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Inhibition of Nifedipine Metabolism in Dogs by Erythromycin: Difference between the Gut Wall and the Liver

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Abstract

The purpose of this study was to evaluate possible interaction of nifedipine with erythromycin or rokitamycin in the intestinal mucosa. Male beagle dogs were orally administered nifedipine (10 mg), with or without oral pre-medication with erythromycin (300 mg), and 300 mg erythromycin or rokitamycin twice a day for 3 days. The experiments were of randomized cross-over design with a two-week wash-out period between dosing regimens.

Erythromycin pre-medication for 3 days resulted in a significant increase in the area under the serum nifedipine concentration–time curve (AUC), whereas the curve for one nifedipine metabolite (M-2) decreased significantly. When the effects of erythromycin on the metabolism of nifedipine were studied using dog liver microsomes it was found that erythromycin significantly inhibited formation of M-2 but not of the metabolite M-1. These results indicate that formation of M-2 from M-1 in the liver might be reduced by erythromycin pre-medication. To avoid possible metabolism in the gut, the dogs were then administered 8 mg nifedipine into the peritoneal cavity, with or without multiple dose pre-treatment with erythromycin for 3 days. After intraperitoneal administration of nifedipine, the maximum concentration (C_{max}) of nifedipine increased significantly.

After pre-administration of erythromycin the relative bioavailability of nifedipine after oral administration was increased compared with injection into the peritoneal cavity. In-vitro study using rat intestinal microsomes and the in-vivo rat intestinal loop technique also showed that pre-administration of erythromycin inhibits nifedipine metabolism in the small intestine.

Nifedipine is a calcium-channel blocker which has been widely used to treat arterial hypertension and other cardiovascular diseases. When nifedipine is administered orally it is almost completely absorbed from the gastrointestinal tract, but its bioavailability is only 45–65% because of pre-systemic metabolism to inactive metabolites (Klaus & Johannes 1983). It is well known that the oxidation of nifedipine is catalysed by members of the cytochrome P450 3A (CYP3A) family (Guengerich et al 1991), and that nifedipine interacts with several drugs (Schlanz et al 1991a, b). We have also reported the interaction of nifedipine with quinidine sulphate owing to inhibition of first-pass metabolism (Tsuruta et al 1995). The use of erythromycin and other macrolide antibiotics has been extended to the treatment of *Helicobacter pylori* infection, a common cause of gastritis and gastric ulcers. Therefore, these drugs are often used in combination with other drugs. However, some macrolide antibiotics might be a source of extensive drug interactions (Liedholm & Nordin 1991; Periti et al 1992). A mechanism has been proposed whereby macrolide antibiotics are *N*-demethylated by CYP3A4 to nitrosoalkanes, which combine with the haem of P450 to form a stable complex, thus rendering the enzyme inactive. The CYP3A4-mediated metabolism of drugs is therefore inhibited by some macrolide antibiotics.

The site of first-pass oxidative metabolism is generally considered to be the liver because of its size, its relatively high level of P450 activity, and its anatomic location relative to the site of absorption. However, recent studies on cyclosporin (Kolars et al 1991) indicate that CYP3A metabolic activity in the intestinal mucosa substantially contributes to the overall first-pass metabolism. Such findings raise the question of whether nifedipine is also subject to significant intestinal metabolism.

To the best of our knowledge, there is no report on the study of the interaction of nifedipine with macrolides by use of liver and intestinal microsomes. The aim of this study was to investigate whether first-pass metabolism of nifedipine is inhibited by macrolide antibiotics, to compare effects of multiple-dose and single-dose erythromycin, and to compare the effects of erythromycin and rokitamycin. We also investigated whether the small intestine is an important site of nifedipine-erythromycin interaction.

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Materials and Methods

Materials

Nifedipine and its metabolites (M-1 and M-2) were generous gifts from Bayer AG (Leverkusen, Germany). Erythromycin and rokitamycin used in-vivo were tablets purchased from Dainippon Pharmaceutical (Osaka, Japan) and Asahi Chemical Industry (Tokyo, Japan), respectively; erythromycin and rokitamycin used in-vitro were generously provided by the respective pharmaceutical companies. Other reagents were of analytical grade or higher.

In-vivo experiments

In the single-dose study 5 male beagle dogs, 9–14 kg, were fasted overnight with free access to water. The next morning the dogs were administered 300-mg erythromycin tablets orally and, 2 h later, a 10-mg nifedipine capsule (Adalat), again orally. Blood samples (700 μL) were collected from the

median antebrachial vein after 0, 0·5, 1, 1·5, 2, 4, 6 and 8 h. In the multiple-dose study, the dogs were administered erythromycin or rokitamycin tablets (300 mg) orally twice a day for 3 days. After the last administration, the procedure described above was followed. In addition, dogs administered erythromycin were administered nifedipine solution (8 mg total) to the abdominal cavity and blood samples were collected after 0, 0·25, 0·5, 1, 1·5, 2, 4 and 6 h.

These experiments were performed in a randomized cross-over design with a two-week wash-out period between regimens.

Preparation of liver and intestinal microsomes

Liver microsomes were prepared from the livers of male beagle dogs according to the method of Kamataki et al (1979). Liver specimens were individually homogenized in sodium phosphate buffer (0·33 M, pH 7·4) containing EDTA (0·167 mM). The homogenate was centrifuged at 105 000 *g* for 1 h, and the pellet was rinsed and suspended in the same buffer to obtain the microsomal suspension.

Intestinal epithelial cells were prepared from fasted male Wistar rats, 300–400 g, according to the method of Wang et al (1989). The cells were isolated from the upper 60 cm of the small intestine using the phosphate buffer described above, and subsequent procedures were the same as described for the liver microsomes.

The amount of protein in each microsomal preparation was determined by the method of Lowry et al (1951). The final microsomal suspension and all samples in these experiments were frozen and stored at −80°C until use.

In-vitro metabolism of nifedipine

The metabolic pathway of nifedipine is shown in Fig. 1. Determination of in-vitro nifedipine metabolism by hepatic microsomes was performed according to the method of Tsuruta et al (1995). The reaction mixture (900 μL) contained microsomal suspension (300 μL, equivalent to 0·6 mg protein), phosphate buffer (300 μL), water (100 μL) with or without erythromycin (20 μM) and NADPH-generating system (i.e. glucose-6-phosphate (80 mM), NADP (8 mM), $MgCl_2$ (60 mM) and glucose-6-phosphate dehydrogenase (10 units mL^{-1}), preincubated at 37°C for 30 min; 200 μL). The reaction was initiated by addition of phosphate buffer (0·5 M, pH 7·4, 100 μL) containing nifedipine (from 0·5 to 8 μM); it was performed at 37°C in a shaking water bath for 20 min, and then terminated by adding the reaction mixture (300 μL) to dichloromethane-*n*-pentane (3:7, v/v; 5 mL) for nifedipine and M-1 or to chloroform for M-2.

All experiments were performed in duplicate, and samples containing nifedipine were carefully protected from light by aluminium foil to prevent photodegradation.

In-vivo metabolism of nifedipine

Male Wistar rats, 300–370 g, were administered erythromycin (3·3 mg kg^{-1} dissolved in lactated Ringer's solution) by oral gavage twice a day for 3 days. On each occasion an equivalent volume of lactated Ringer's solution was administered to the control group by oral gavage. After the last administration the rats were fasted overnight with free access to water. The next morning erythromycin (3·3 mg kg^{-1}) or lactated Ringer's solution was administered orally. After 2 h, the rats were anaesthetized by intraperitoneal injection of pentobarbital (50 mg kg^{-1}). The animals were kept warm by means of a heating pad and lamp and the small intestine was exposed by a midline abdominal incision. A 45-cm length of the small intestine 1 cm from the pyloric end of the stomach was looped and fitted with a 3-way stopcock. A 10-mL sample of nifedipine (50 μg mL^{-1})-supplemented lactated Ringer's solution maintained at 37°C was introduced into the closed loop. Blood was collected continuously after the introduction of nifedipine into the intestinal loop at 1, 2, 3, 6, 10, 15, 20 and 30 min according to the method of Wang et al (1989) with a minor modification. Heparinized blood (1000 μL, 20 units mL^{-1}) from another rat was then injected into the jugular vein after

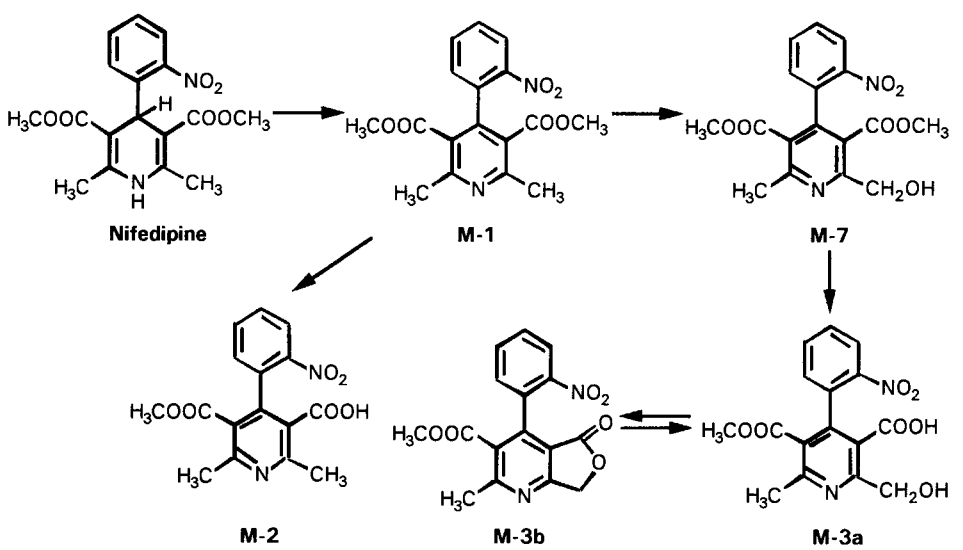

FIG. 1. Metabolic pathways of nifedipine.

each collection of the portal blood, and 300 μL of solution from the intestinal loop was collected at 0, 15 and 30 min.

Analysis

Nifedipine, M-2 (Roosemalen et al 1991) and M-1 (Guengerich et al 1986) were determined by HPLC (Shimadzu (Kyoto, Japan), model LC-6A) with UV detection.

Calculations

The area under the serum concentration–time curve (AUC) was calculated by the trapezoidal rule. Relative bioavailability was determined by comparing the normalized AUC_{0-6} with the doses administered orally and intraperitoneally. Mean residence time (MRT) was calculated by model-independent statistical moment analysis (Yamaoka et al 1978). Enzyme kinetics were analysed by Lineweaver–Burke plots. Values of the Michaelis–Menten constant, K_m, and the maximum rate of metabolism, V_{max}, were assessed by least-squares linear regression.

Statistical analysis

Results are expressed as mean ± s.e. Paired analysis or the unpaired Student's *t*-test were used to compare two groups. For multiple group analysis variance in data was initially confirmed by one-way analysis of variance, and the location of this variance was determined using Dunnett's procedure to determine the confidence level for the difference between each treatment mean and the control mean. A P value < 0.05 was considered as indicative of significance.

Results and Discussion

Some macrolide antibiotics inhibit drug metabolism in the liver by formation of complexes between their metabolites and cytochrome P450. Periti et al (1992) reported that macrolide antibiotics can be classified according to their types of drug interaction and that erythromycin belongs to a group which forms nitrosoalkanes and consequently an inactive cytochrome P450-metabolite complex whereas rokitamycin belongs to a group which does not inactivate cytochrome P450 and does not modify the pharmacokinetics of other drugs. However, Marre et al (1993) reported that an in-vitro study on interaction between cyclosporin A and macrolide antibiotics showed that rokitamycin was a more potent inhibitor of cyclosporin metabolism than erythromycin. Thus, we investigated whether these macrolide antibiotics interact with nifedipine during metabolism in the liver and the gut wall.

Fig. 2 shows the time courses of the serum concentrations of nifedipine and its metabolite (M-2) with or without pre-treatment with erythromycin or rokitamycin after oral administration to dogs. Multiple pre-treatment with erythromycin resulted in a significant increase in the AUC for nifedipine compared with that obtained after administration of nifedipine alone (259.0 ± 32.7 compared with 156.0 ± 17.7 ng h mL^{-1}, $P < 0.05$), whereas the AUC for metabolite M-2 was significantly reduced (1898 ± 84 compared with 3444 ± 811 ng h mL^{-1}, $P < 0.05$). There was no significant difference between the serum levels of nifedipine and M-2 among the groups receiving multiple pre-treatment with rokitamycin, single pre-treatment with erythromycin or nifedipine alone. Therefore, it seems that only multiple pre-treatment with erythromycin significantly inhibits first-pass metabolism of nifedipine. Thus, rokitamycin might be suitable for concomitant administration in man because of the absence of drug interaction.

In the past, the first-pass metabolism of nifedipine has been mainly attributed to CYP3A4 which was thought to be present only in hepatocytes (Guengerich et al 1991). However, it is now recognized that CYP3A4 is distributed extensively in the small intestine, where it is responsible for significant first-pass metabolism of orally administered drugs (Wang et al 1989; Krishna & Klotz 1994; Gomez et al 1995). Thus it is now assumed that nifedipine, cyclosporin, midazolam and triazolam are metabolized not only in the liver but also at the intestinal

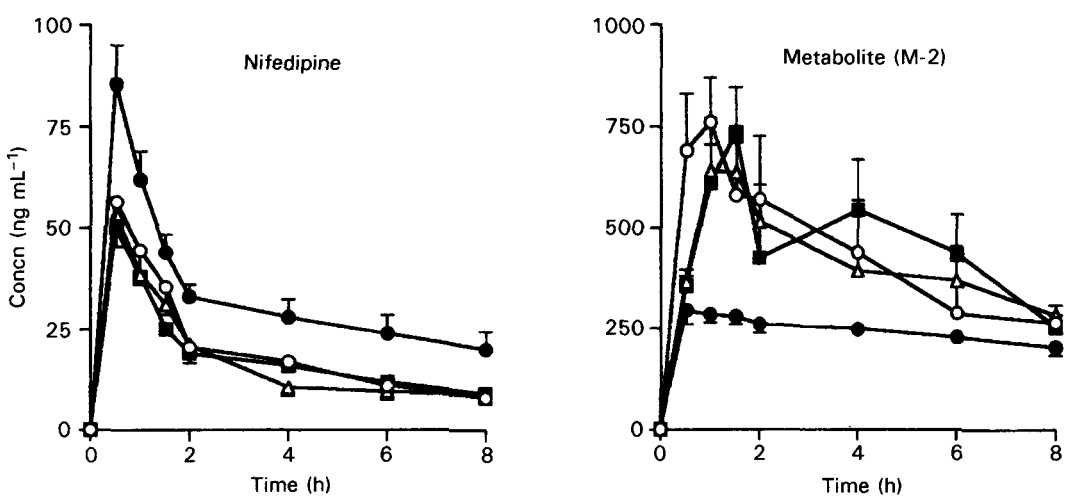

FIG. 2. Effect of co-administration of macrolide antibiotics on serum concentrations of nifedipine and M-2 after oral administration of nifedipine (10 mg) in beagle dogs. Data are expressed as the mean ± s.e.m. (n = 5). ○ Nifedipine alone, △ with multiple doses of rokitamycin, ● with multiple doses of erythromycin, ■ with a single dose of erythromycin.

Table 1. Pharmacokinetic parameters for nifedipine and metabolite M-2 after intraperitoneal administration of nifedipine (8 mg) with or without multiple pre-treatment with oral erythromycin in beagle dogs.

	Nifedipine		Metabolite M-2	
Parameter	Alone	With erythromycin	Nifedipine alone	With erythromycin
Maximum concentration (ng mL^{-1})	120·7 ± 33·8	235·0 ± 31·6*	786·3 ± 203	351·1 ± 45*
Mean residence time (h)	2·31 ± 0·12	1·77 ± 0·18	2·79 ± 0·10	2·85 ± 0·17
Area under the serum nifedipine concentration–time curve from 0 to 6 h (ng h mL^{-1})	263·4 ± 31·8	308·0 ± 21·1	2399 ± 594	1646 ± 238*

Data are expressed as means ± s.e.m. (n = 5). *Significantly different from the control group (unpaired *t*-test).

Table 2. Effect of erythromycin on the kinetic parameters of nifedipine oxidation by liver microsomes from beagle dogs.

	Nifedipine		Metabolite M-2	
Parameter	Alone	With erythromycin	Nifedipine alone	With erythromycin
Michaelis–Menten constant (μM)	8·12 ± 1·18	8·78 ± 2·25	2·50 ± 0·63	2·98 ± 0·77
Maximum rate of metabolism (nmol $mg^{-1}min^{-1}$)	0·19 ± 0·03	0·25 ± 0.06	0·08 ± 0·005	0·04 ± 0·011*
Maximum rate of metabolism/Michaelis–Menten constant (mL mg^{-1} min^{-1})	0·03	0·03	0·03	0·01

Data are expressed as means ± s.e.m. (n = 3). *Significantly different from the control group (unpaired *t*-test).

wall (Rashid et al 1995). In a previous report (Tsuruta et al 1997) we also suggested the possibility that first-pass metabolism of nifedipine can occur in the rat intestine.

C_{max}, MRT and AUC_{0-6} values after intraperitoneal injection are listed in Table 1. C_{max} for nifedipine after multiple pre-treatment with erythromycin was significantly ($P < 0·05$) greater than that for nifedipine alone (235·0 ± 31·6 ng mL^{-1} compared with 120·7 ± 33·86 ng mL^{-1}), and C_{max} and AUC_{0-6} for M-2 (351·1 ± 45·0 ng mL^{-1} and 1646 ± 238 ng h mL^{-1}, respectively) after multiple pre-treatment with erythromycin were significantly ($P < 0·05$) reduced. There was no significant difference between MRT values obtained after multiple pre-treatment with erythromycin and after administration of nifedipine alone.

The effect of erythromycin on the formation of M-1 and M-2 by metabolism of nifedipine by dog liver microsomes was determined. The enzyme kinetic parameters (i.e. K_m and V_{max}) determined from Lineweaver–Burk plots are listed in Table 2. Formation of M-1 was not inhibited by addition of erythromycin. On the other hand, V_{max} for the formation of M-2 was significantly ($P < 0·05$) different; the mean V_{max} in the presence of erythromycin was about one half that for nifedipine alone (0·04 ± 0·011 and 0·08 ± 0·005 nmol mg^{-1} min^{-1}, respectively). These results indicate that multiple dose pre-medication with erythromycin might inhibit nifedipine metabolism in hepatocytes.

The relative bioavailability of nifedipine after oral administration of nifedipine capsule (Adalat) compared with administration into the peritoneal cavity was 42·5 ± 3·5% (calculated from data shown in Fig. 2 and Table 1). Considering that nifedipine is almost completely absorbed in the gastrointestinal tract (Klaus & Johannes 1983), this suggests that nifedipine can be metabolized in the enterocytes in the small intestine. Further, the relative bioavailability of nifedipine was increased by multiple dose pre-treatment with erythromycin (57·2 ± 8·2%). These results indicate that interaction of nifedipine and erythromycin might also occur in the enterocytes of the gut. Bailey et al (1996) recently reported that erythromycin probably reduced felodipine biotransformation at the gut wall and in the liver, although they did not measure concentrations of felodipine and its metabolite in the portal vein.

To confirm nifedipine metabolism in the enterocytes, we performed an in-vitro study using rat intestinal microsomes and the in-vivo intestinal loop technique in rats. The results are shown in Fig. 3, Table 3 and Fig. 4. Fig. 3 and Table 3 show the effect of pre-treatment with erythromycin on the formation of M-1 and M-2 from nifedipine in rat intestinal microsomes. Pre-treatment with erythromycin reduced the extent of metabolism of nifedipine to M-1 and M-2. Fig. 4 shows the rates of appearance of nifedipine and M-2 in the portal vein in rats after administration of nifedipine into the intestinal loop with or without pre-treatment with erythromycin. Rates of appearance of nifedipine after pre-treatment with erythromycin were close to those of the control. However, the rates of appearance of M-2 in the portal vein appreciably decreased with multiple doses of erythromycin. The presence of M-2 in the portal blood indicates that nifedipine is metabolized by intestinal microsomes. Furthermore, a decrease in the formation of M-2 suggests that nifedipine metabolism in the enterocyte of the gut might be inhibited by pre-treatment with multiple doses of erythromycin.

In conclusion, it was confirmed that nifedipine is metabolized not only in the liver but also in the intestine, and that its metabolism is inhibited by multiple doses of erythromycin. In contrast with erythromycin, rokitamycin appears to have little effect on nifedipine metabolism even when the drug is administered repeatedly. Possible usefulness of rokitamycin in man remains to be confirmed.

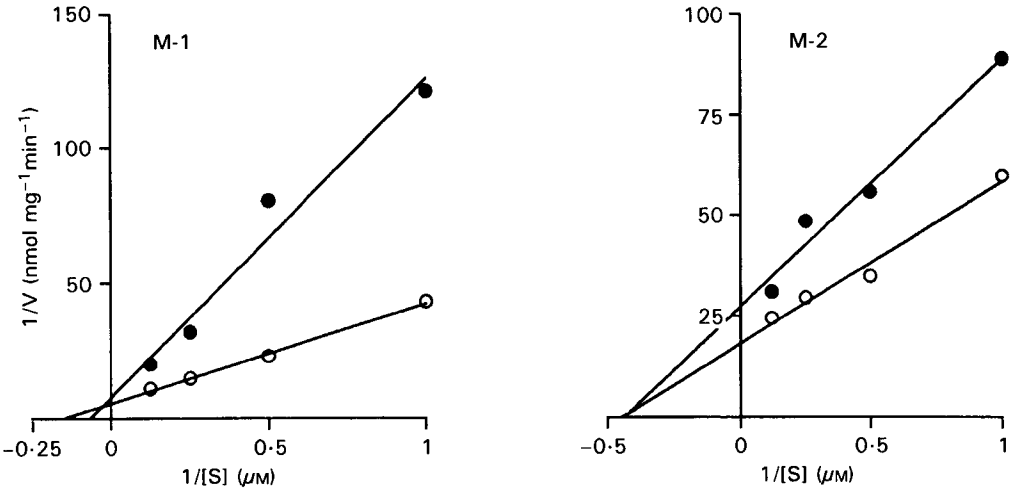

FIG. 3. Effect of erythromycin on the formation of nifedipine metabolites (M-1 and M-2) by rat intestinal microsomes. ○ Nifedipine alone, ● with erythromycin.

Table 3. Effect of erythromycin on the kinetic parameters of nifedipine oxidation by rat intestinal microsomes.

	Nifedipine		Metabolite M-2	
Parameter	Alone	With erythromycin	Nifedipine alone	With erythromycin
Michaelis–Menten constant (μM)	9·58 ± 2·16	29·4 ± 3·72	2·27 ± 0·25	3·43 ± 0·62
Maximum rate of metabolism (nmol mg^{-1} min^{-1})	0·24 ± 0·03	0·46 ± 0·23	0·06 ± 0·002	0·05 ± 0·003
Maximum rate of metabolism/Michaelis–Menten constant (mL mg^{-1} min^{-1})	0·03	0·02	0·03	0·01

Data are expressed as means ± s.e.m. (n = 3).

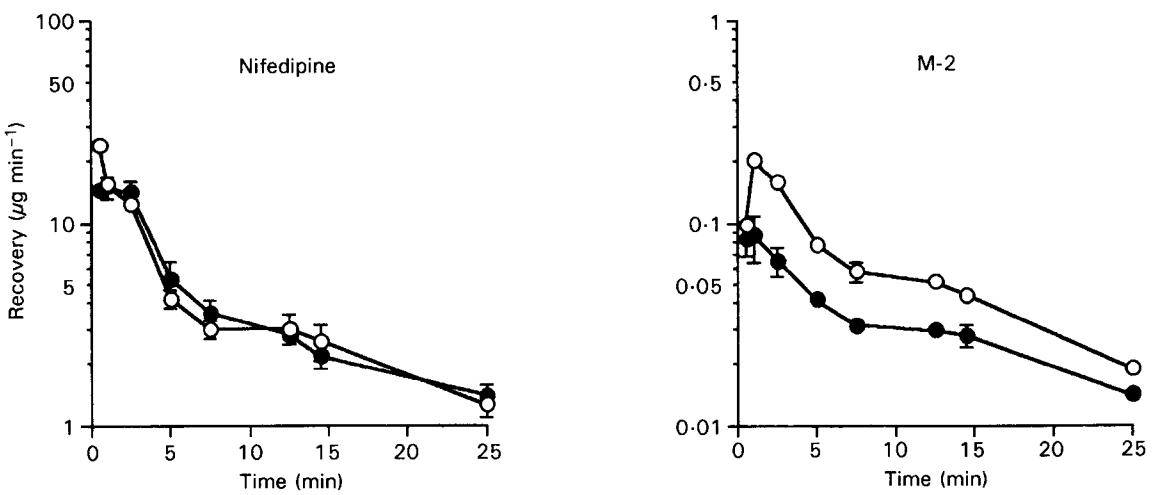

FIG. 4. Nifedipine and metabolite M-2 recovered in portal venous blood after intestinal administration of nifedipine (0·5 mg) after pre-treatment with multiple doses of erythromycin in rats. Data are expressed as the mean ± s.e.m. (n = 4). ○ Nifedipine alone, ● with multiple doses of erythromycin.

References

Bailey, D. G., Bend, J. B., Arnold, M. O., Tran, L. T., Spence, J. D. (1996) Erythromycin-felodipine interaction: magnitude, mechanism, and comparison with grapefruit juice. Clin. Pharmacol. Ther. 60: 25–33

Gomez, D. Y., Wacher, V. J., Tomlanovich, S. J., Hebert, M. F., Benet, L. Z. (1995) The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. Clin. Pharmacol. Ther. 58: 15–19

Guengerich, F. P., Martin, M. V., Beaune, P. H., Kremers, P., Wolff, T., Waxman, D. J. (1986) Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. J. Biol. Chem. 261: 5051–5060

Guengerich, F. P., Brian, W. R., Iwasaki, M., Sari, M. A., Baarnhielm, C., Berntsson, P. (1991) Oxidation of dihydropyridine calcium-channel blockers and analogues by human liver cytochrome P-450 3A4. J. Med. Chem. 34: 1838–1844

Kamataki, T., Sugiura, M., Yamazoe, Y., Kato, R. (1979) Purification and properties of cytochrome P-450 and NADPH-cytochrome c (P-450) reductase from human liver microsomes. Biochem. Pharmacol. 28: 1993–2000

Klaus, D. R., Johannes, S. (1983) Pharmacokinetics and metabolism of nifedipine. Hypertension 5 (Suppl. II): 18–24

Kolars, J. C., Awni, W. N., Merion, R. M., Watkins, P. B. (1991) First-pass metabolism of cyclosporine by the gut. Lancet 338: 1488–1490

Krishna, D. R., Klotz, U. (1994) Extrahepatic metabolism of drugs in humans. Clin. Pharmacokinet. 26: 144–160

Liedholm, H., Nordin, G. (1991) Erythromycin-felodipine interaction. DICP, Ann. Pharmacother. 25: 1007–1008

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Marre, F., Sousa, G., Orloff, A. M., Rahmani, R. (1993) In-vitro interaction between cyclosporin A and macrolide antibiotics. Br. J. Clin. Pharmacol. 35: 447–448

Periti, P., Mazzei, T., Mini, E., Novelli, A. (1992) Pharmacokinetic drug interaction of macrolides. Clin. Pharmacokinet. 23: 106–131

Rashid, T. J., Martin, U., Clarke, H., Waller, D. G., Renwick, A. G., George, C. F. (1995) Factors affecting the absolute bioavailability of nifedipine. Br. J. Clin. Pharmacol. 40: 51–58

Roosemalen, M. C. M., Soons, P. A., Funaki, T., Breimer, D. (1991) High-performance liquid chromatographic determination of the polar metabolites of nifedipine in plasma, blood and urine. J. Chromatogr. 565: 516–522

Schlanz, K. D., Myre, S. A., Bottorff, M. B. (1991a) Pharmacokinetic interactions with calcium channel antagonists (Part I). Clin. Pharmacokinet. 21: 344–356

Schlanz, K. D., Myre, S. A., Bottorff, M. B. (1991b) Pharmacokinetic interactions with calcium channel antagonists (Part II). Clin. Pharmacokinet. 21: 448–460

Tsuruta, S., Matsumura, K., Tsuruta, S., Imanura, K., Arimori, K., Nakano, M. (1995) The mechanism of pharmacokinetic interaction between nifedipine and quinidine. Jpn J. Clin. Pharmacol. Ther. 26: 793–801

Tsuruta, S., Nakamura, K., Arimori, K., Nakano, M. (1997) Effects of erythromycin, clarithromycin and rokitamycin on nifedipine metabolism in rats. Biol. Pharm. Bull. 20: 411–416

Wang, S. X., Sutfin, T. A., Baarnhielm, C., Regard, C. (1989) Contribution of the intestine to the first-pass metabolism of felodipine in the rat. J. Pharmacol. Exp. Ther. 250: 632–636

Yamaoka, N., Nakagawa, T., Uno, T. (1978) Statistical moments in pharmacokinetics. J. Pharmacokin. Biopharm. 6: 547–558