JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1403–1407

Received December 19, 2007
Accepted June 11, 2008
DOI 10.1211/jpp/60.10.0017
ISSN 0022-3573

Communication

Reduced prehepatic extraction of nicardipine in the presence of pioglitazone in rats

Jun-Shik Choi, Young-Youp Koh, Joong-Hwa Chung, Dong-Hyun Choi and Hyo-Kyung Han

Abstract

This study investigated the effect of pioglitazone on the pharmacokinetics of oral and i.v. nicardipine in rats. Pharmacokinetic parameters were determined after nicardipine was administered orally (12 mg kg^{-1}) or i.v. (4 mg kg^{-1}) with or without a single dose of oral pioglitazone (0.3 or 1.0 mg kg^{-1}). Compared with the control group given nicardipine alone, coadministration of pioglitazone significantly decreased the total plasma clearance of orally administered nicardipine (by 40.4–46.3%, $P < 0.05$) and significantly increased the area under the plasma concentration–time curve (by 81.8–96.3%) and the peak plasma concentration, C_{max} (by 56.5–66.8%). T_{max} and the terminal plasma half-life of nicardipine were not affected, however. Coadministration of oral pioglitazone did not affect the pharmacokinetics of i.v. nicardipine, implying that pioglitazone may mainly decrease the prehepatic extraction of nicardipine during intestinal absorption. In conclusion, pioglitazone significantly enhanced the oral bioavailability of nicardipine in rats by reducing its presystemic clearance.

Introduction

Nicardipine, a dihydropyridine calcium channel antagonist, exhibits highly potent coronary and peripheral vasodilation activity by blocking the influx of extracellular calcium across cell membranes (Sorkin & Clissold 1987; Pepine & Lambert 1990). Nicardipine is arterioselective and effective for the treatment of hypertension, myocardial ischaemia, and vasospasm in surgical patients (Kishi et al 1984; Tobias 1995).

Nicardipine is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration but its bioavailability is low because of a marked first-pass effect (Higuchi & Shiobara 1980). Nicardipine is metabolized mainly by cytochrome P450 (CYP) 2C8, CYP2D6 and CYP3A4, suggesting that the pharmacokinetic interactions of nicardipine with various substrates of these CYPs should be evaluated in-vivo (Nakamura et al 2005). In particular, as patients with hypertension frequently develop other conditions and often have to take several drugs, there is considerable potential for interactions. Indeed, there have been a number of reports of significant pharmacokinetic and pharmacodynamic drug interactions associated with calcium channel blockers (Rosenthal & Ezra 1995). Since drug–drug interactions may lead to a high risk of side-effects or reduced therapeutic effect, it is important to evaluate the potential for drug interactions in combination therapy.

Pioglitazone, an agonist at the peroxisome proliferator-activated receptor, is a novel hypoglycaemic agent for the treatment of type 2 diabetes (Lehmann et al 1995; Chilcott et al 2001). Pioglitazone is well absorbed and is mainly metabolized by CYP2C8 and CYP3A4 (Hanefeld 2001; Sahi et al 2003; Jaakkola et al 2006a). Previous reports have indicated pharmacokinetic interactions between pioglitazone and other drugs such as rifampicin and gemfibrozil (Deng et al 2005; Jaakkola et al 2006b). Jaakkola et al (2006b) reported that rifampicin substantially reduced the systemic exposure of pioglitazone, probably by inducing CYP2C8. Deng et al (2005) reported that gemfibrozil greatly increased the plasma concentration of pioglitazone by inhibition of its metabolism. Given that both pioglitazone and nicardipine can interact with CYP3A4 and CYP2C8, there is a high chance for a drug interaction between these two drugs; however, the effects of pioglitazone on the pharmacokinetics of nicardipine have not been reported. The aim of this study was to investigate the effect of pioglitazone on the pharmacokinetics of oral and i.v. nicardipine in rats.

College of Medicine, Chosun University, 375 Seosuk-dong, Dong-Gu, Gwangju, Korea

Young-Youp Koh, Joong-Hwa Chung, Dong-Hyun Choi

BK21 project team, College of Pharmacy, Chosun University, 375 Seosuk-dong, Dong-Gu, Gwangju, Korea

Jun-Shik Choi, Hyo-Kyung Han

Correspondence: Hyo-Kyung Han PhD, College of Pharmacy, Chosun University, 375 Seosuk-dong, Dong-Gu, Gwangju, Korea.
E-mail: hkhan@chosun.ac.kr

Materials and Methods

Materials

Nicardipine, nimodipine and pioglitazone were purchased from Sigma Chemical Co. (St Louis, MO, USA). HPLC-grade acetonitrile and hexane were purchased from Merck (Darmstadt, Germany). All other chemicals were of reagent grade and were used without further purification.

Animal studies

Male Sprague–Dawley rats, 7–8 weeks old and weighing 270–300 g, were purchased from the Dae Han Laboratory Animal Research Company (Choongbuk, Korea). Standard rat chow diet (No. 322-7-1) was purchased from Superfeed Company (Gangwon, Korea).

Animals had free access to commercial rat chow and tap water. They were maintained at a temperature of 22 ± 2°C with a 12 h light–dark cycle and a relative humidity of 50–60%. The rats were acclimated to these conditions for at least 1 week before the experiment. Rats were fasted for at least 18 h before the experiment but were allowed free access to tap water.

On the day of experiment, the rats were divided into six groups of six. Three groups received oral nicardipine (12 mg kg^{-1}) alone (control) or with 0.3 or 1.0 mg kg^{-1} oral pioglitazone. The other three groups received an i.v. dose of nicardipine (4 mg kg^{-1}), alone (control) or with 0.3 and 1.0 mg kg^{-1} oral pioglitazone. Pioglitazone was administered 30 min before nicardipine.

Blood samples were collected via the femoral artery at 0, 0.017, 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h for i.v. studies, and at 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h for oral studies. Blood samples were centrifuged at 13 000 rev min^{-1} for 5 min and the plasma separated and stored at −40°C until analysis.

HPLC assay

Plasma concentrations of nicardipine were determined by the HPLC assay reported by Eastwood et al (1990) with a slight modification. Briefly, 50 μL 2 μg mL^{-1} nimodipine as an internal standard, 20 μL 2 M sodium hydroxide solution and 1.2 mL tert-butylmethylether:hexane (75:25 v/v) were added to 0.2 mL of the plasma sample. The mixture was then stirred for 2 min and centrifuged at 13 000 rev min^{-1} for 10 min. One mL of the organic layer was transferred to a clean test tube and evaporated at 35°C under a stream of nitrogen. The residue was dissolved in 200 μL mobile phase and centrifuged at 13 000 rev min^{-1} for 5 min. A 50 μL sample of the supernatant was injected onto the HPLC system, which consisted of two solvent delivery pumps (Model LC-10AD), a UV detector, a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD) (Shimadzu Co., Tokyo, Japan). Chromatographic separations were achieved using a Symmetry C_{18} column (4.6 × 150 mm, 5 μm, Waters Corp., Milford, MA, USA) and a μBondapak C_{18} HPLC precolumn (10 μm, Waters Corp.). The mobile phase was acetonitrile:0.015 KH_2PO_4 (60:40, v/v, pH 4.5) with 2.8 mM triethylamine, delivered at a flow rate of 1.5 mL min^{-1}. Chromatography was performed at 30°C, set by the HPLC column temperature controller. Detection was at 254 nm. The retention times of nicardipine and the internal standard were 7.8 and 4.2 min, respectively. The intra- (n = 5) and interday (n = 5) coefficients of variation were less than 15%. The detection limit of nicardipine in rat plasma was 10 ng mL^{-1}.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using WinNonlin version 5.2 software (Pharsight Corporation, Mountain View, CA, USA). The elimination rate constant (K_{el}) was estimated from the slope of the terminal phase of the log plasma concentration–time profile fitted by the method of least squares; the terminal half-life ($t_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach C_{max} (T_{max}) of nicardipine in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration–time curve (AUC) from time zero to the time of last measured concentration (C_{last}) (AUC_{0-t}) was calculated by the linear trapezoidal rule. The AUC from time zero to infinity ($AUC_{0-\infty}$) was determined from the AUC_{0-t} plus the extrapolated area determined by C_{last}/K_{el}. Total plasma clearance (CL) was calculated from dose/AUC. The relative bioavailability (RB) of nicardipine was estimated from the ratio of AUCs in the presence and absence of pioglitazone, multiplied by 100.

Statistical analysis

Data are presented as mean ± s.d. Statistical analysis was conducted using one-way analysis of variance followed by a-posteriori testing with Dunnett's correction. Differences were considered to be significant at a level of $P < 0.05$.

Results and Discussion

The mean plasma concentration–time profiles of nicardipine following oral administration to rats in the presence and the absence of pioglitazone are shown in Figure 1. The mean pharmacokinetic parameters of nicardipine were summarized in Table 1. As shown in Figure 1, coadministration of a single oral dose of pioglitazone (0.3 or 1.0 mg kg^{-1}) significantly enhanced the oral exposure of nicardipine in rats compared with the control group given nicardipine alone but there was no significant change in T_{max} or $t_{1/2}$ of nicardipine. The AUC of nicardipine increased by 81.8–96.3% and C_{max} by 56.5–66.8% in the presence of pioglitazone. Consequently, the RB of nicardipine increased by 1.81–1.96-fold with the co-administration of pioglitazone. Moreover, pioglitazone (0.3 and 1.0 mg kg^{-1}) significantly decreased the total plasma clearance (CL/F) of nicardipine by 40.4–46.3%, which could lead to the enhanced exposure to nicardipine in the presence of pioglitazone.

The i.v. pharmacokinetic profiles of nicardipine in the presence and the absence of pioglitazone are shown in Figure 2 and the pharmacokinetic parameters are summarized in

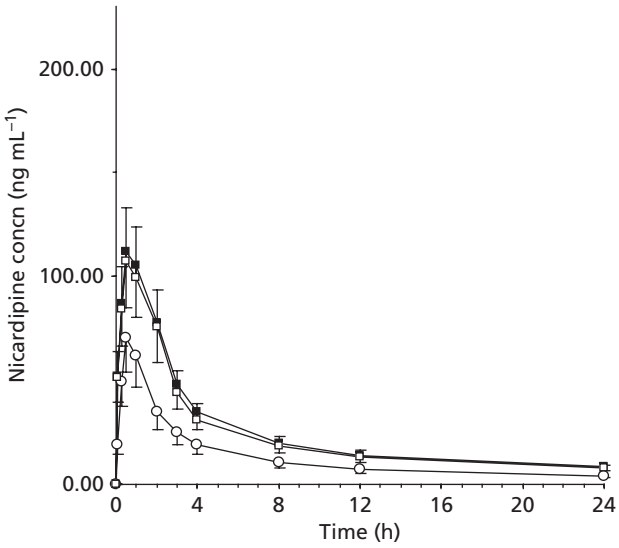

Figure 1 Mean plasma concentration–time profiles of nicardipine after oral administration (12 mg kg^{-1}) to rats alone (open circles) and in the presence of pioglitazone 0.3 mg kg^{-1} (filled squares) or 1.0 mg kg^{-1} (open squares). Data are mean ± s.d. (n = 6).

Table 2. Pioglitazone had no effect on the pharmacokinetic profiles of i.v. nicardipine, although it had a significant effect on the bioavailability of orally administered nicardipine, suggesting that pioglitazone reduces the intestinal extraction of nicardipine rather than hepatic extraction.

There is a growing body of evidence that CYPs in enterocytes contribute significantly to first-pass metabolism and the oral bioavailability of various drugs. For instance, fentanyl undergoes substantial metabolism in the small intestinal relative to that in the liver (Labroo et al 1997), and 50% of orally administered ciclosporin is metabolized in the small intestine (Hebert 1997). The predominant CYP in the intestine is CYP3A4; alterations in the expression and/or activity of intestinal CYP3A4 may lead to significant drug–drug interactions (de Waziers et al 1990; Kaminsky & Fasco 1991). Gut-wall enzymes therefore represent an important site for drug interactions after oral administration of CYP3A4 substrates, as exemplified by the pharmacokinetic interaction between grapefruit juice and ciclosporin (Ducharme et al 1995).

Nicardipine is also a substrate of CYP3A4 and thus the inhibition of gastrointestinal CYP3A4 by concomitantly administered drugs could significantly alter the pharmacokinetics of nicardipine following oral administration. This is well supported by the findings from our present study as well as previous reports (Uno et al 2000; Kubota et al 2003). Kubota et al (2003) reported that oral administration of *Ginkgo biloba* extract significantly increased hepatic CYP content and significantly decreased both the systemic exposure to nicardipine and its hypotensive effects in rats. Uno et al (2000) assessed the relative role of the intestinal and hepatic metabolism of nicardipine during first-pass extraction with and without intake of grapefruit juice. In their studies, grapefruit juice significantly increased the mean oral bioavailability of nicardipine and the available fraction of the dose absorbed unmetabolized at the gut whereas the pharmacokinetic parameters of nicardipine after i.v. administration were not affected by intake of grapefruit juice. Thus, their studies indicated that the gut was the major presystemic extraction site of nicardipine in humans (Uno et al 2000). In parallel, the present study also demonstrated that pioglitazone, a substrate of CYP2C8 and CYP3A4, significantly enhanced the oral exposure of nicardipine without changing its systemic clearance, suggesting that pioglitazone may effectively reduce the prehepatic extraction of nicardipine. However, this result seems to contradict previous reports by Kajosaari et al (2006) but support those of others (Uno et al 2000; Kubota et al 2003). Kajosaari et al (2006) reported that pioglitazone did not increase the plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide. The explanation for this discrepancy is not yet clear. Given that (i) the gut is the major presystemic extraction site of nicardipine (Uno et al 2000), and (ii) pioglitazone is well absorbed across the gut wall but shows extensive plasma protein binding (Krietter et al 1994; Hanefeld 2001), the concentration of pioglitazone available to CYP enzymes should be much higher in enterocytes than in hepatocytes. Thus, pioglitazone may have a more profound effect on drugs undergoing substantial intestinal metabolism such as nicardipine. Furthermore, considering that only very low amounts of CYP2C proteins are detected in duodenum while CYP3A4 is highly expressed in the small intestine (de Waziers et al 1990; Kaminsky & Fasco 1991), pioglitazone may particularly affect the intestinal CYP3A4-mediated metabolism of nicardipine in rats.

Table 1 Pharmacokinetic parameters of nicardipine after oral administration (12 mg kg^{-1}) to rats in the presence and the absence of oral pioglitazone (0.3 or 1.0 mg kg^{-1})

	Nicardipine alone	**Nicardipine + pioglitazone**	
		0.3 mg kg^{-1}	1.0 mg kg^{-1}
$AUC_{0-\infty}$ (ng h mL^{-1})	374 ± 68.7	735 ± 237*	681 ± 257*
C_{max} (ng mL^{-1})	72.6 ± 16.9	121 ± 45.4*	114 ± 30.8*
T_{max} (h)	0.6	0.5	0.6
CL/F (mL min^{-1} kg^{-1})	550 ± 97.3	296 ± 88.0*	328 ± 113*
$t_{1/2}$ (h)	9.5 ± 1.8	11 ± 3.1	10 ± 1.6
RB (%)	100	196	181

$AUC_{0-\infty}$, area under the plasma–concentration time curve from time zero to infinity; C_{max}, maximum plasma concentration; T_{max}, time of C_{max}; CL/F, total plasma clearance; $t_{1/2}$, elimination half-life; RB, relative bioavailability. Data are mean ± s.d. (n = 6). *$P < 0.05$ vs nicardipine alone.

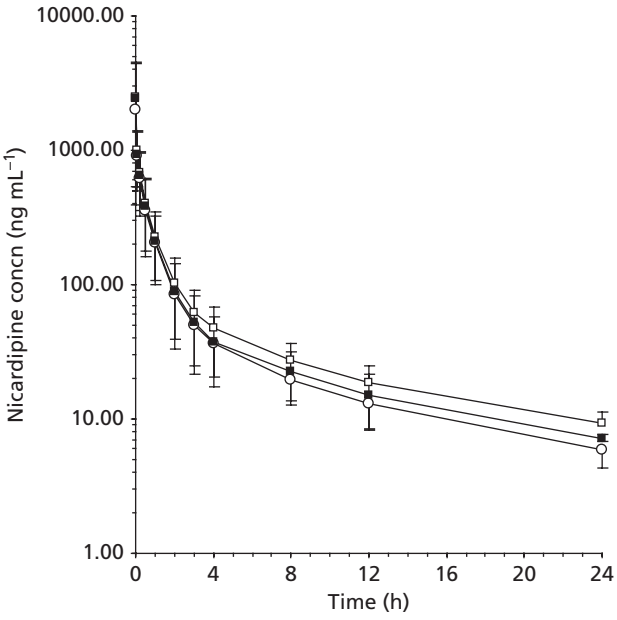

Figure 2 Mean plasma concentration–time profiles of nicardipine after i.v. administration (4 mg kg^{-1}) to rats, alone (open circles) and in the presence of pioglitazone 0.3 mg kg^{-1} (open squares) or 1.0 mg kg^{-1} (filled squares). Data are mean ± s.d. (n = 6).

Table 2 Pharmacokinetic parameters of nicardipine after i.v. (4 mg kg^{-1}) to rats in the presence and the absence of oral pioglitazone (0.3 or 1.0 mg kg^{-1})

	Nicardipine alone	Nicardipine + pioglitazone	
		0.3 mg kg^{-1}	1.0 mg kg^{-1}
$AUC_{0-\infty}$ (ng h mL^{-1})	1150 ± 360	1430 ± 303	1250 ± 263
C_{max} (ng mL^{-1})	64.0 ± 24.5	48.6 ± 10.8	55.6 ± 12.9
T_{max} (h)	8.1 ± 2.8	8.8 ± 1.5	8.8 ± 1.0

$AUC_{0-\infty}$, area under the plasma–concentration time curve from time zero to infinity; C_{max}, maximum plasma concentration; T_{max}, time of C_{max}. Data are mean ± s.d. (n = 6).

Although nicardipine is a substrate of P-glycoprotein (P-gp), the pharmacokinetic interaction observed in the present study is unlikely to be due to inhibition of P-gp. Nicardipine is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration (Higuchi & Shiobara, 1980; Delchier et al 1988). Also, in Caco-2 cells, the basolateral-to-apical and apical-to-basolateral permeability ratios for nicardipine were close to unity, and were not affected by the addition of a P-gp inhibitor (Lentz et al 2000). Thus, given the quite high passive permeability of nicardipine, intestinal efflux transporters probably do not affect the intestinal absorption of nicardipine.

Taken together, the data indicate that the oral pharmacokinetics of nicardipine could be altered by the concomitant use of pioglitazone via inhibition of prehepatic extraction. The present study indicates a potential interaction between nicardipine and pioglitazone, the clinical significance of which requires further evaluation in clinical studies.

Conclusion

Pretreatment with a single dose of oral pioglitazone significantly enhanced the oral bioavailability of nicardipine in rats but did not affect the i.v. pharmacokinetics of nicardipine.

References

Chilcott, J., Tappenden, P., Jones, M. L., Wight, J. P. (2001) A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus. *Clin. Ther.* **23**: 1792–1823

Delchier, J. C., Guerret, M., Vidon, N., Dubray, C., Lavene, D. (1988) Influence of digestive secretions and food on intestinal absorption of nicardipine. *Eur. J. Clin. Pharmacol.* **34**: 165–171

Deng, L. J., Wang, F., Li, H. D. (2005) Effect of gemfibrozil on the pharmacokinetics of pioglitazone. *Eur. J. Clin. Pharmacol.* **61**: 831–836

de Waziers, I., Cugnenc, P. H., Yang, C. S., Leroux, J. P., Beaune, P. H. (1990) Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther.* **253**: 387–394

Ducharme, M. P., Warbasse, L. H., Edwards, D. J. (1995) Disposition of intravenous and oral cyclosporine after administration with grapefruit juice. *Clin. Pharmacol. Ther.* **57**: 485–491

Eastwood, R. J., Galustian, C., Bhamra, R. K., Holt, D. W. (1990) High-performance liquid chromatographic method for the measurement of nicardipine in plasma or serum. *J. Chromatogr.* **530**: 463–468

Hanefeld, M. (2001) Pharmacokinetics and clinical efficacy of pioglitazone. *Int. J. Clin. Pract.* **121**(Suppl.): 19–25

Hebert, M. F. (1997) Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv. Drug Deliv. Rev.* **27**: 201–214

Higuchi, S., Shiobara, Y. (1980) Comparative pharmacokinetics of nicardipine hydrochloride, a new vasodilator, in various species. *Xenobiotica* **10**: 447–454

Jaakkola, T., Laitila, J., Neuvonen, P. J., Backman, J. T. (2006a) Pioglitazone is metabolized by CYP2C8 and CYP3A4 in vitro: potential for interactions with CYP2C8 inhibitors. *Basic Clin. Pharmacol. Toxicol.* **99**: 44–51

Jaakkola, T., Backman, J. T., Neuvonen, M., Laitila, J., Neuvonen, P. J. (2006b) Effect of rifampicin on the pharmacokinetics of pioglitazone. *Br. J. Clin. Pharmacol.* **61**: 70–78

Kajosaari, L. I., Jaakkola, T., Neuvonen, P. J., Backman, J. T. (2006) Pioglitazone, an in vitro inhibitor of CYP2C8 and CYP3A4, does not increase the plasma concentrations of the CYP2C8 and CYP3A4 substrate repaglinide. *Eur. J. Clin. Pharmacol.* **62**: 217–223

Kaminsky, L. S., Fasco, M. J. (1991) Small intestinal cytochromes P450. *Crit. Rev. Toxicol.* **21**: 407–422

Kishi, Y., Okumura, F., Furuya, H. (1984) Haemodynamic effects of nicardipine hydrochloride: studies during its use to control acute hypertension in anaesthetized patients. *Br. J. Anaesth.* **56**: 1003–1007

Krietter, P. A., Colletti, A. E., Doss, G. A., Miller, R. R. (1994) Disposition and metabolism of the hypoglycemic agent pioglitazone in rats. *Drug Metab. Dispos.* **22**: 625–630

Kubota, Y., Kobayashi, K., Tanaka, N., Nakamura, K., Kunitomo, M., Umegaki, K., Shinozuka, K. (2003) Interaction of Ginkgo biloba extract (GBE) with hypotensive agent, nicardipine, in rats. *In Vivo* **17**: 409–412

Labroo, R. B., Paine, M. F., Thummel, K. E., Kharasch, E. D. (1997) Fentanyl metabolism by human hepatic and intestinal cytochrome P450 3A4: implications for interindividual variability in disposition, efficacy, and drug interactions. *Drug Metab. Dispos.* **25**: 1072–1080

Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., Kliewer, S. A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.* **270**: 12953–12956

Lentz, K. A., Polli, J. W., Wring, S. A., Humphreys, J. E., Polli, J. E. (2000) Influence of passive permeability on apparent P-glycoprotein kinetics. *Pharm. Res.* **17**: 1456–1460

Nakamura, K., Ariyoshi, N., Iwatsubo, T., Fukunaga, Y., Higuchi, S., Itoh, K., Shimag, N., Nagashima, K., Yokoi, T., Yamamoto, K., Horiuchi, R., Kamataki, T. (2005) Inhibitory effects of nicardipine to cytochrome P450 (CYP) in human liver microsomes. *Biol. Pharm. Bull.* **28**: 882–885

Pepine, C. J., Lambert, C. R. (1990) Cardiovascular effects of nicardipine. *Angiology* **41**: 978–986

Rosenthal, T., Ezra, D. (1995) Calcium antagonists. Drug interactions of clinical significance. *Drug Saf.* **13**: 157–187

Sahi, J., Black, C. B., Hamilton, G. A., Zheng, X., Jolley, S., Rose, K. A., Gilbert, D., LeCluyse, E. L., Sinz, M. W. (2003) Comparative effects of thiazolidinediones on in vitro P450 enzyme induction and inhibition. *Drug Metab. Dispos.* **31**: 439–446

Sorkin, E. M., Clissold, S. P. (1987) Nicardipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in the treatment of angina pectoris, hypertension and related cardiovascular disorders. *Drugs* **33**: 296–345

Tobias, J. D. (1995) Nicardipine: applications in anesthesia practice. *J. Clin. Anesth.* **7**: 525–533

Uno, T., Ohkubo, T., Sugawara, T., Higashiyama, A., Motomura, S., Ishizaki, T. (2000) Effects of grapefruit juice on the stereoselective disposition of nicardipine in humans: evidence for dominant presystemic elimination at the gut site. *Eur. J. Clin. Pharmacol.* **56**: 643–649