifically bound benidipine, which was dissolved in the biological membrane and could not be displaced by BayK8644, began binding to the calcium channels again after removal of BayK8644. Because benidipine is a hydrophobic compound ($\log D_{7.4} = 3·81$) and weakly cationic with a pK_a of 7·34 under physiological conditions, it has a high partition coefficient in the lipid bilayer (Nosaka & Ishii 1991), behaviour similar to that of other hydrophobic dihydropyridines (Mason et al 1989; Micheli et al 1991) and its binding to calcium channels would occur after dissolution in the biological membrane. In this study the concentrations of benidipine in mesenteric artery involved specific binding to the target site and non-specific binding to the lipid bilayer. Thus, rapid elevation of the concentration of benidipine in the mesenteric artery could be interpreted mainly as being derived from non-specific binding before specific binding. That the concentration observed in the mesenteric artery was over 10 times higher than the estimated concentration in the effective compartment supports this hypothesis. Benidipine bound non-specifically to membrane would act as a reservoir surrounding the calcium channels and contribute partially to the duration of its pharmacological activity, as is observed for other lipophilic drugs such as amlodipine and lacidipine (van Zwieten & Pfaffendorf 1993). Our results indicate that the effective compartment of benidipine, assumed from the slow onset and long-lasting effect of this compound, actually does exist.

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