Effects of Glipizide on the Pharmacokinetics of Carvedilol after Oral and Intravenous Administration in Rats

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Abstract

This study was designed to investigate the effects of glipizide on the pharmacokinetics of carvedilol after oral or intravenous administration of carvedilol in rats. Clinically carvedilol and glipizide can be prescribed for treatment of cardiovascular diseases as the complications of diabetes, and then, Carvedilol and glipizide are all substrates of CYP2C9 enzymes. Carvedilol was administered orally or intravenously without or with oral administration of glipizide to rats. The effects of glipizide on cytochrome P450 (CYP) 2C9 activity and P-gp activity were also evaluated. Glipizide inhibited CYP2C9 activity in a concentration-dependent manner with 50% inhibition concentration (IC₅₀) of 18 μM. Compared with the control group, the area under the plasma concentration-time curve (AUC) was significantly increased by 33.0%, and the peak concentration (Cₘₐₓ) was significantly increased by 50.0% in the presence of glipizide after oral administration of carvedilol. Consequently, the relative bioavailability (R.B.) of carvedilol was increased by 1.13- to 1.33-fold and the absolute bioavailability (A.B.) of carvedilol in the presence of glipizide was increased by 36.8%. After intravenous administration, compared to the control, glipizide could not significantly change the pharmacokinetic parameters of carvedilol. Therefore, the enhanced oral bioavailability of carvedilol may mainly result from inhibition of CYP2C9-mediated metabolism rather than both P-gp-mediated efflux in the intestinal or in the liver and renal elimination of carvedilol by glipizide.

Key Words: Carvedilol, Glipizide, CYP2C9, P-gp, Pharmacokinetics, Rats

INTRODUCTION

Glipizide is a sulfonylurea antidiabetics with the treatment of type 2 diabetes mellitus and has a duration of action of up to 24 hours. The usual initial dose is 2.5 to 5 mg daily given as a single dose about 30 minutes before breakfast (Kradjan et al., 1995).

Glipizide is readily absorbed from the gastrointestinal tract with peak plasma concentrations occurring 1 to 3 h after a single dose. It is extensively bound to plasma proteins and has a half-life of approximately 2 to 4 hours. It is metabolized mainly in the liver and excreted chiefly in the urine, largely as inactive metabolites (Wahlin-Boll et al., 1982).

Furthermore, the effects of glipizide on cytochrome P450 (CYP) 2C9 activity and P-gp activity were also evaluated using CYP inhibition assays. Carvedilol is an arylenatemine and has nonspecific β- and α₁ adrenergic blocking effects (Bristow et al., 1992). Carvedilol also reduces the release of endothelin and directly scavenges free radicals of oxygen (Feuerstein et al., 1997). It is used to treat systemic arterial hypertension (Cournot et al., 1992; Lund-Johansen et al., 1992) and congestive heart failure (DasGupta et al., 1991) and is purported to improve exercise capacity (Cleland et al., 1996; Hampton, 1996) and longevity in humans (Bristow et al., 1996).

Carvedilol is well absorbed from the gastrointestinal tract, but is subject to considerable first-pass metabolism in the intestinal and/or liver (McTavish et al., 1993; Morgan, 1994). Carvedilol is more than 98% bound to plasma proteins. Carvedilol is metabolized by both oxidation and conjugation pathways in the liver into some metabolites (Neugebauer et al., 1987; Neugebauer and Neubert, 1991). The oxidation pathways are mainly catalyzed by CYP2C9 enzymes in human (McTavish et al., 1993; Morgan, 1994; Oldham and Clarke, 1997), and then CYP2D6 is responsible for the formation of 4'-hydroxy carvedilol and 5'-hydroxy carvedilol, and both metabolites are excreted into urine (Neugebauer and Neubert, 1991). Since carvedilol is a substrate of both CYP2C9 en...
zymes and P-gp (Bart et al., 2005), the modulation of CYP enzyme activities may cause the significant changes in the pharmacokinetic profile of carvedilol.

There are a few interactions between glipizide and other drugs (Connacher et al., 1987; Arauz-Pacheco et al., 1990; Kivisto and Neuvonen, 1991; Kradjan et al., 1994; Niemi et al., 2001).

Clinically carvedilol and glipizide can be prescribed for treatment of cardiovascular diseases as the complications of diabetes. However, pharmacokinetic interaction between glipizide and carvedilol has not been reported in vivo. Therefore, the present study aims to investigate the effect of glipizide on the CYP2C9 activity, P-gp activity and pharmacokinetics of carvedilol after oral and intravenous administration in rats.

MATERIALS AND METHODS

Chemicals and apparatus

Carvedilol, glipizide and nimodipine [an internal standard for high-performance liquid chromatograph (HPLC) analysis for carvedilol] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). HPLC grade acetonitrile was acquired from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade.

Apparatuses used in this study were a HPLC equipped with a Waters 1515 isocratic HPLC Pump, a Waters 717 plus autosampler and a Waters™ 474 scanning fluorescence detector (Waters Co., Milford, MA, USA), a HPLC column temperature controller (Phenomenex Inc., CA, USA), a Branson® Ultrasonic Cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA) and a high-speed micro centrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats of 7-8 weeks of age (weighing 270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and given free access to a commercial rat chow diet (No. 322-7-1; Superfeed Research Co. (Choongbuk, Republic of Korea) and given free access to tap water. Each animal was anaesthetized lightly with a gentle stream of nitrogen gas at 35°C. The residue was reconstituted in a 150 μl of the mobile phase and centrifuged (13,000 rpm, 5 min). The resulting mixture was then vigorously vortex-mixed for 5 min and centrifuged at 13,000 rpm for 5 min. A 50-μl aliquot of the supernatant was injected into the HPLC system. Chromatographic separations were achieved using a Chromolith Performance (RP-18e, 100×4.6 mm) column from Merck (Darmstadt, Germany). The mobile phase consisted of 0.01M disodium hydrogen phosphate (pH 3.5, adjusted with phosphoric acid)-acetonitrile (75:24.3, v/v). The flow rate of the mobile phase was maintained at 2.0 ml/min. Chromatography was performed at 25°C, which was regulated by an HPLC column temperature controller. The fluocrescence detector was operated at an excitation wavelength of 240 nm with an emission wavelength of 340 nm. The retention times at a flow rate of 2 ml/min were as follows: carvedilol at 8.076 min internal standard at 9.305 min. The lower limit of quantification for carvedilol in rat plasma was 10 ng/ml. The coefficient of the variation of carvedilol was less than 14.3%.

CYP 2C9 inhibition assay

The assays of inhibition on human 2C9 enzyme activities were performed in a multiwell plate using CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi et al., 1997). Briefly, human CYP enzymes were obtained from baculovirus-infected insect cells. CYP substrates (7-MFC for CYP2C9) were incubated with or without test compounds in the enzyme/substrate contained buffer consisting of 1 pmol of P450 enzyme and NADPH generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM MgCl₂) in a potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45 min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) set at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP2C9 was run on the same plate and produced 99% inhibition. All experiments were performed in du-