JPP Journal of Pharmacy and Pharmacology

JPP 2002, 54: 821–825

Received November 19, 2001
Accepted February 15, 2002
ISSN 0022-3573

Influence of nicardipine and nifedipine on plasma carvedilol disposition after oral administration in rats

Nobuo Hokama, Norio Hobara, Masayuki Sakai, Hiromasa Kameya, Susumu Ohshiro and Matao Sakanashi

Abstract

The effect of two kinds of 1,4-dihydropyridine calcium-channel blockers, nicardipine hydrochloride and nifedipine, on the disposition of carvedilol, was studied in rats. Blood samples were assayed for carvedilol levels using solid-phase extraction and high-performance liquid chromatography. The plasma carvedilol concentration was found to be significantly higher, and the area under the concentration–time curve up to 24 h ($AUC_{0\rightarrow 24}$) was 6.7 and 3.0 times higher after simultaneous oral administration of 20 mg kg^{-1} carvedilol with 40 mg kg^{-1} nicardipine hydrochloride, or with 40 mg kg^{-1} nifedipine, respectively, than after administration of carvedilol alone. The pharmacokinetic interaction between carvedilol and dihydropyridine calcium-channel blockers is thought to be attributable to vasodilator-induced changes in hepatic first-pass metabolism, inhibition in the absorption barrier by P-glycoprotein and in the metabolism of carvedilol.

Introduction

Carvedilol, (±)-1-(carbazol-4-yloxy)-3-{[2-(o-methoxyphenoxy) ethyl] amino}-2-2-propanol is a nonselective β-blocking agent with vasodilating properties that are attributed mainly to its blocking activity on α_1-receptors (Sponer et al 1987; Ruffolo et al 1990). It undergoes a first-pass effect with hepatic elimination and has an absolute bioavailability of about 25%. Dihydropyridine calcium-channel blockers (Ca-channel blockers) lower blood pressure by a selective action on vascular smooth muscle in the resistance vessels. Clinically the combination of a Ca-channel blocker and a β-blocker is very effective in the treatment of hypertension (Hansson et al 1985).

Pharmacokinetic interaction between these drugs is possible, however, since both are high-clearance drugs dependent on liver metabolism and blood flow. Furthermore, recent studies suggest that the combination of a lipophilic β-blocker with a Ca-channel blocker results in pharmacokinetic interactions (McLean et al 1985). A pharmacokinetic interaction between carvedilol and Ca-channel blockers has not been reported, therefore we investigated the possibility of pharmacokinetic interaction between carvedilol and nicardipine hydrochloride or nifedipine in rats.

Department of Hospital Pharmacy, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207, Aza Uehara, Nishihara-cho, Okinawa 903-0215, Japan

Nobuo Hokama, Norio Hobara, Masayuki Sakai, Hiromasa Kameya, Susumu Ohshiro

Department of Pharmacology, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207, Aza Uehara, Nishihara-cho, Okinawa 903-0215, Japan

Matao Sakanashi

Correspondence: N Hokama, Department of Hospital Pharmacy, School of Medicine, Faculty of Medicine, University of the Ryukyus, 207, Aza Uehara, Nishihara-cho, Okinawa 903-0215, Japan.

Materials and Methods

Chemicals

Carvedilol (lot no. 44857800) was kindly donated by Daiichi Pharmaceutical Co. Ltd (Japan). Two kinds of Ca-channel blockers, nicardipine hydrochloride (Perdipine tablets; Yamanouchi Pharmaceutical Co. Ltd) and nifedipine (Adalat-L10; Bayer Yakuhin, Ltd) were purchased for the preparations. Ethyl *p*-hydroxybenzoate was used as the internal standard. Methanol and distilled water were used for high-performance liquid chromatography (HPLC), and other reagents were of analytical grade.

Animals

Male Sprague–Dawley rats (260–311 g, n = 18) were housed three or four to a plastic-walled cage (26 × 36 × 25 cm), and had unlimited access to food and water except for 12 h before and during the experiment. The ambient temperature and humidity were kept at 23–25°C and 55 ± 15%, respectively. The rats were maintained on a 12-h light–dark cycle (light on at 0800 h).

Nicardipine hydrochloride and nifedipine were administered to the rats at a dose of 40 mg kg^{-1} in 0.5% sodium carboxymethylcellulose (CMC-Na) suspension through a nasal catheter. CMC-Na 0.5% suspension was administered to separate groups of rats as control. Immediately after administration of Ca-channel blockers and 0.5% CMC-Na suspension, carvedilol was administered to the rats at a dose of 20 mg kg^{-1}. The rats were anaesthetized with diethyl ether before oral administration. To determine the concentration–time profile of plasma carvedilol, ca. 60-μL blood samples were obtained from the tail vein at 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after oral administration of carvedilol. For plasma separation from the blood sample, a haematocrit-centrifuge (himac CT12, HITACHI) was used.

The animals used in this study were handled in accordance with the Guidelines for Animal Experimentation of the University of the Ryukyus, and the experimental protocol was approved by the Animal Care and Use Committee of this institution.

Detection of carvedilol

For the extraction of carvedilol and internal standard, a Bond Elut (1 mL volume, lot no. 062217, Varian) solid-phase extraction column containing octylsilica was used. The column was pre-washed twice with 1 mL of methanol, followed by washings with 1 mL of distilled water. Then 500 μL of 20 mM KH_2PO_4 (pH 4.0), 20 μL of plasma containing carvedilol and 15 μL of 100 μg mL^{-1} internal standard solution were added to the column. Distilled water (0.5 mL) followed by 0.5 mL of 20 mM KH_2PO_4 (pH 2.5) were passed through the column. Carvedilol and the internal standard retained on the column were eluted with 500 μL of elution solvent (methanol–20 mM KH_2PO_4 pH 2.5, 90:10). Twenty microlitres of this solution was injected into the HPLC column.

Plasma carvedilol concentrations were determined by HPLC (pump-type LC-6A, Shimadzu) with a spectrofluorometric detector (Type RF-530, Shimadzu), and were calculated using a data module (Type C-R3A, Shimadzu). A stainless-steel column packed with octadecyl silica (Shim-pack CLC-ODS, 150 mm × 6.0 mm i.d., 5 μm particle size, Shimadzu) was maintained at 40°C. The mobile phase was methanol–50 mM KH_2PO_4 pH 2.5, 60:40 v/v, and the flow-rate was 1.0 mL min^{-1}. Measurements were made at an excitation wavelength of 247 nm and an emission wavelength of 344 nm. The analytical mean recovery of carvedilol averaged 94.2%. The detection limit was 3.63 ng mL^{-1} in the plasma. The coefficients of variation of the mean reproducibility were 2.70–7.51% for the within-day assay, and 5.01% for the between-day assay (7 days), indicating that the analytical method was effective for the determination of plasma carvedilol levels (Hokama et al 1999).

Analysis of data

The maximum plasma drug concentrations (C_{max}) and the time to reach maximum concentrations (T_{max}) were determined. The area under the concentration–time curve up to 24 h ($AUC_{0\rightarrow 24}$) was calculated using the trapezoidal rule. Plasma half-life ($t_{1/2}$) was determined by linear regression using the log plasma concentration measured at 2, 3, 4, 8, 12 and 24 h after oral administration. The Mann–Whitney U-test was used to test for drug interactions. Assessment of statistical significance was based on the conventional level of $P < 0.05$. All results are given as mean ± s.d.

Results

Figure 1 shows the mean plasma concentrations of carvedilol when given alone and in combination with nicardipine hydrochloride in the rats. The plasma carvedilol concentrations were significantly higher at

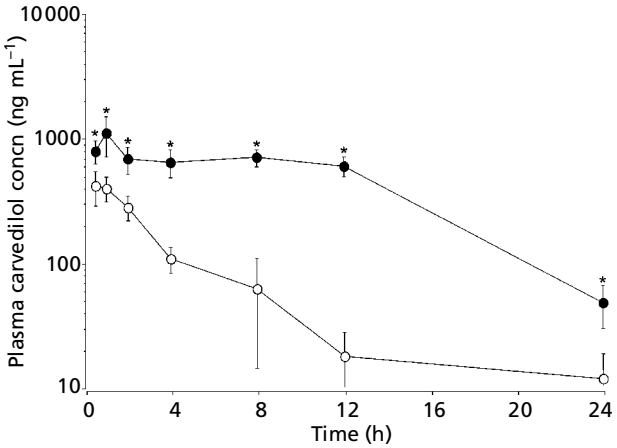

Figure 1 Plots of average plasma carvedilol concentration against time after oral administration of 20 mg kg^{-1} carvedilol to rats in the presence (●) or absence (○) of 40 mg kg^{-1} nicardipine hydrochloride. Values are the means±s.d. of results from 4 or 5 rats. *$P < 0.05$ vs absence of nicardipine.

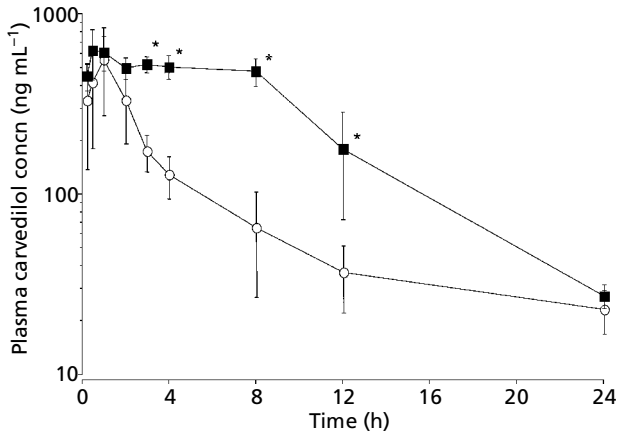

Figure 2 Plots of average plasma carvedilol concentration against time after oral administration of 20 mg kg^{-1} carvedilol to rats in the presence (■) or absence (○) of 40 mg kg^{-1} nifedipine. Values are the means±s.d. of results from 4 or 5 rats. *$P < 0.05$ vs absence of nifedipine.

30 min ~ 24 h after simultaneous oral administration of carvedilol with nicardipine than after carvedilol alone. The mean pharmacokinetic parameters are given in Table 1. The disposition of carvedilol is adequately described by a two-compartment model (Hokama et al 1999). When carvedilol was given in combination with nicardipine, C_{max} and $AUC_{0\rightarrow 24}$ significantly increased compared with carvedilol alone. No significant difference in either T_{max} or $t_{1/2}\alpha$ was detected.

Figure 2 shows the mean plasma concentrations of carvedilol when given alone and in combination with nifedipine in the rats. The plasma carvedilol concentrations were significantly higher than the control levels at 3–12 h after simultaneous oral administration of carvedilol with nifedipine. The values of T_{max}, C_{max}, $t_{1/2}$ and $AUC_{0\rightarrow 24}$ are shown in Table 2. The carvedilol $AUC_{0\rightarrow 24}$ increased when nifedipine was co-administered. There was no significant change in T_{max} or $t_{1/2}\alpha$. The $t_{1/2}\beta$ in combination with Ca-channel blockers could not be determined because of enterohepatic circulation (Fujimaki & Hakusui 1989).

Discussion

It has been reported that there is a little pharmacokinetic interaction between β-blocking agents and dihydropyridine Ca-channel blockers (Smith et al 1987; Elliot et al 1991). In this experiment, the results showed that the plasma carvedilol concentrations and $AUC_{0\rightarrow 24}$ for carvedilol were significantly higher after simultaneous oral administration of 20 mg kg^{-1} carvedilol with 40 mg kg^{-1} nicardipine hydrochloride, and with 40 mg kg^{-1} nifedipine than after carvedilol alone. This suggested that Ca-

Table 1 Carvedilol pharmacokinetic parameters after oral administration of carvedilol with or without nicardipine hydrochloride to rats.

Treatment	n	T_{max} (h)	C_{max} (ng mL^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0\rightarrow 24}$ (μg h mL^{-1})
Carvedilol	5	0.80±0.27	435.3±126.2	1.93±0.39	41.7±31.3	1.82±0.29
Carvedilol with nicardipine	4	0.90±0.22	1120.0±400.4*	2.00±1.38		12.13±1.05*

Carvedilol 20 mg kg^{-1} in 0.5% CMC-Na suspension or carvedilol 20 mg kg^{-1} with nicardipine 40 mg kg^{-1} in 0.5% CMC-Na suspension was administered via nasal catheter to rats. Data are mean values±s.d. T_{max}, time to reach C_{max}; C_{max}, maximum plasma concentration; $t_{1/2}\alpha$, apparent elimination half-life in distribution phase; $t_{1/2}\beta$ apparent elimination half-life in elimination phase; AUC, area under the plasma concentration–time curve. *$P < 0.05$ vs carvedilol alone.

Table 2 Carvedilol pharmacokinetic parameters after oral administration of carvedilol with or without nifedipine to rats.

Treatment	n	T_{max} (h)	C_{max} (ng mL^{-1})	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	$AUC_{0\rightarrow 24}$ (μg h mL^{-1})
Carvedilol	5	0.90±0.22	554.8±281.1	2.05±1.05	20.9±7.4	2.15±0.72
Carvedilol with nifedipine	4	0.75±0.29	675.1±175.1	2.96±1.26		6.53±1.41*

Carvedilol 20 mg kg^{-1} in 0.5% CMC-Na suspension or carvedilol 20 mg kg^{-1} with nifedipine 40 mg kg^{-1} in 0.5% CMC-Na suspension was administered via nasal catheter to rats. Data are mean values±s.d. T_{max}, time to reach C_{max}; C_{max}, maximum plasma concentration; $t_{1/2}\alpha$, apparent elimination half-life in distribution phase; $t_{1/2}\beta$, apparent elimination half-life in elimination phase; AUC, area under the plasma concentration–time curve. *$P < 0.05$ vs carvedilol alone.

channel blockers increase the systemic bioavailability of carvedilol.

The mechanism of this interaction is not completely understood. Another vasodilator, hydralazine, increases the plasma concentrations of both propranolol (McLean et al 1980) and metoprolol (Jack et al 1982). Also lipophilic basic drugs such as carvedilol undergo extensive first-pass metabolism after oral administration (Möllendoff et al 1987). Clearly, it is possible that any Ca-channel blocker which acts as a vasodilator may influence liver blood flow. Therefore, the explanation for the increased plasma concentrations of carvedilol in combination with Ca-channel blocker may be a reduction in first-pass loss.

However, dihydropyridine Ca-channel blockers have been shown to inhibit cytochrome P450-mediated metabolism both in-vitro and in-vivo (Drobitch et al 1997), therefore they have the potential to inhibit the metabolism of a large number of drugs. Recently, it has been reported that carvedilol is metabolized in human liver microsomes by the P450s CYP2D6, CYP1A2, CYP2E1, CYP2C9 and CYP3A4 (Oldham & Clarke 1997). On the other hand, dihydropyridine Ca-channel blockers are metabolized by the human liver and are a typical substrate of CYP3A4. The explanation for the increased plasma concentrations of carvedilol in combination with Ca-channel blockers may thus also be an inhibition of the metabolism of carvedilol.

Moreover, it is generally known that the Ca-channel blockers (e.g. nifedipine) inhibit P-glycoprotein. P-glycoprotein is considered to play a role as an absorption barrier by transporting several drugs from intestinal cells into the lumen, and it has been reported that some β-blockers are transported out of cells by P-glycoprotein (Terao et al 1996). Although there is no evidence that carvedilol is transported out of cells by P-glycoprotein, possibly the increased plasma concentrations of carvedilol in combination with Ca-channel blockers may also be due to an inhibition in the absorption barrier of carvedilol by the P-glycoprotein. The mechanisms of this interaction are not known and could include a change in hepatic blood flow or change in the activity of metabolic enzymes and absorption barrier by the P-glycoprotein. Thus the pharmacokinetic interaction between Ca-channel blockers and carvedilol could be complex.

It has been reported (Hakusui et al 1989) that the pharmacokinetic parameters of carvedilol, T_{max}, C_{max}, $AUC_{0\rightarrow 72}$ and $t_{1/2}\beta$ were 1 h, 713 ng mL^{-1}, 7.57 μg h mL^{-1} and 29.8 h, respectively, after administration of 10 mg kg^{-1} carvedilol in the rat. These results are comparable with the pharmacokinetic parameters obtained in this study. The doses of each therapeutic agent employed in this study were set in consideration of the dosage used in animal experiments when each therapeutic agent was being developed as a medicine. For example, in an investigation of the effect of carvedilol on the mean blood pressure in spontaneously hypertensive rats (SHR), carvedilol was administered orally at 10–30 mg kg^{-1} (Hashimoto et al 1991), and in an examination of the effect of nicardipine on mean blood pressure in SHR, nicardipine was administered orally at 3–30 mg kg^{-1} (Richer et al 1985; Takenaka et al 1985).

Moreover, a two-phasic peak appeared in the plasma carvedilol concentration–time curve when carvedilol was given in combination with nicardipine and nifedipine. However, this peak was not observed in the group given carvedilol alone. Biliary excretion of carvedilol amounted to 84.9% (Fujimaki & Hakusui 1989) of the orally administered amount. Enterohepatic circulation (Fujimaki & Hakusui 1989) was taking place, and 40% of the amount excreted in bile was reabsorbed in the rats. The amount of the drug excreted into bile is closely related to metabolism, therefore the amount of carvedilol excreted into bile is directly proportional to the carvedilol concentrations. It is suggested that the excretion of carvedilol into bile increases because the plasma carvedilol concentration increases when it is

given in combination with Ca-channel blockers, and a two-phasic peak appears. Therefore, the carvedilol $t_{1/2}\beta$ could not determined.

Carvedilol is a racemic compound, and both the *R*- and the *S*-enantiomers of carvedilol are metabolized in human liver microsomes, the *S*-enantiomer being metabolized faster than the *R*-enantiomer, although the same P-450 enzymes seem to be involved in each case (Oldham & Clarke 1997). After oral administration of *R*,*S*-carvedilol to humans, the AUC values for the *R*-(+)-enantiomer were found to be 2.8 times greater than those for the more active *S*-(−)-enantiomer (Fujimaki et al 1990). The nonselective β-blocking activity resides mainly in the *S*-enantiomer of carvedilol, while the α-blocking activity is shared by the *R*- and *S*-enantiomers (Nichols et al 1989). Carvedilol is used clinically as a racemic mixture of both enantiomers. If pharmacokinetic interaction takes place between carvedilol and dihydropyridine Ca-channel blockers, the ratio of α-blocking activity and β-blocking activity, reported as 1:8, may change because of the above reason. It will be necessary to perform further studies and to determine the mechanisms.

References

Drobitch, R. K., McLellan, R. A., Renton, K. W. (1997) Amlodipine inhibits rat microsomal cytochrome P450-mediated drug biotransformation. *J. Pharm. Sci.* **86**: 1501–1503

Elliot, H. L., Meredith, P. A., Mcnally, C., Reid, J. L. (1991) The interactions between nisoldipine and two β-adrenoceptor antagonists – atenolol and propranolol. *Br. J. Clin. Pharmacol.* **32**: 379–385

Fujimaki, M., Hakusui, H. (1989) Studies on the metabolic fate of carvedilol (III). Biliary excretion and enterohepatic circulation in rats. *Xenobio. Metabol. Dispos.* **4**: 667–671

Fujimaki, M., Murakoshi, Y., Hakusui, H. (1990) Assay and disposition of carvedilol enantiomers in humans and monkeys: evidence of stereoselective presystemic metabolism. *J. Pharm. Sci.* **79**: 568–572

Hakusui, H., Fujimaki, M., Esumi, Y., Takaichi, M., Saitou, T., Ootsuki, M., Miyake, T., Honda, N. (1989) Studies of the metabolic fate of carvedilol (I). Pharmacokinetics in rat following a single oral dose. *Xenobio. Metabol. Dispos.* **4**: 637–649

Hansson, B. G., Lyngstam, G., Lyngstam, O., Ryder, L., Thilen, U. (1985) Antihypertensive effect of felodipine combined with beta-blockade. *Drugs* **29** (suppl. 2): 131–135

Hashimoto, H., Kanda, A., Hubo, H., Tanaka, M. (1991) Features of the acute hypotensive action of carvedilol and its ameliorating effect on myocardial ischemia. *J. Cardiovasc. Pharmacol.* **18** (suppl. 4): S22–S28

Hokama, N., Hobara, N., Kameya, H., Ohshiro, S., Sakanashi, M. (1999) Rapid and simple micro-determination of carvedilol in rat plasma by high-performance liquid chromatography. *J. Chromatogr. B* **732**: 233–238

Jack, D. B., Kendall, M. J., Dean, S., Laugher, S., Zaman, R., Tenneson, M. E. (1982) The effect of hydralazine on the pharmacokinetics of three different beta-adrenoceptor antagonists: metoprolol, nadolol and acebutolol. *Biopharm. Drug Dispos.* **3**: 46–54

McLean, A. J., Skews, H., Bobik, A., Dudley, F. J. (1980) Interaction between oral propranolol and hydralazine. *Clin. Pharmacol. Ther.* **27**: 726–732

McLean, A. J., Knight, R., Harrison, P. M., Harper, R. W. (1985) Clearance-based oral drug interaction between verapamil and metoprolol and comparison with atenolol. *Am. J. Cardiol.* **55**: 1628–1629

Möllendoff, E. V., Reiff, K., Neubenbauer, G. (1987) Pharmacokinetics and bioavailability of carvedilol, a vasodilating beta-blocker. *Eur. J. Clin. Pharmacol.* **33**: 511–513

Nichols, A. J., Sulpizio, A. C., Ashton, D. J., Hieble, J. P., Ruffolo, R. R. (1989) The interaction of the enantiomers of carvedilol with α1- and β1-adrenoceptors. *Chirality* **1**: 265–270

Oldham, H. G., Clarke, S. E. (1997) *In vitro* identification of the human cytochrome P450 enzymes involved in the metabolism of R(+)- and S(−)-carvedilol. *Drug Metab. Dispos.* **25**: 970–977

Richer, C., Lefevre-Borg, F., Cadilhac, M., Cavero, I., Giudicelli, J. F. (1985) Nicardipine: experimental antihypertensive action and interactions with the alpha-adrenergic sympathetic system. *J. Pharmacol.* **16**: 45–58

Ruffolo, R. R., Gellai, M., Hieble, J. P., Willette, R. N., Nichols, A. J. (1990) The pharmacology of carvedilol. *Eur. J. Clin. Pharmacol.* **38**: S82–S88

Smith, S. R., Wilkins, M. R., Jack, D. B., Kendall, M. J., Laugher, S. (1987) Pharmacokinetic interactions between felodipine and metoprolol. *Eur. J. Clin. Pharmacol.* **31**: 575–578

Sponer, G., Bartsch, W., Strein, K., Muller-Beckmann, B., Bohm, E. (1987) Pharmacological profile of carvedilol as a β-blocking agent with vasodilating and hypotensive properties. *J. Cardiovasc. Pharmacol.* **9**: 317–327

Takenaka, T., Asano, M., Shiono, K., Shibasaki, M., Inagaki, O. (1985) Cardiovascular pharmacology of nicardipine in animals. *Br. J. Clin. Pharmacol.* **20** (suppl. 1): 7S–22S

Terao, T., Hisanaga, E., Sai, Y., Tamai, I., Tsuji, A. (1996) Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J. Pharm. Pharmacol.* **48**: 1083–1089