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Possibility of Partial Absorption of Nicardipine by Routes Other Than the Hepato-portal System After Oral Administration in Rats

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Abstract

The systemic availability of nicardipine after different routes of administration has been examined in rats, with particular attention to differentiating oral absorption from intestinal and hepatic metabolism. The quantities of nicardipine and its metabolite were determined by capillary column gas chromatography.

A linear relationship was shown between the hepatic first-pass effect and dose after hepato-portal administration of nicardipine; the hepatic first-pass effect was calculated to be approximately 80%. However, the availability after oral and rectal administration was found to be more than twice that observed after hepato-portal administration. Partial avoidance of the hepatic first-pass effect after oral and rectal administration are estimated to be 37·3% and 35·2%, respectively, assuming that all absorbed molecules pass through the liver.

These findings suggest that the absorption of nicardipine after oral administration also occurs by routes other than the hepato-portal system.

Nicardipine hydrochloride has been used for the treatment of angina, hypertension and cerebrovascular disease (Sorkin & Clissold 1987). The systemic availability of nicardipine after oral administration has been shown to be dose-dependent in rats (Higuchi & Shiobara 1980a) and in man (Graham et al 1985; Wagner et al 1987). These results suggest saturation of the pre-systemic elimination process, because intestinal absorption of nicardipine after oral administration has been reported to be complete both in rats (Higuchi et al 1977) and man (Delchier et al 1988). The assay method used was not specific to nicardipine, and the combined concentrations of nicardipine and its pyridine metabolite were measured in the rat study. Pharmacokinetic analysis of the data derived from this method could be misleading because of the low vasodilative activity of the pyridine metabolite, which was only 1/300 that of nicardipine (Wu et al 1987). To differentiate between the contributions of intestinal and hepatic absorption to overall nicardipine bioavailability, further animal studies were performed using a newly devised, specific and rapid simultaneous microdetermination of nicardipine and its pyridine metabolite by capillary column gas chromatography (Watari et al 1990).

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Materials and Methods

Animal studies and drug administration

Male Wistar rats, 240–289 g, were fasted overnight before the experiment; cannulation was performed under light ether anaesthesia, and the experiment was performed with conscious animals after recovery from the anaesthesia. All cannulated rats were kept in a supine position on restraining plates.

For collection of urine and bile, cannulas with polyethylene tubing were inserted into the bladder (PE-200) and bile duct (PE-10). For blood collection, cannulas (PE-50) were inserted into the femoral artery. For intravenous administration a cannula (PE-50) was inserted into the femoral vein, into which a bolus of 0·5, 1·0 or 2·0 mg kg^{-1} was injected. For hepato-portal administration a dose of 2·0 or 4·0 mg kg^{-1} was infused into the hepato-portal vein with a syringe (30 gauge) over a period of 2·5 or 5 min. For rectal administration a dose of 4·0 mg kg^{-1} was injected into the rectum with a top-round syringe, and the anus was closed with a clip to prevent leakage. For oral administration a dose of 4·0 mg kg^{-1} was intubated into the

stomach. For intra-arterial administration a cannula (PE-50) was inserted into the left carotid artery, into which a bolus of 2·0 mg kg^{-1} was injected.

Blood samples were taken at different intervals, depending on the individual animals, after intravenous administration. For intra-arterial administration the sampling times were 2·5, 5, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min. For 2·0 mg kg^{-1} hepato-portal administration the sampling times were 2·5, 5, 10, 20, 30, 40, 60, 80, 100 and 120 min and for 4·0 mg kg^{-1} hepato-portal administration the sampling times were 2·5, 5, 10, 20, 30, 40, 60, 90, 120, 150 and 180 min. For oral administration the sampling times were 0·25, 0·5, 0·75, 1, 1·5, 2, 3, 4, 5 and 6 h. For rectal administration the sampling times were 0·25, 0·5, 0·75, 1, 1·5, 2, 3, 4, 5 and 6 h.

The drug solution was prepared by diluting the original solution in ethanol with normal saline (containing 10% ethanol for intravenous administration at a dose of 0·5 mg kg^{-1} and 20% for administration by other routes). The effects of co-administration of ethanol were not investigated because the amounts were fairly small compared with the body weights of the animals. Volumes of the drug solution administered were from 0·5 to 2 mL kg^{-1}.

Plasma–protein binding and blood–plasma ratio

After intravenous administration of nicardipine, blood samples (2·0–2·5 mL) from the femoral artery were drawn into the heparinized tube at arbitrary times. A sample (50 μL) was taken for determination of the concentration of the drug in whole blood; the remaining samples were immediately centrifuged to obtain plasma, which was submitted both for measurement of the unbound plasma concentration using an ultrafiltration technique (MPS-1; Amicon, Beverly, MA) and for determination of the plasma concentration. All samples were then immediately frozen at −30°C until assay. The adsorption of the drug by the MPS-1 apparatus was negligible. The method for microdetermination of nicardipine and its pyridine metabolite is described elsewhere (Watari et al 1990); the limit of detection is 5 ng mL^{-1}.

Data analysis

The areas under the curve (AUC), calculated by the curve-fitting (a biexponential equation) and trapezoidal methods were in good agreement. The trapezoidal method was used to determine AUC in this study.

Moment analysis was performed according to the method of Yamaoka (1984). The mean absorption time (MAT) and the variance of the absorption time

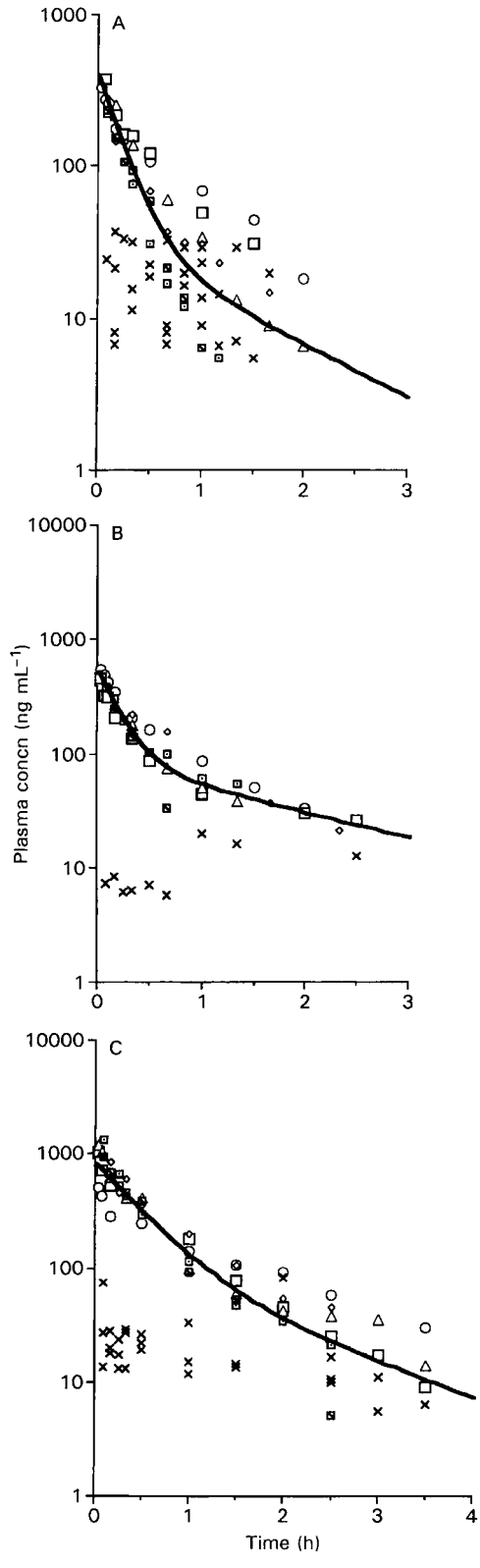

Figure 1. Time-courses of nicardipine and its pyridine metabolite in plasma after intravenous administration of (A) 0·5, (B) 1·0 and (C) 2·0 mg kg^{-1} nicardipine. Each symbol represents individual data from each of six rats; the concentrations of the pyridine metabolite are designated ×. Solid lines are the calculated values for nicardipine.

(VAT) of the drug after non-instantaneous input (ni) were calculated according to equations 1 and 2:

$$MAT = MRT_{ni} - MRT_{i.v.} \quad (1)$$

$$VAT = VRT_{ni} - VRT_{i.v.} \quad (2)$$

where MRT is the mean residence time and VRT the variance of the residence time. The systemic availability (F) of nicardipine was calculated from equation 3:

$$F = ((AUC_{ni}/AUC_{i.v.})/(dose_{ni}/dose_{i.v.})) \times 100 \quad (3)$$

The fraction (F_{nh}) passing through the non-hepatic route after rectal or oral administration was calculated as (De Boer et al 1979):

$$F_{nh} = ((F_{rectal}(\text{or } F_{oral}) - F_{pv})/(1 - F_{pv})) \times 100 \quad (4)$$

where F_{rectal} or F_{oral} are the systemic availability after rectal or oral administration and F_{pv} is the systemic availability after hepato-portal vein infusion.

For multiple comparisons, one-way analysis of variance and the Tukey–Kramer test were done successively on Yukms Stat Light software (Tokyo, Japan).

Results

Plasma levels after intravenous administration

Figure 1 shows the time-courses of the plasma concentration of nicardipine and its pyridine metabolite after intravenous injection of 0·5, 1·0 and 2·0 mg kg^{-1} of the drug. The plasma concentrations of nicardipine seemed to decrease in a biexponential fashion, that is, the disposition of nicardipine could be described by a two-compartment model; this is similar to results obtained from studies on dogs, monkeys and man (Higuchi & Shiobara 1980a).

There was no significant difference among doses for dose-normalized AUC, blood clearance (CL), MRT and VRT (Table 1). Therefore, the disposition of nicardipine in rats apparently shows a linear relationship with doses ranging from 0·5 to 2·0 mg kg^{-1}.

Table 1. Dose-normalized area under the plasma concentration–time curve on a per milligram basis of nicardipine, blood clearance, systemic availability, and parameters of moment analysis.

Route	Dose (mg kg^{-1})	Area under the curve $\times 10^{-3}$* (ng min mL^{-1})	Clearance† (mL min^{-1} kg^{-1})	Mean residence time‡ (min)
Intravenous	0·5	16·0 ± 2·1	70·1 ± 9·2	29·9 ± 6·1
	1·0	12·9 ± 1·5	86·3 ± 11·3	58·7 ± 13·9
	2·0	15·8 ± 0·8	65·6 ± 3·5	48·9 ± 7·7
Intra-arterial	2·0	14·2 ± 1·5	76·8 ± 8·5	88·3 ± 13·6
Hepato-portal	2·0	3·32 ± 0·5	366 ± 70·8	62·9 ± 9·3**
	4·0	3·14 ± 0·5	373 ± 65·5	82·1 ± 9·2**
Oral	4·0	7·59 ± 0·6	139 ± 11·9	506 ± 249
Rectal	4·0	7·34 ± 0·4	142 ± 7·9	411 ± 132

Route	Dose (mg kg^{-1})	Variance of residence times§ $\times 10^{-3}$	Systemic availability¶ (%)	Mean absorption time¶ (min)	Variance of absorption time/(Mean absorption time)²¶
Intravenous	0·5	1·4 ± 0·4			
	1·0	6·4 ± 3·9			
	2·0	3·7 ± 0·9			
Intra-arterial	2·0	12·4 ± 3·3			
Hepato-portal	2·0	5·9 ± 2·0	22·3	17·1	7·2
	4·0	10·4 ± 3·1	21·7	36·3	5·0
Oral	4·0	622 ± 495	50·9	460	2·9
Rectal	4·0	252 ± 182	49·3	365	1·9

Values are means ± s.e.m. for five or six rats. Statistical differences were evaluated by one-way analysis of variance followed by the Tukey–Kramer test.

*The oral, rectal or hepato-portal values were significantly different from the intravenous and intra-arterial values, respectively ($P < 0·01$). †The oral or rectal values were significantly different from the hepato-portal value ($P < 0·01$), which was significantly different from the intravenous and intra-arterial values, respectively ($P < 0·01$). ‡The oral or rectal values were significantly different from the intravenous value ($P < 0·01$) and the hepato-portal value ($P < 0·05$). The oral value was significantly different from the intra-arterial value ($P < 0·05$). §The oral value was significantly different from the intravenous value ($P < 0·05$). ¶The mean value of the area under the curve, the mean residence time, or the variance of the mean residence time after three doses of intravenous administration was used for calculation. **The mean residence time is the ((area under the moment curve)/(the area under the curve)) – mean input time (Watari & Benet 1989).

Blood clearance was converted from plasma clearance by applying the ratio of the drug concentration in whole blood to that in plasma (derived from Figure 4). Blood clearance after intravenous administration ranged from 65·6 to 86·3 mL min^{-1} kg^{-1}, which should be almost equivalent to the hepatic clearance, because the excretion of nicardipine into the urine and bile was negligible, as shown in Table 2.

Plasma levels after other routes of administration

On the basis of the lack of dose-dependency for nicardipine disposition for intravenous doses ranging from 0·5 to 2·0 mg kg^{-1}, other routes of administration were investigated with various doses of nicardipine, taking the plasma concentrations into consideration. Figure 2 shows the time-courses of the plasma concentrations of nicardipine and its pyridine metabolite after injection of 2·0 mg kg^{-1} nicardipine into the left carotid artery, and hepato-portal infusion of 2·0 mg kg^{-1} nicardipine over 2·5 min, and 4·0 mg kg^{-1} nicardipine over 5 min. The plasma concentrations of nicardipine and its pyridine metabolite after the 2·0 mg kg^{-1} intra-arterial injection were very similar to those after the 2·0 mg kg^{-1} intravenous injection (Figure 1C). In contrast, throughout the investigation the plasma concentrations of nicardipine after hepato-portal infusion were noticeably lower than after intravenous and intra-arterial injection. These findings indicate a pronounced hepatic first-pass effect for nicardipine after hepato-portal administration.

Figure 3 shows the time-courses of the plasma concentrations of nicardipine and its pyridine metabolite after oral administration of 4·0 mg kg^{-1} and rectal administration of 4·0 mg kg^{-1} of nicardipine. The profiles of the time-courses of the unchanged drug in plasma after rectal administration are similar to that of prolonged-release medication. The maximum plasma concentrations after oral administration were within 15 to 30 min.

After oral and hepato-portal administration a considerable amount of the pyridine metabolite was detectable in plasma whereas only a small amount was detected after rectal administration. The ratios

Table 2. Fraction recovered from urine and bile during the 7 h after intravenous administration of nicardipine (2·0 mg kg^{-1}).

	Nicardipine (%)	Pyridine metabolite (%)
Urine	0·004 ± 0·004	n.d.
Bile	0·073 ± 0·012	0·013 ± 0·003

Values are mean ± s.e.m. of results from six rats. The values for urine include zero values for measurements below the sensitivity of the assay. n.d., not detected.

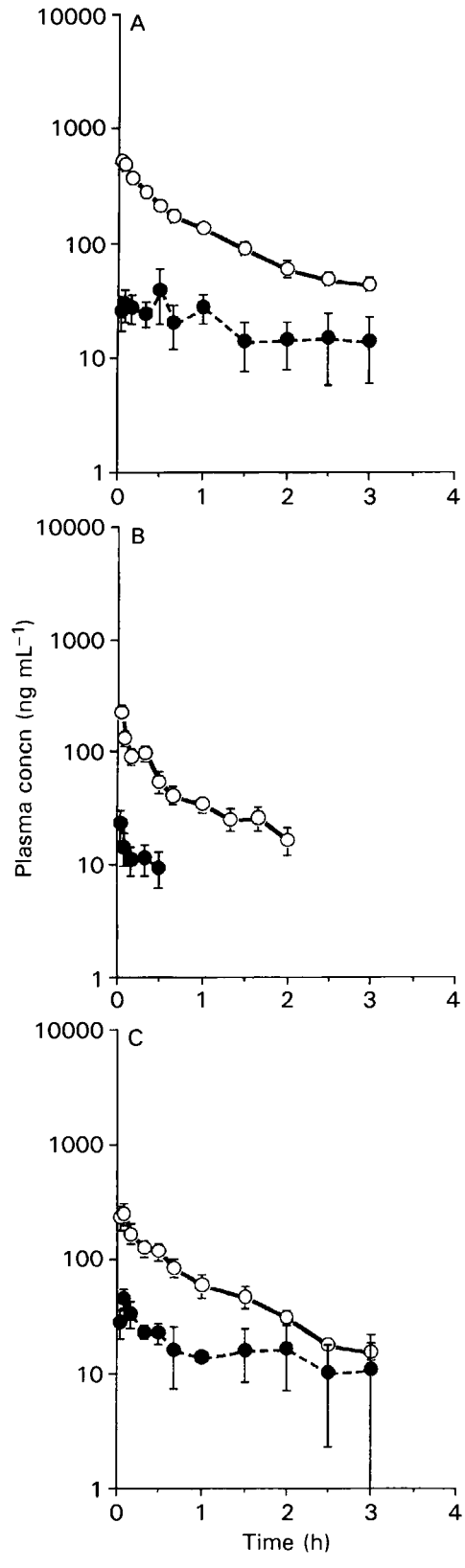

Figure 2. Time-courses of nicardipine (○) and its pyridine metabolite (●) in plasma after (A) administration of 2·0 mg kg^{-1} nicardipine into the left carotid artery, and infusion into the hepato-portal vein of (B) 2·0 and (C) 4·0 mg kg^{-1} nicardipine. Each point is the mean ± s.e.m. of results from six rats.

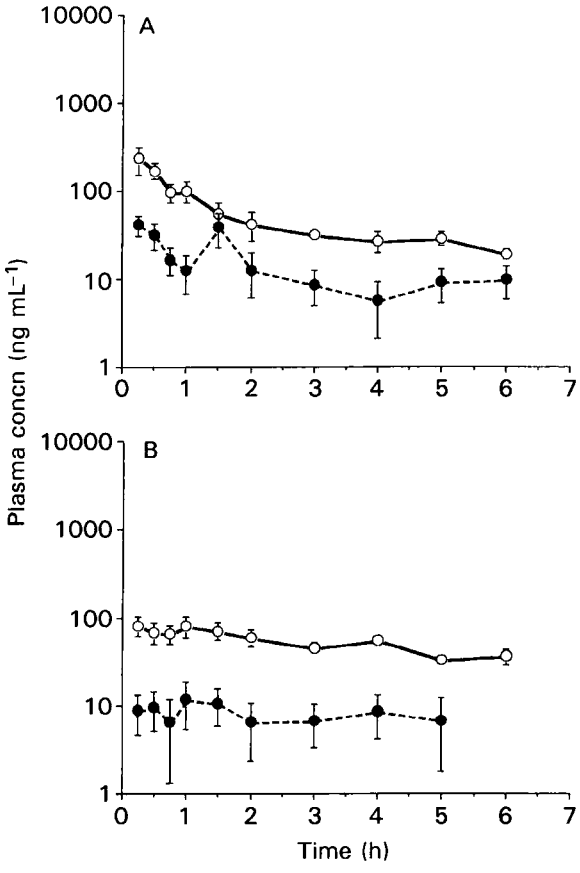

Figure 3. Time-courses of nicardipine (○) and its pyridine metabolite (●) after (A) oral administration of 4·0 mg kg^{-1} nicardipine and (B) rectal administration of 4·0 mg kg^{-1} nicardipine. Each point is the mean ± s.e.m. of results from five (A) or six (B) rats.

$AUC_{pyridine\ metabolite}/AUC_{nicardipine}$ calculated from the mean plasma concentrations until the final samplings were 15·0, 27·6, 26·0 and 14·5% for intra-arterial (2·0 mg kg^{-1}), hepato-portal (4·0 mg kg^{-1}), oral and rectal administration, respectively.

Plasma protein binding and blood-plasma ratio

Figure 4A shows the plasma-unbound fraction of nicardipine. As no significant slope is observed against the abscissa, the unbound fraction in plasma is assumed to be constant at 0·030 ± 0·025 (mean ± s.d., n = 18) for a wide range of drug concentrations, indicating that nicardipine binds extensively to plasma proteins, with plasma binding estimated as 0·970. This value is comparable with those obtained in an in-vitro study (more than 90% bound to the plasma proteins; Higuchi & Shiobara (1980b)).

The blood–plasma concentration ratio for nicardipine was also found to be constant at 0·975 ± 0·183 (n = 29) (Figure 4B) over a wide range of drug concentrations in the blood. The result is in agreement with the concentration-independent binding of nicardipine to the plasma proteins.

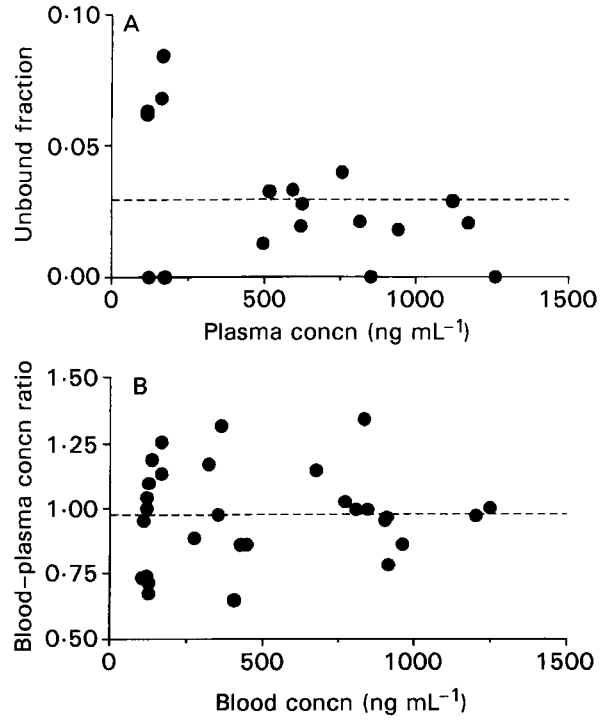

Figure 4. (A) Dependence of plasma unbound fraction on plasma concentration and (B) blood–plasma concentration ratio on blood concentration.

Systemic availability of nicardipine and moment analysis

Table 1 shows the systemic availability and parameters of moment analysis for nicardipine after different routes of administration. Analysis of variance revealed that the pharmacokinetic parameters for the five routes of administration were significantly different for AUC, CL, MRT ($P < 0.01$), and VRT ($P < 0.05$). The Tukey–Kramer test subsequently identified specific discrimination in the data (Table 1).

The dose-normalized AUC after intravenous injection were almost the same as after intra-arterial injection, indicating the absence of a pulmonary first-pass effect for nicardipine. The values of systemic availability (F) were 21·7% for hepato-portal vein infusion, 50·9% for oral administration, and 49·3% for rectal administration (Table 1); that is, the F values after oral and rectal administration are more than twice those after hepato-portal vein infusion. In addition, the F value of 50·9% obtained after oral administration of 4·0 mg kg^{-1} was in approximate agreement with the corresponding 45·1% for a dose of 5·0 mg kg^{-1} reported by Higuchi & Shiobara (1980a). The fraction (F_{nh}), the dose ratio which partially avoided the hepato-portal system according to equation 4, was estimated to be 37·3% or 35·2% after oral or rectal administra-

tion, respectively, assuming all of the molecules absorbed passed through the liver.

As shown in Table 1, the values of MAT after oral and rectal administration are much larger than those of MRT after intravenous administration. This indicates that the absorption kinetics after oral and rectal administration were consistent with the flip-flop phenomenon. Much larger values of VRT were obtained after oral and rectal administration than after other routes of administration, which also indicates prolonged residence of the drug in the body, i.e. extremely slow absorption after oral and rectal administration.

The VAT/MAT^2 ratio, however, varied with drug-mixing conditions at the dosing site. The ratio is close to unity when complete mixing of the drug is achieved at the dosing site and steady-state absorption can be expected (Yamaoka 1984). At ratios below unity, the absorption process might be described as a catenary step. When it behaves like a mamillary system, the ratio is above unity. It therefore appears that absorption after oral and rectal administration occurs in a mamillary manner because the value of the VAT/MAT^2 ratio is above unity.

Discussion

For intravenous doses ranging from 0·5 to 2·0 mg kg^{-1} no dose-dependency was observed for nicardipine disposition. The percentage fraction of the pyridine metabolite to nicardipine fluctuated from 1·4 to 27·9% in plasma and from 8·02 to 46·8% in whole blood. No concentration-dependency was found for the plasma-unbound fraction and the blood–plasma ratio of nicardipine (Figure 4). Therefore, competitive interaction between nicardipine and its pyridine metabolite with plasma proteins should be less prominent in the in-vivo concentration range.

Almost no unchanged nicardipine is excreted in the urine and bile, and no substantial pulmonary first-pass effect is present, because the dose-normalized AUCs are nearly the same for intravenous and intra-arterial administration. Therefore, the blood clearance should correspond to the hepatic clearance, which is within the range of hepatic blood flow (60–93 mL min^{-1} kg^{-1}) (Pang & Gillette 1978; Boxenbaum 1980; Anderson et al 1987), irrespective of the extensive binding of the drug to plasma protein. Thus, the metabolism of nicardipine appears to be non-restrictive in the liver (Wilkinson & Shand 1975; MacKichan 1984).

There was no non-linearity for hepato-portal vein infusion of this dose (Table 1) and the hepatic first-pass effect was calculated to be approximately 80% of input dose. Intriguingly, the F values after oral and rectal administration were more than twice those after hepato-portal vein infusion. Assuming that all the molecules absorbed flow through the liver, the partial avoidance of the hepatic first-pass effect would be 37·3 or 35·2% after oral or rectal administration, respectively. This is supposedly an indication that an unidentified absorption process after oral administration results in enhanced availability.

Although the values of availability after oral and rectal administration are very similar, the ratio $AUC_{\text{pyridine metabolite}}/AUC_{\text{nicardipine}}$ after rectal administration was lower than after oral administration and was comparable with that after intra-arterial administration (Figures 2 and 3). This also indicates partial avoidance of hepatic first-pass metabolism of nicardipine after rectal administration. Usually after rectal administration the superior haemorrhoidal vein conducts blood via the inferior mesenteric vein into the portal vein. In the lower part of the rectum there are inferior and middle haemorrhoidal veins through which blood passes directly into the inferior vena cava (De Boer et al 1979). This might explain how the partial avoidance of the hepatic first-pass effect occurs after rectal administration.

An explanation of why, after oral administration, the F value is more than twice that observed after hepato-portal vein infusion and the partial avoidance of the hepatic first-pass effect, irrespective of the considerable amount of pyridine metabolite in the plasma, remains to be elucidated. A reported non-linearity in the intestinal metabolism of enterocytes (Pichard et al 1990; Kolars et al 1992) should be effective in reducing the F value and should rule out its contribution to the increased F value after oral administration. Therefore, it is likely that the transport of nicardipine into the enterocytes is limited for some unknown reason. Further, an exclusive system of drugs such as P-glycoprotein in the intestine might be active in preventing the incorporation of nicardipine into the enterocytes. As a possible explanation it is considered that the lymph might play an important role in the absorption process, resulting in large values of MAT and VRT (Table 1) (Ohkubo et al 1997), because nicardipine has a relatively large molecular weight (479·5 for nicardipine base; Supersaxo et al (1990)). These considerations are consistent with our observation that the ratio $AUC_{\text{pyridine metabolite}}/AUC_{\text{nicardipine}}$ after oral administration is similar to that after hepato-portal administration.

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