BIOPHARMACEUTICS & DRUG DISPOSITION, VOL. 18:6, 509–522 (1997)

EXTRAHEPATIC FIRST-PASS METABOLISM OF NIFEDIPINE IN THE RAT

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ABSTRACT

The peroral (po) bioavailability of nifedipine is reported to range from about 45 to 58% in the rat; this compares favourably to human beings. The metabolism of nifedipine is similar in rats and humans (oxidation of the dihydropyridine ring), with the liver believed to be solely responsible for the systemic clearance of the drug and the observed first-pass effect after po dosing. The purpose of this study was to determine whether intestinal metabolism also contributes to the first-pass elimination of nifedipine in the rat. The systemic availabilities of nifedipine doses given by po, intracolonic (ic), and intraperitoneal (ip) routes of administration were compared to that for an intravenous (iv) dose (in each case a dose of 6 mg kg^{-1} was given) using adult male Sprague–Dawley rats (249–311 g, $n = 6$ or 7/group). The geometric mean of systemic nifedipine plasma clearance after iv dosing was 10·3 mL min^{-1} kg^{-1}. The nifedipine blood-to-plasma ratio was found to be about 0·59. Therefore, the systemic blood clearance of nifedipine was about 17·5 mL min^{-1} kg^{-1}; which, compared to the hepatic blood flow of rats (55 to 80 mL min^{-1} kg^{-1}) showed that nifedipine is poorly extracted by the liver ($0{\cdot}22 \leqslant E_H \leqslant 0{\cdot}32$). The mean absolute bioavailabilities of the po, ip, and ic doses were 61, 90, and 100%, respectively. Assuming complete absorption of the extravascular nifedipine doses these results indicate that, in addition to hepatic extraction, substantial first-pass elimination of nifedipine occurs within the wall of the small intestine but not the colon of the rat. ©1997 by John Wiley & Sons, Ltd.

KEY WORDS: extravascular dosing; first-pass metabolism; high-performance liquid chromatography; nifedipine; pharmacokinetics; rat

INTRODUCTION

The pharmacokinetics of the calcium channel antagonist nifedipine have not yet been fully characterized in rats or human beings, one reason being the difficulty measuring plasma nifedipine concentrations in the low-nanogram range with sufficient specificity, sensitivity, and accuracy to give good

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CCC 0142–2782/97/060509–14$17.50

Received 17 June 1996
Accepted 14 September 1996

pharmacokinetic parameter estimates after nifedipine dosing. Although specific and sensitive analytical methods for nifedipine are now available — typically using high-performance liquid chromatography (HPLC) or gas chromatography[1–3] — several questions remain to be answered regarding the absorption and disposition of this drug, including the following: (i) does the gastrointestinal (GI) tract contribute to the first-pass effect observed after peroral (po) dosing?; (ii) what is the underlying cause of interindividual and intraindividual variability in nifedipine systemic availability (F) after po dosing?; and (iii) what mechanisms are involved in modifying F and clearance by various foods and drugs? — including the poorly understood interaction between grapefruit juice and nifedipine (and other 1, 4-dihydropyridine calcium channel antagonists).[4–7]

In rats and humans, nifedipine is well absorbed from the gut lumen ($\geqslant 90\%$).[8–14] After absorption, nifedipine is metabolized by oxidative mechanisms — involving cytochrome P450 (CYP) 3A isozymes — to a pharmacologically inactive nitropyridine analogue, which is subsequently metabolized to more polar compounds.[15–17] The liver is considered to be the principal organ responsible for systemic plasma clearance of nifedipine[18,19] and, hence, is thought to be responsible for the pronounced 'first-pass' effect observed after po nifedipine administration in the rat ($0{\cdot}45 \leqslant F \leqslant 0{\cdot}58$)[20,21] and humans ($0{\cdot}45 \leqslant F \leqslant 0{\cdot}68$)[11,12,20,22] — although it has been suggested that the gut wall may also contribute to the presystemic elimination of nifedipine.[23–26] In human beings, hepatic plasma clearance of nifedipine is reported to account for only 65% of systemic plasma clearance suggesting extrahepatic sites of metabolism.[25] Interestingly, systemic plasma nifedipine clearance in the rat (about 2·4–10 mL min^{-1} kg^{-1})[20,21,26–28] is relatively low compared to hepatic blood flow (Q_H, about 55–80 mL min^{-1} kg^{-1}),[29,30] suggesting a low nifedipine extraction ratio through the liver. A review of the data from an isolated perfused rat liver experiment, conducted by Scherling *et al.*,[9] shows that unchanged nifedipine can still be detected in the perfusate even after about 12 passes through the liver, further indicating low nifedipine extraction by this organ. Thus, hepatic extraction alone cannot account for the relatively poor po bioavailability of nifedipine in the rat — suggesting that the rat may be a good model to investigate presystemic nifedipine gut wall biotransformation.

This study was undertaken to determine whether intestinal metabolism contributes to the first-pass elimination of nifedipine in the male Sprague–Dawley rat. Standard pharmacokinetic measures (including systemic availability) of nifedipine given by intravenous (iv), po, intracolonic (ic), and intraperitoneal (ip) routes were compared after dosing in adult male Sprague–Dawley rats. In addition, the whole-blood-to-plasma concentration ratio of nifedipine (B/P_r) in the rat was measured to calculate systemic blood clearance and therefore estimate the hepatic extraction ratio (E_H) using literature values of Q_H.

MATERIALS AND METHODS

Chemicals

Nifedipine was purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). Nisoldipine (internal standard, IS) was kindly provided by Miles Canada Inc. (Etobicoke, ON, Canada). Other drugs were as follows: heparin (Hepalean®, 1000 U.S.P. units mL^{-1}) was supplied by Organon Teknika Inc. (Toronto, ON, Canada); sodium pentobarbital (Somnotol®, 65 mg mL^{-1}) was purchased from MTC Pharmaceuticals (Cambridge, ON, Canada). All other chemicals and solvents were either reagent or HPLC grade and obtained from commercial suppliers.

Animals and surgical cannulation procedure

Adult male Sprague–Dawley rats (initially weighing 240–300 g) were obtained from the Biosciences Animal Service (University of Alberta, Edmonton, AB, Canada) and housed for at least 2 d in a clean room and fed food and water *ad libitum*. On the day before dosing experiments, rats were moved to the laboratory and subjected to surgical cannulation. Under anaesthesia induced by sodium pentobarbital (65 mg kg^{-1}) a small longitudinal incision was made in the skin of each rat over the right jugular vein, which was then made accessible by clearing the surrounding tissues. The vein was catheterized with Silastic® laboratory tubing (0·635 mm i.d., 1·194 mm o.d., Dow Corning Corp., Midland, MI, U.S.A.) containing heparinized (100 IU mL^{-1}) normal saline, and fixed in place with two nonabsorbable surgical sutures (Surgical Suture USP, Cyanamid Canada, Montreal, QC, Canada). Each cannula was terminated with a long piece of polyethylene tubing (PE-50, i.d. 0·58 mm, o.d. 0·965 mm, Clay Adams, Parsippany, NJ, U.S.A.) and the free end exteriorized to the dorsal side of the neck (in total, the dead volume of jugular catheter was about 0·1 mL). The exposed areas were then closed using nonabsorbable surgical suture. The animal was allowed to recover for the next 16–20 h in a metabolic cage and provided only with drinking water for the remainder of the experiment (i.e., animals were fasted before and during each experiment). The implanted cannulas provided a site for iv administration of nifedipine (if necessary) and permitted frequent blood sampling from each rat.

Pharmacokinetic study: experimental design

All drug preparation, dosing, and collection of blood samples was performed under sodium lamps to prevent nifedipine photodegradation.[31] A nifedipine dosing solution (5 mg mL^{-1} in polyethylene glycol-400) was prepared the day before each experiment — to allow sufficient time for drug solubilization — and

wrapped in aluminum foil to protect the solution from light and stored at room temperature. Each conscious rat received a single dose of nifedipine ($6\,mg\,kg^{-1}$) by a predetermined route of administration, as described below. Blood draws (0·25 mL samples) were made at predetermined times using heparinized 1 mL syringes connected to the jugular cannula. The blood volume drawn was immediately replaced with an equal volume of normal saline. Blood samples were centrifuged (Beckman Microfuge E, Beckman Instruments, Palo Alto, CA, U.S.A.) at 15 000 rpm for 4 min and the plasma samples obtained were placed in 1·5 mL poly(propylene) microfuge tubes, stored in light-resistant bags and kept at $-20\,^{\circ}C$ until needed for analysis.

Intravenous administration. Six rats (249–300 g) received an iv dose of nifedipine through the jugular vein cannula (given over 4 min to prevent injury to the animal that could result from excessively high nifedipine plasma concentrations). The cannula was immediately rinsed with 0·5 mL normal saline and blood samples were taken at −5, 5, 8, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min — the start of the 4 min nifedipine infusion period was designated as 0 min.

Intraperitoneal administration. Six rats (264–293 g) received an ip dose of nifedipine given over about 10 s using a short 26G needle (length, 12·7 mm) inserted into the left caudal area of the abdomen. Blood samples were drawn at −5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min.

Peroral administration. Six rats (260–297 g) received a po dose of nifedipine given over about 10 s using an oral feeding tube. Blood samples were drawn at −5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min.

Intracolonic administration. Seven rats (257–299 g) received an ic dose of nifedipine given over about 10 s through a 15 cm length of PE-100 tubing (i.d., 0·86 mm, o.d. 1·52 mm, Clay Adams, Parsippany, NJ, U.S.A.) the entire length of tubing was inserted rectally after lubricating the outer surface of the tube with polyethylene glycol-300. Blood samples were drawn at −5, 2, 5, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420, and 480 min.

Whole-blood-to-plasma concentration ratio: experimental design

Three stock solutions were prepared by dissolving known amounts of nifedipine in 100 mL methanol ($20\,\mu g\,mL^{-1}$, $500\,\mu g\,mL^{-1}$, and $2\,mg\,mL^{-1}$ solutions, respectively). The B/P_r was measured after adding nifedipine to freshly collected heparinized blood — yielding low ($200\,ng\,mL^{-1}$), medium ($5\,\mu g\,mL^{-1}$), and high ($20\,\mu g\,mL^{-1}$) concentrations ($n = 5$/concentration) — obtained from a separate group of male Sprague–Dawley rats (280–311 g). Methanol concentrations in the blood samples were limited to 1% (v/v) by

using an appropriate stock solution concentration (described above). The blood samples were gently mixed and incubated at 37 °C for 30 min to allow for distribution of the drug into erythrocytes. The concentration of nifedipine was determined in 0·05–0·1 mL samples of blood and plasma obtained after centrifugation.

Chromatography

Unknown nifedipine concentrations in rat plasma and blood were determined by means of a previously reported HPLC method (lower limit of quantitation, 5 ng mL^{-1})[2] using 0·05–0·2 mL samples diluted to 1·0 mL with HPLC grade water. All analyses were conducted under sodium lamps to prevent photodegradation of nifedipine. Calibration curve standards (containing 5–2000 ng of nifedipine) were prepared by adding a known amount of the drug to 0·1 mL blank rat plasma and diluting to 1·0 mL as described above. The calibration curve best-fit regression line was calculated using a $1/\chi^2$ weighting factor (where χ corresponds to the amount of nifedipine added; r^2 values greater than 0·99 were always obtained; interday and intraday variability were less than 10%). An analytical quality control in-run sample (10, 150, or 900 ng; duplicate or triplicate samples for each run) was placed between every six unknown test samples. The analyses were performed with the following instrumentation: a model 600E solvent delivery system, a model 717 autosampler; a model 486 tunable UV/VIS absorbance detector (set at 350 nm); and an NEC 486–33 MHz computer running Millennium 2010 Chromatography Manager software Version 1.1 (Waters, Mississauga, ON, Canada).

Pharmacokinetic analysis

Standard pharmacokinetic parameters obtained from each of the individual rat plasma concentration–time profiles were calculated by non-compartmental methods using the computer program WinNonlin Standard Edition Version 1.0 (Scientific Consulting, Apex, NC, U.S.A.) — area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule from time zero (t_0) to the time to reach peak concentration (t_{max}), and the logarithmic trapezoidal rule applied from t_{max} to the time of the last observable concentration (t_{last}), followed by extrapolation to infinity (i.e., C_{tlast}/k_{el}).

Statistical analysis

All the calculated pharmacokinetic parameters — except t_{max} — were assumed to follow a log-normal distribution, and were log-transformed before analysis. The pharmacokinetic parameter estimates obtained were expressed as either geometric or arithmetic means with their corresponding

95% confidence intervals, as appropriate. The pharmacokinetic parameters obtained were further analysed by independent measures oneway analysis of variance (ANOVA), and a *post hoc* test (Duncan's multiple-range test) was used to determine where, if any, differences occurred. A value of $p \leqslant 0{\cdot}05$ was considered statistically significant. The computer program SPSS for Windows Version 6.1 (SPSS, Chicago, IL, U.S.A.) was used for all the statistical tests. For the purposes of generating plasma concentration–time profiles, plasma nifedipine concentrations were expressed as arithmetic means $\pm$ standard deviation (s).

RESULTS AND DISCUSSION

The mean ($\pm s$) plasma nifedipine concentration–time profiles obtained in this study after iv, ip, po, and ic dosing of nifedipine (6 mg kg^{-1}) to separate groups of rats are shown in Figure 1. The individual profiles obtained after iv dosing displayed a distributive phase consistent with a drug known to display multi-compartmental characteristics. Individual profiles from extravascular dosing typically showed a rapid rise in plasma nifedipine concentrations, after which levels declined monoexponentially. However, plasma concentration–time profiles obtained after po dosing were found to be more variable, perhaps due to rate-limiting absorption from the GI tract in some of the rats.

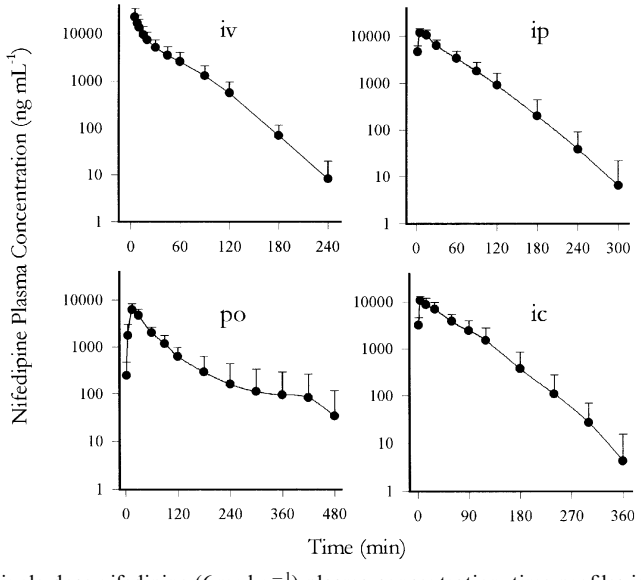

Figure 1. Single-dose nifedipine (6 mg kg^{-1}) plasma concentration–time profiles in male Sprague–Dawley rats after iv, ip, po, and ic administration. Data are presented as arithmetic mean values $\pm s$ ($n = 6$ or 7)

Standard pharmacokinetic parameters calculated from the data obtained in this study are shown in Table 1 and significant differences, where found, are noted. Interestingly, statistical analysis of the clearance values obtained after extravascular administration (i.e., po, ic, ip) showed that only po clearance was statistically different from systemic (iv) clearance. The values for mean absorption time and mean residence time (see Table 1) showed that nifedipine was rapidly absorbed and eliminated in this study. Nifedipine was found to have a relatively high volume of distribution at steady state (V_{ss}, see Table 1) indicating that, despite high plasma protein binding (about 99% in rat plasma, unpublished observations from our laboratory), the drug is very highly bound to extravascular tissues in the rat.

Little or no unchanged nifedipine is found in the excreta (urine and bile) of rats; hence, the drug is cleared from plasma via biotransformation, presumably by the liver.[9,20] Therefore, systemic clearance may closely reflect hepatic clearance; however, this statement assumes that no other extrahepatic elimination route exists.[20] The mean systemic nifedipine clearance found in this study (Table 1) is similar to that given by Boje *et al.*,[28] who report a mean ($\pm s$) value of $9{\cdot}1 \pm 6{\cdot}9\,\mathrm{mL\,min^{-1}\,kg^{-1}}$ (from a $2\,\mathrm{mg\,kg^{-1}}$ iv dose) in six fasted male Sprague–Dawley rats (200–350 g). Several other reports give values ranging from about 2·4 to $10\,\mathrm{mL\,min^{-1}\,kg^{-1}}$.[20,21,26,27] Thus, although the

Table 1. Pharmacokinetic parameters describing the absorption and disposition of nifedipine given by several routes of administration in the rat. Where appropriate, data are expressed as geometric means with corresponding 95% CI; except for t_{max} which is presented as the arithmetic mean with 95% CI ($n = 6$ or 7 rats per group)

Parameters	iv	ip	po	ic
AUC ($\mu g\,mL^{-1}\,min$)	584 (470–724)[a]	523 (354–772)[a]	354 (284–441)[b]	582 (451–751)[a]
C_{max} ($ng\,mL^{-1}$)	25 663 (20 535–32 070)[c]	13 098 (10 787–15 907)[d]	5884 (3331–10 397)[e]	12 257 (10 278–14 615)[d]
t_{max} (min)	—	7·5 (4.6–10.4)[f]	16.7 (9.5–23.8)[g]	8.6 (6.3–10.8)[f]
$t_{1/2\lambda}$ (min)	21·4 (18·2–25·2)[h]	21·3 (14·4–31·5)[h]	29·6 (20·6–42·5)[h]	30·5 (21·0–44·4)[h]
MRT (min)	31.8 (26·4–38·2)[i]	41·4 (32·2–53·2)[i,j]	62.9 (34·2–115·6)[j]	50·6 (40·5–63·2)[j]
MAT (min)	—	9·6	31·1	18·8
Cl ($mL\,min^{-1}\,kg^{-1}$)	10·3 (8·3–12·7)[k]	—	—	—
Cl/F ($mL\,min^{-1}\,kg^{-1}$)	—	11·5 (7·8–16·9)[k]	17·0 (12·9–22·4)[l]	10·3 (8·3–12·8)[k]
V_{ss} ($mL\,kg^{-1}$)	326 (287–372)	—	—	—
F	1·0	0·90	0·61	1·0

[a–l] Identical superscript characters identify non-significant differences ($p > 0{\cdot}05$).

Table 2. Whole-blood-to-plasma concentration ratio of nifedipine in the rat

Expected blood concentration	Actual blood concentration measured[a]	Blood-to-plasma ratio (B/P_r)[a]
200 ng mL^{-1}	245·6 ± 29·8 ng mL^{-1}	0·60 ± 0·05
5 μg mL^{-1}	4·9 ± 0·6 μg mL^{-1}	0·60 ± 0·06
20 μg mL^{-1}	18·6 ± 0·8 μg mL^{-1}	0·56 ± 0·05

[a]Data are expressed as means ± s (n = 5 samples).

systemic clearance of nifedipine in the rat is somewhat variable, nonetheless, clearance of the drug is low relative to literature values of Q_H for this animal (about 55 to 80 mL min^{-1} kg^{-1}).[29,30]

To calculate the nifedipine E_H from systemic plasma clearance — assuming the liver is the sole eliminating organ — it is necessary to estimate the hepatic blood clearance (Cl_b) of nifedipine using the B/P_r (see Materials and Methods). Hence, a study to determine the B/P_r of nifedipine in the rat was undertaken and the results are shown in Table 2. The B/P_r measured at low, medium, and high nifedipine concentrations (spanning the concentration range typically found in the rat plasma samples of the pharmacokinetic study) was relatively constant with a mean value of about 0·59. A low B/P_r value (i.e., $<1·0$) indicates that the drug has a high affinity for plasma — indeed nifedipine is highly bound to rat plasma proteins as stated above — and given the value obtained very little, if any, nifedipine is present in the haematocrit.

Hepatic nifedipine blood clearance (Cl_{Hb}) was calculated to be about 17·5 mL min^{-1} kg^{-1}, using the expression

$$Cl_{Hb} = Cl_{Hp}\frac{[plasma]}{[blood]} = \frac{Cl_{Hp}}{B/P_r} \quad (1)$$

where Cl_{Hp} is the hepatic plasma clearance. The value of E_H can be derived from the expression

$$E_H = \frac{Cl_{Hb}}{Q_H} \quad (2)$$

From equation (2), E_H in the rat was calculated to be in the range of 0·22–0·32 using Q_H values of 55 and 80 mL min^{-1} kg^{-1}, respectively.

The systemically available fraction (F) of a po dose is determined from the expression

$$F = F_L F_G F_H \quad (3)$$

where F_L is the fraction of intact (not decomposed) drug absorbed from the gut lumen, F_G is the fraction of the absorbed dose escaping destruction within the

walls of the GI tract and passing into the portal vein, and F_H is the fraction of hepatically available drug escaping liver extraction (see Figure 2).[32] In rat, the absorption of nifedipine from the GI tract is rapid and relatively complete (>90%).[8] In addition, to our knowledge, nifedipine has not been shown to decompose in the lumen of the GI tract of any animal species although this mechanism has been proposed to explain the poor bioavailability of nifedipine in rats and human beings.[33] Hence, assuming F_L and F_G are unity, the predicted F of the drug was calculated from

$$F = F_H = 1 - E_H \tag{4}$$

which gave a range of 0·68–0·78 (using the values of E_H determined above). In this study, F was found to be lower (Table 1) than that predicted above, indicating that extrahepatic first-pass metabolism may be involved, although incomplete absorption of intact drug from the GI tract cannot be completely ruled out (i.e., nifedipine degradation could potentially occur in the rat GI tract). Similarly, Kondo and Sugimoto[20] report that nifedipine F after intraduodenal administration in male Wistar rats averages between 0·52 and 0·57 — in the same study systemic nifedipine clearance averages only

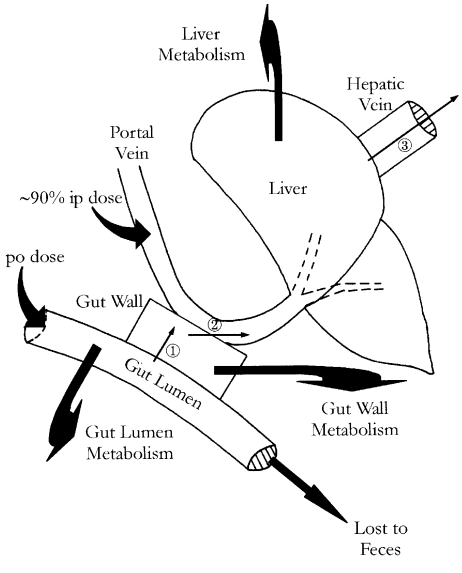

Figure 2. An illustration showing the potential sites of drug loss and drug metabolism that can limit the systemic availability of pharmaceutical compounds dosed by po and ip routes of administration. ①, fraction of the (intact) drug dose absorbed from the gut lumen (F_L); ②, fraction of the drug dose absorbed into the hepatoportal circulation ($F_L F_G$), where F_G is the fraction of the absorbed dose that passes into the portal vein; ③, fraction of the drug dose systemically available ($F_L F_G F_H$), where F_H is the fraction of the hepatically available drug that passes into the hepatic vein

5·2 mL min^{-1} kg^{-1} (using iv doses of 0·025–0·1 mg kg^{-1}) from which F values of 0·84–0·89 would be predicted.

Comparison of po and ip routes of administration of a drug allows an indirect method for estimating the relative contribution of the intestine to first-pass metabolism (see Figure 2).[34,35] Similarly, comparison of po and ic routes of administration allows an indirect method for estimating the relative contribution of the small intestine *versus* the colon to first-pass extraction of a drug.

Administration via the po route subjects a drug to potential first-pass elimination by the gut and liver, whereas the majority (>90%) of an ip dose of a drug is absorbed into the hepatoportal circulation and only undergoes hepatic extraction.[34,35] After ip dosing, the resulting value for F can be used to estimate E_H by rearrangement of equation (4). In this study F was 0·90 after ip dosing (Table 1); hence, E_H is estimated as 0·10, which is lower than the range reported above using equation (2). However, the variability in systemic plasma clearance between rats produces a degree of uncertainty in the estimation of E_H and could account for the discrepancy found. Alternatively, this result could suggest that hepatic blood clearance of nifedipine (estimated as 5·5–8 mL min^{-1} kg^{-1} using the E_H value calculated from the ip data) is somewhat smaller than systemic blood clearance in the rat (17·5 mL min^{-1} kg^{-1}), implying the presence of another extrahepatic elimination route in addition to first-pass gut metabolism. Nevertheless, the results confirm that the poor po bioavailability of nifedipine cannot be entirely accounted for by hepatic extraction.

The colon, including the caecum and rectum, in the adult white rat is about 21–27 cm in length.[36,37] Considering the total combined lengths of the proximal colon (5·5–6·5 cm), major flexure (3–3·5 cm), distal colon (4·5–6·5 cm), and rectum (3–3.5 cm),[36] a 15 cm length of PE-100 tubing was inserted rectally, when needed, into one group of adult male Sprague–Dawley rats so that nifedipine could be delivered into the proximal colon (i.e., for the ic dosing experiments).

The majority of a nifedipine dose administered *via* the rat proximal colon (ic dosing) would be expected to be absorbed into the hepatoportal circulation and undergo hepatic extraction; however, a significant portion of the dose could escape hepatic first-pass metabolism by spreading to the rectum where it could be absorbed directly into the general circulation.[20] In addition, metabolic activity of CYP isoenzymes within the gut wall is generally higher in the duodenum and jejunum than the ileum and colon of humans and rats.[23,38–40] Hence, nifedipine F after ic dosing would be expected to be exceed F after po dosing, and be equal to or greater than F after ip dosing assuming complete absorption of intact drug. In fact, in this study F was unity after ic dosing (see Table 1), suggesting no or little colonic wall metabolism (as expected) but also, surprisingly, that hepatic extraction was too small to be detected or was nonexistent. This may have arisen due to a large portion of the ic dose

bypassing hepatic extraction (i.e., rectal absorption), or because hepatic presystemic extraction was too small to be detected in the rats used in the study given the relatively large variability observed in nifedipine clearance. Alternatively, this result may be further evidence of a small liver extraction ratio and hepatic clearance of nifedipine and suggest the presence of another extrahepatic elimination route besides first-pass gut metabolism. This result also suggests that none of the ic nifedipine dose was lost in the faeces — i.e., completely absorbed intact from the colon.

Until relatively recently it was believed that only the liver, and not the intestinal mucosa, contained a sufficient amount of CYP enzymes to affect drug bioavailability.[34,41] However, metabolic and pharmacokinetic studies with such drugs as cyclosporine, L-dopa, and flurazepam show that significant biotransformation can occur within enterocytes of the intestinal wall of rats and humans.[38,42–45]

To date, conflicting evidence exists for gut wall metabolism of 1, 4-dihydropyridine calcium channel antagonists in rats. Wang *et al.*[35] showed that felodipine, a second-generation 1, 4-dihydropyridine calcium channel antagonist, undergoes substantial first-pass elimination by the intestine of male Sprague–Dawley rats. However, Flinois *et al.*[46] showed that *in vitro* and *in vivo* metabolism of oxodipine, another 1, 4-dihydropyridine calcium channel antagonist, was negligible in rats. Gut wall metabolism has also been speculated to contribute to the first-pass elimination of nifedipine in human beings.[4–7,25,47,48] Most of the available evidence comes from studies in which nifedipine is coadministered with grapefruit juice, showing increased bioavailability of nifedipine after po but not iv dosing.[4–7,48] Considering that the mechanism responsible for the interaction of grapefruit juice with 1, 4-dihydropyridine compounds has not been fully established, the rat may be a useful model with which to study this effect and will be the subject of a future report.

The CYP catalysed oxidation of nifedipine in the rat is mediated by the isozymes 3A and 2C in the rat (also referred to by the common names PCN-E and UT-A, respectively); whereas in human beings nifedipine is oxidized solely by CYP 3A.[49] Hence, this and other potential differences in the absorption and disposition of nifedipine mean the results of this study may not necessarily be extrapolated to human beings. However, the intestinal mucosa in humans may partially contribute to the metabolism of nifedipine and may explain some of the intersubject and intrasubject variability in systemic availability of this drug and could play a role in some observed food and drug interactions.

In conclusion, the results of this study showed that nifedipine F in the rat is affected by both gut wall (small intestine) and hepatic presystemic extraction after po administration. Relatively low extraction of nifedipine by the rat liver was confirmed and no evidence of first-pass metabolism within the wall of the proximal colon was observed after ic dosing. These findings suggest that the rat is a suitable model with which to investigate CYP mediated metabolism of

drugs in the intestinal mucosa and to further study the mechanism(s) of various food and drug interactions.

ACKNOWLEDGMENTS

John S. Grundy is the recipient of a Pharmacy Manufacturers Association of Canada–Health Research Foundation/Medical Research Council of Canada (PMAC–HRF/MRC) studentship.

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