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Carvedilol increases ciclosporin bioavailability by inhibiting P-glycoprotein-mediated transport

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Abstract

Carvedilol is often used to treat hypertension and for prophylaxis in vascular sclerosis in renal transplant recipients, who require concomitant treatment with ciclosporin. However, there are few reports regarding the pharmacokinetic interactions between carvedilol and ciclosporin. We have investigated the potential effects of carvedilol on the pharmacokinetics of ciclosporin, and examined the inhibitory effects of carvedilol on P-glycoprotein-mediated transcellular transport using Caco2 cells. Ciclosporin alone or with carvedilol was orally or intravenously administered to rats. The oral administration of carvedilol (10 mg kg^{-1}) with ciclosporin (10 mg kg^{-1}) increased the whole blood concentration of ciclosporin. When ciclosporin (3 mg kg^{-1}) was intravenously administered with carvedilol (3 mg kg^{-1}), there was no difference in the whole blood ciclosporin concentration between administration with and without carvedilol. Co-administration with carvedilol increased ciclosporin bioavailability from 33% to 70%. In Caco2 cells, carvedilol caused a concentration-dependent increase in the intracellular accumulation of ciclosporin, and its effect was comparable with that of verapamil. Carvedilol considerably raised the concentration of ciclosporin in the blood and this interaction was associated with the absorption phase of ciclosporin. This interaction was caused by the inhibition of P-glycoprotein-mediated transport by carvedilol in the intestine.

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Introduction

Carvedilol is a nonselective β-blocking agent which has vasodilating properties that are attributed mainly to its blocking activity at α_1-receptors. Since carvedilol is used in the treatment of mild to moderate hypertension and does not affect renal perfusion and filtration, it can be given to renal transplant recipients (Heitmann et al 2002). In addition, carvedilol has been found to inhibit the proliferation and migration of vascular smooth muscle cells (Patel et al 1995; Park et al 2006). This pharmacological effect may be useful for renal transplant recipients because chronic rejection is associated with the abnormal proliferation and migration of vascular smooth muscle, and fibrosis is a common feature of transplant vascular sclerosis (Ishii et al 2005). For these reasons, there are many cases where carvedilol and immunosuppressive agents could and should be used concomitantly.

Carvedilol, when absorbed orally, is extensively metabolized and then excreted primarily into the bile (Gehr et al 1999). Ciclosporin, the principal immunosuppressant used in organ transplant patients and employed to treat a variety of autoimmune diseases, is extensively metabolized in the liver and eliminated mainly by biliary excretion (Lindholm 1991). Both drugs have been shown to be transported by P-glycoprotein, which plays a significant role in the oral absorption and excretion of xenobiotics (Saeki et al 1993; Kakumoto et al 2003). These points should be taken into account when considering factors affecting drug–drug interactions, and specifically the pharmacokinetics of ciclosporin. Indeed, we have had firsthand experience of increased ciclosporin blood levels after administering carvedilol concomitantly to a renal transplant patient: the patient's AUC_{0-4}/dose increased 24%. However, there are few reports describing the pharmacokinetic interaction between carvedilol and ciclosporin, and the mechanisms behind the interaction are not clear.

The purpose of this study was to examine the potential effects of carvedilol on the pharmacokinetics of ciclosporin. Furthermore, the inhibitory effects of carvedilol on

P-glycoprotein-mediated transport were investigated using a human colon adenocarcinoma cell line.

Materials and Methods

Materials

Ciclosporin and carvedilol were kindly provided by Novartis Pharma K. K. (Tokyo, Japan) and Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan), respectively. Verapamil, Dulbecco's modified Eagle medium (DMEM), fetal bovine serum, Triton X-100, and trypsin were purchased from Sigma-Aldrich (St Louis, MO). HEPES, penicillin, streptomycin, and nonessential amino acids were purchased from Gibco BRL (Grand Island, NY). All other chemicals used were of the highest purity available.

Animal study

All experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University Graduate School of Medicine. Male Wistar rats (280–320 g; Japan SLC Inc., Shizuoka, Japan) were used for the pharmacokinetic study. Although food was withdrawn overnight before the experiment, rats were always allowed free access to water. One day before drug administration, the rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (25 mg kg^{-1}). A silastic catheter was inserted into the right jugular vein to allow the collection of blood samples and as a route for intravenous administration. Doses of ciclosporin either alone or with carvedilol were orally (ciclosporin 10 mg kg^{-1}; carvedilol 10 mg kg^{-1}) or intravenously (ciclosporin 3 mg kg^{-1}; carvedilol 3 mg kg^{-1}) administered. These dosage adjustments were required due to the poor absorption of ciclosporin (27 ± 18% (Ptachcinski et al 1985)). Oral administration was accomplished by inserting a water-lubricated curved blunt stainless-steel catheter into the oesophagus. Blood samples were collected using heparinized syringes at 1, 2, 4, 6, 8, and 12 h post-dose and were analysed for ciclosporin using a fluorescence polarization immunoassay with a TDX analyser (Abbott Laboratories, Chicago, IL). Ciclosporin blood concentrations were fitted to a one-compartment model with first-order absorption and elimination using Prism 4 for Windows software (GraphPAD Software Inc, San Diego, CA).

Cell culture and intracellular accumulation study

P-glycoprotein function was studied in the epithelial layer of a human colon adenocarcinoma cell line, Caco-2, which demonstrates a P-glycoprotein-dependent intestinal absorptive cell phenotype. Caco-2 cells at passage 40 were obtained from Riken Gene Bank (Tsukuba, Japan). They were cultured in DMEM containing 10% fetal bovine serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1}-streptomycin, and 100 μM nonessential amino acids. The adhesive cells were harvested at 80% confluence by exposure to a trypsin-EDTA solution (0.25% trypsin and 0.02% EDTA in a phosphate-buffered solution).

Intracellular accumulation was studied according to a procedure described by Zhu et al (2006). In brief, Caco-2 cells were seeded at a density of 1×10^5 cells mL^{-1}/well in 24-well plates, and the culture medium was changed every two days until the experiment was initiated. After reaching confluence, Caco-2 cells were pre-incubated at 37°C for 30 min with serum-free DMEM containing 25 mM HEPES, pH 7.4. Ciclosporin and either carvedilol or verapamil were added to the culture medium to a final concentration of 0.1 μM for ciclosporin, and 0.02, 0.2, 0.6, 2 or 20 μM for carvedilol and verapamil. The solution contained no more than 0.1% dimethyl sulfoxide, and was discarded after 90-min incubation. The cells were washed three times with ice-cold phosphate-buffered saline and permeabilized with a 1% Triton X-100-containing buffer. The ciclosporin concentrations in the buffer were analysed using the method described above but with a different calibration curve. The amount of ciclosporin in each sample was standardized, with the protein content determined using a BCA protein assay kit (Pierce, Rockford, IL). The degree of inhibition observed at the different carvedilol or verapamil concentrations was estimated based on a 50% inhibitory concentration (IC50) determined with an inhibitory sigmoid E_{max} model using non-linear regression curve fitting; the results were analysed using Prism 4 for Windows software (GraphPAD Software Inc, San Diego, CA).

Statistical analysis

All data are presented as the mean ± s.d. The individual differences of ciclosporin concentrations with and without carvedilol at each sampling time were performed using a Mann–Whitney U-test. The differences of each pharmacokinetic parameter were also established using the same test, with $P < 0.05$ being taken as significant. All statistical analyses were performed with StatView 4.5 (Abacus Concepts Inc., Berkeley, CA).

Results

Pharmacokinetic study

Figure 1 shows the whole blood concentration–time curves of ciclosporin after oral (ciclosporin 10 mg kg^{-1}, Figure 1A) and intravenous (ciclosporin 3 mg kg^{-1}, Figure 1B) administration, with and without carvedilol (i.v. 3 mg kg^{-1}; p.o. 10 mg kg^{-1}). Co-administration of carvedilol significantly ($P < 0.05$) increased the whole blood concentration of ciclosporin following oral administration. When ciclosporin was intravenously administered with carvedilol, there was no difference in the whole blood ciclosporin concentration to when administered without carvedilol. The pharmacokinetic parameters of ciclosporin with and without carvedilol are shown in Table 1. Co-administration of carvedilol resulted in a decrease in clearance (CL) and a 2-fold increase in AUC and C_{max} compared with administration of ciclosporin alone when the drugs were taken orally. The pharmacokinetic parameters (AUC and CL) were not altered when carvedilol was administered intravenously. Since the increase in AUC was linear with ciclosporin dose, co-administration with carvedilol showed

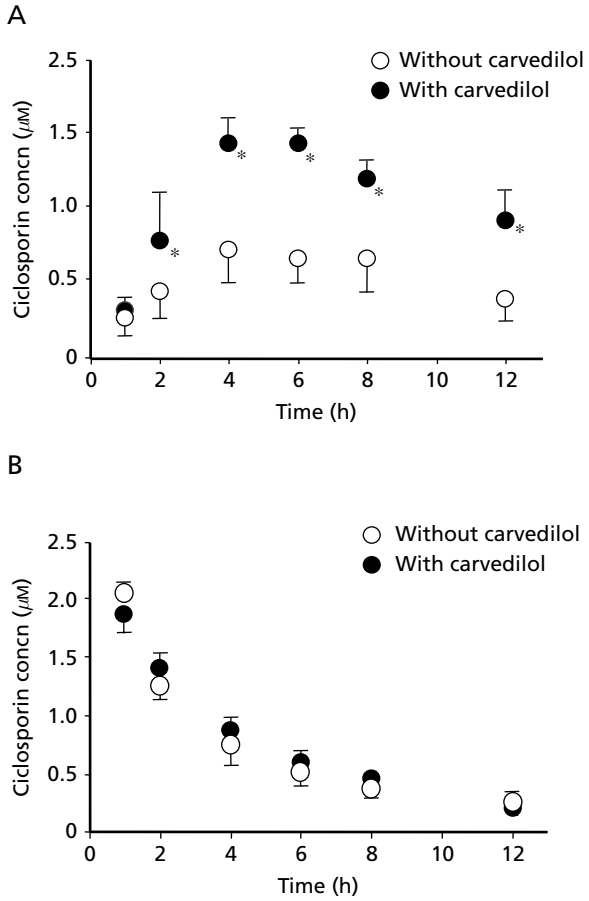

Figure 1 Whole blood concentration–time curves of ciclosporin after (A) 10 mg kg^{-1} orally administered with or without carvedilol or (B) 3 mg kg^{-1} intravenously administered with or without carvedilol. Each point represents the mean ± s.d. of four rats. *$P < 0.05$, significantly different from administration without carvedilol.

Table 1 Effect of carvedilol on pharmacokinetic parameters of ciclosporin

Parameter	Oral administration		Intravenous administration	
	Control	Carvedilol	Control	Carvedilol
AUC (μM h)	9.8 ± 2.7	23.6 ± 5.7	9.1 ± 1.7	10.2 ± 1.4
	$P < 0.01$		$P = 0.17$	
k_a (h^{-1})	0.17 ± 0.04	0.21 ± 0.06		
	$P = 0.38$			
k_{el} (h^{-1})	0.17 ± 0.06	0.17 ± 0.03	0.27 ± 0.08	0.23 ± 0.01
	$P = 0.46$		$P = 0.17$	
Vd/F (L kg^{-1})	4.25 ± 0.51	2.25 ± 0.13		
	$P < 0.01$			
Vd (L kg^{-1})			1.00 ± 0.40	1.10 ± 0.09
			$P = 0.47$	
CL/F (L h^{-1} kg^{-1})	0.71 ± 0.15	0.39 ± 0.03		
	$P < 0.01$			
CL (L h^{-1} kg^{-1})			0.27 ± 0.13	0.25 ± 0.03
			$P = 0.56$	
C_{max} (μM)	0.72 ± 0.10	1.42 ± 0.12		
	$P < 0.01$			

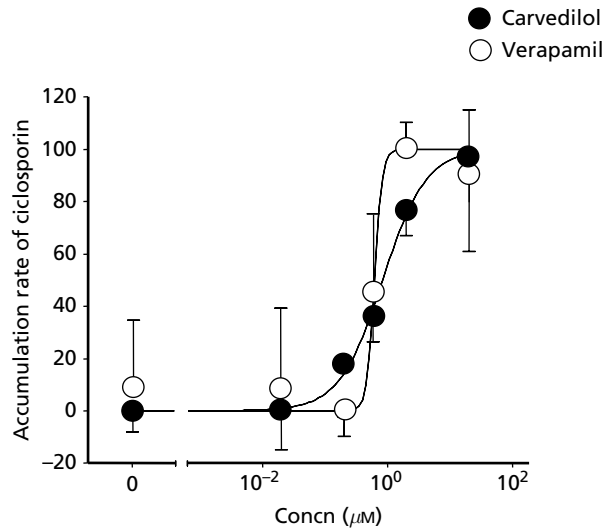

Figure 2 Concentration-dependent accumulation rate of 0.1 μM ciclosporin by carvedilol and verapamil in Caco-2 cells. The IC50 values of carvedilol and verapamil were 0.83 and 0.62 μM, respectively. Each point represents the mean ± s.d. of four data points.

an increase in bioavailability of ciclosporin from 32.6% to 70.1%. The elimination rate constants were not altered by carvedilol administered either orally or intravenously. These results suggested that the increase in the AUC and C_{max} of ciclosporin induced by carvedilol via the oral route was mainly due to enhanced absorption of ciclosporin in the gastrointestinal tract, and not to inhibited elimination via the biliary tract or to hepatic metabolism.

Intracellular accumulation study

Ciclosporin is a substrate of P-glycoprotein and is transported across Caco-2 cell membranes. Since Caco-2 cells exhibit features typical of intestinal epithelial cells (Sambuy et al 2005), these cells have been used widely as a standard model for intestinal efflux, and for investigating the mechanisms of absorption of several classes of drugs. We evaluated the inhibitory effect of carvedilol on the efflux of ciclosporin using the intracellular accumulation study. As shown in Figure 2, the accumulation of ciclosporin was markedly increased in the presence of carvedilol, the effects of which were dependent on concentration. This concentration-dependent accumulation of ciclosporin was also found with verapamil in Caco-2 cells. The IC50 value of carvedilol (0.831 μM) was comparable with that of verapamil (0.616 μM).

Discussion

Padi & Chopra (2002) reported that ciclosporin induced oxidative stress and that the resultant renal dysfunction was prevented by carvedilol, which has potent antioxidative effects. These results indicated that co-administration of carvedilol

with ciclosporin was useful not only for the treatment of hypertension and the inhibition of muscular smooth muscle cell proliferation, but also for the prevention of ciclosporin-induced nephrotoxicity. However, there are few clinical reports regarding the interaction between ciclosporin and carvedilol, and the precise mechanism of this interaction has yet to be elucidated. Therefore, we have investigated the effect of carvedilol on the pharmacokinetics of ciclosporin in rats and in the Caco-2 cell line (which expresses P-glycoprotein). Previous reports indicated that blood levels of ciclosporin increased when carvedilol was introduced, and thus the dose of ciclosporin had to be decreased by 20% (Kaijser et al 1997) or 10% (Bader et al 2005). Kaijser et al (1997) speculated that cytochrome P450 enzymes contributed to this interaction. Indeed, ciclosporin is metabolized by CYP3A4, which plays a dominant role in the metabolic elimination of many drugs (Lamba et al 2002). Drugs which are substrates of CYP3A4, including calcium channel blockers, macrolide antibiotics, and antifungal azoles, have been shown to clinically increase the concentrations of ciclosporin in blood (Guan et al 1996; Koselj et al 1994). Carvedilol undergoes extensive metabolism by CYP2D6, CYP3A4, CYP1A2, CYP2E1 and CYP2C9, while CYP2D6 is the rate-limiting enzyme for the overall disposition (Gehr et al 1999; Graff et al 2001). CYP3A4 has a minor influence on the disposition of carvedilol, because the increase in metabolic clearance after treatment with rifampicin, which markedly induced the expression of CYP3A4, contributed only slightly to the increase in total body clearance (Giessmann et al 2004). Therefore, it was presumed that the probability of a drug–drug interaction dependent on CYP3A4 was unlikely. Figure 1B indicates that carvedilol did not interact with ciclosporin during the elimination phase, indicating that the absorption of ciclosporin was increased by carvedilol, as shown in Figure 1A.

The activity of cytochrome P450 affects not only drug elimination, but also the bioavailability of many drugs. Several studies have shown that intestinal CYP3A4 was responsible for the first pass metabolism of orally administered ciclosporin (Gomez et al 1995; Wu et al 1995). However, if carvedilol were to inhibit intestinal CYP3A4, it would also inhibit liver CYP3A4 activity. Therefore, it was quite unlikely that this was the mechanism behind the interaction. On the other hand, Bader et al (2005) suggested that carvedilol influenced ciclosporin levels through its effects on P-glycoprotein in cardiac transplant recipients. We designed an in-vitro study to evaluate the inhibitory effects of carvedilol on ciclosporin transport. Several in-vitro models have been proposed to determine the inhibitory effect or substrate specificity of P-glycoprotein. In this study, we examined the inhibitory effects of carvedilol and verapamil on the intracellular uptake of the P-glycoprotein substrate ciclosporin in Caco-2 cells. The Caco-2 cell line has been used extensively as a model of the intestinal barrier and absorptive properties of the intestinal mucosa. Caco-2 cells express many kinds of transporter proteins such as multidrug resistance-associated proteins, P-glycoprotein, peptide transporters, organic anion transporters, and organic cation transporters (Katsura & Inui 2003). Previous reports indicated that the pharmacokinetics of ciclosporin were related to the expression level of enterocyte P-glycoprotein in renal transplant patients (Lown et al 1997). Additionally, the basolateral to apical transport of ciclosporin is much greater across human MDR1 cDNA-transfected porcine kidney epithelial LLC-PK1 cells than in LLC-PK1 cells (Adachi et al 2001). Therefore, P-glycoprotein is mainly responsible for the cellular extrusion of ciclosporin. Verapamil is also a well established probe that is routinely used to identify compounds that have the potential to inhibit P-glycoprotein.

Carvedilol has been found to be a substrate of P-glycoprotein, which can be considered as another mechanism of drug–drug interaction (Kakumoto et al 2003; Bart et al 2005). Kakumoto et al (2003) indicated that carvedilol inhibited the treatment of anticancer drugs mediated by P-glycoprotein, and that the inhibitory effect was similar to that of verapamil. The results of our study using Caco-2 cells also showed that carvedilol inhibited the transport of ciclosporin mediated by P-glycoprotein, and that this inhibitory effect was comparable with that of verapamil. After long-term oral administration of carvedilol (25 mg/day) to healthy subjects, the peak plasma concentration in extensive metabolizers and poor metabolizers of CYP2D6 was nearly 0.1 and 0.15 μM, respectively (Giessmann et al 2004). This concentration was almost the 20% inhibitory concentration for the transport of ciclosporin obtained in this study. Therefore, there is a possibility of interaction between ciclosporin and carvedilol in the clinical setting.

Kakumoto et al (2003) speculated that the antioxidative effect of carvedilol caused the inhibitory effect on P-glycoprotein-mediated transport. Indeed, it has been reported that high levels of reactive oxygen species, resulting in severe cellular oxidative stress, increased the expression of MDR1 genes (Ziemann et al 1999). However, this was unlikely because in our study only 90-min incubation with carvedilol inhibited the transport of ciclosporin across Caco-2 cells, and a previous study demonstrated a significant increase in mdr1b mRNA expression after two days of treatment with H_2O_2 (Ziemann et al 1999). Further study is needed to clarify the mechanism.

Ciclosporin is also known to inhibit P-glycoprotein-mediated transport (Chiou et al 2002). Carvedilol is not usually trapped in the brain because P-glycoprotein in the blood–brain barrier extrudes carvedilol from the brain. Bart et al (2005) indicated that the amount of carvedilol taken up in the rat brain was increased after pretreatment with ciclosporin. Although we did not monitor the concentration of carvedilol in blood in this study, there is a possibility that carvedilol blood concentration, and also the distribution of carvedilol in the brain, could be increased after co-treatment with ciclosporin. There have been no studies regarding the interaction between carvedilol and ciclosporin in man which focus on carvedilol, but we should be aware of such cases and monitor clinical symptoms closely.

Conclusion

Co-administration with carvedilol increased ciclosporin bioavailability. By using Caco-2 cells, we found that carvedilol caused a concentration-dependent increase in the intracellular accumulation of ciclosporin, and its effect was comparable with that of verapamil. This study has shown that carvedilol

considerably raised the concentration of ciclosporin in blood, and that this interaction was associated with the absorption phase of ciclosporin caused by the inhibition of P-glycoprotein-mediated transport by carvedilol in the intestine.

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