Inhibitory and Stimulative Effects of Amiodarone on Metabolism of Carvedilol in Human Liver Microsomes

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It was reported that coadministration of amiodarone with carvedilol increased the serum concentration to dose (C/D) ratio of S-carvedilol in patients with heart failure, but not of R-carvedilol. The aim of the present study was to investigate the effect of amiodarone and its metabolite on the metabolism of R- and S-carvedilol in human liver microsomes (HLM). Oxidation of carvedilol in HLM was evaluated in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), whereas glucuronidation was evaluated in the presence of uridine 5′-diphosphate (UDP)-glucuronic acid. The oxidation and glucuronidation activities of HLM for S-carvedilol were approximately 2- and 4-fold greater, respectively, than those for R-carvedilol. In the presence of amiodarone (50 μM) and/or desethylamiodarone (25 μM), the oxidation activity for R- and S-carvedilol decreased significantly. In contrast, the glucuronidation activity for R-carvedilol was increased 1.6- and 1.4-fold by amiodarone and desethylamiodarone, respectively, whereas the glucuronidation of S-carvedilol was only slightly changed by amiodarone and desethylamiodarone. These results suggested that inhibition of S-carvedilol oxidation by amiodarone and/or desethylamiodarone is implicated in the increased C/D ratio of S-carvedilol associated with coadministration of amiodarone. On the other hand, the stimulative effect of amiodarone and/or desethylamiodarone on the glucuronidation of R-carvedilol may compensate for the inhibitory effect of those on R-carvedilol oxidation.

Key words carvedilol; amiodarone; oxidation; glucuronidation; human liver microsome

Carvedilol is a β-adrenoceptor antagonist that has been clinically used to treat chronic heart failure as well as hypertension, angina pectoris, and cardiac arrhythmia.1) Orally administered carvedilol undergoes stereoselective first-pass metabolism, and the blood concentration of S-enantiomer with high β-blocking activity is approximately one-half of that of R-enantiomer with low β-blocking activity.2,3) Both enantiomers are mostly eliminated by hepatic metabolism, with renal excretion accounting for only 0.3% of the administered dose.4) Carvedilol is metabolized extensively via aliphatic side-chain oxidation, aromatic ring oxidation, and conjugation pathways.5) Oldham and Clarke reported that the oxidative metabolism of carvedilol is mediated by cytochrome P450 (CYP) 2D6, 2C9, 3A4, and 1A2 in microsomes from human lymphoblastoid cells expressing human CYP.5) In addition, our previous findings indicated that R-carvedilol is metabolized mainly by CYP2D6 and partly by CYP1A2, 2C9, and 3A4, and that S-carvedilol is metabolized mainly by CYP1A2 and partly by CYP2C9, 2D6, and 3A4.7–10) On the other hand, Ohno et al. found that uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT) 2B7, 2B4, and 1A1 are capable of catalyzing the glucuronidation of carvedilol using microsomes from insect cells expressing human UGT.11) In addition, they have demonstrated that glucuronidation of R-carvedilol is mediated by UGT1A1 and 2B4, and that glucuronidation of S-carvedilol is mediated by UGT2B7 and 2B4.11)

In 2005, Fukumoto et al. reported the effect of amiodarone on the stereoselective pharmacokinetics of carvedilol in patients with heart failure.12) That is, they demonstrated that the mean serum concentration to dose (C/D) ratio of S-carvedilol in 54 patients who took amiodarone concomitantly with carvedilol was 2-fold higher than that in 52 patients who took carvedilol alone. On the other hand, there was no significant difference in the mean C/D values of R-carvedilol between the two groups.12) They speculated that the oxidative metabolism of only S-carvedilol might be markedly inhibited by coadministration of amiodarone; however, the effects of amiodarone and/or its metabolite on the oxidation and glucuronidation of carvedilol enantiomers are still unresolved.12)

In the present study, to evaluate the mechanism responsible for the interaction between carvedilol and amiodarone, we investigated inhibitory and stimulative effects of amiodarone and desethylamiodarone on the metabolism of R- and S-carvedilol in human liver microsomes (HLM). The oxidation of carvedilol in HLM was evaluated in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), whereas glucuronidation was evaluated in the presence of UDP-glucuronic acid (UDPGA).

MATERIALS AND METHODS

Materials Carvedilol was kindly supplied by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). NADPH was obtained from Kohjin Co., Ltd. (Tokyo, Japan). UDPGA trisodium salt, amiodarone hydrochloride, furafylline, sulfaphenazole, and quinidine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Ketoconazole and zidovudine were obtained from Wako Pure Chemicals (Osaka, Japan). Desethylamiodarone was purchased from SPI-BIO (Massy Cedex, France). Bilirubin and hydroxyeicosachydroic acid were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other chemicals were of the highest purity available. Pooled HLM from 20 donors (coded as #18888) and two kinds of CYP2D6-deficient single donor HLM (coded as HH31 and HH35) were purchased from BD Biosciences (Woburn, MA, U.S.A.).

Oxidation of R- and S-Carvedilol in HLM Oxidation...
of R- and S-carvedilol in HLM was evaluated in the presence of NADPH, as described previously. That is, the incubation mixture (final volume 100 µl) consisted of 1 µM racemic carvedilol, 1.0 mg/ml microsomal proteins, 2 mM NADPH, and 50 mM potassium phosphate buffer (pH 7.4). Carvedilol was dissolved in a mixture of dimethylformamide and acetone, and the final concentrations of dimethylformamide and acetone in the mixture were 0.05% and 0.02% (v/v), respectively. The reaction was allowed to run for 15 min at 37°C. The metabolized amount of R- and S-carvedilol was calculated by subtracting the amount remaining in the sample from the amount applied. We evaluated the effect of amiodarone (50 µM), desethylamiodarone (25 µM), furafylline (25 µM), sulfaphenazole (10 µM), quinidine (1 µM), and ketoconazole (1 µM) on the oxidation of carvedilol.

**Glucuronidation of R- and S-Carvedilol in HLM** Glucuronidation of R- and S-carvedilol in HLM was evaluated in the presence of UDPGA as described previously. That is, the incubation mixture (final volume 100 µl) consisted of 1 µM racemic carvedilol, 1.0 mg/ml microsomal proteins, 10 mM MgCl2, 2 mM UDPGA, 25 µg/ml aminohexicin, and 50 mM Tris–HCL buffer (pH 7.4). The reaction was allowed to run for 25 min at 37°C. The metabolized amount of R- and S-carvedilol was calculated by subtracting the amount remaining in the sample from the amount applied. We evaluated the effect of amiodarone (50 µM), desethylamiodarone (25 µM), bilirubin (250 µM), hydoxycholic acid (250 µM), and zidovudine (1 mM) on the glucuronidation of carvedilol.

**Assay of Carvedilol Enantiomers** The amount of carvedilol in the samples was measured using chiral high performance liquid chromatography (HPLC) as described by Saito et al. with minor modifications. Briefly, carvedilol was extracted from the samples (0.1 ml) with 5 ml diethyl ether after alkalization in 3 ml of 0.1 M Britton–Robinson buffer (pH 8.5). The organic phase was transferred and evaporated dry in a water bath at 45°C. The residue was dissolved in 500 µl mobile phase, and 50 µl was injected into the HPLC column. The HPLC system consisted of an LC-10A_P Liquid Chromatograph Series (Shimadzu, Kyoto, Japan) with a model RF-10A XL fluorescence detector (Shimadzu) and CHIRALPAK® AD-H column (25 cm×2 mm i.d.; 5 µm particle size; Daicel Chemical Industries, Tokyo, Japan). The mobile phase consisted of 73% hexane, 27% isopropanol, and 0.1% (v/v) diethylamine. Flow rate was 0.3 ml/min and column temperature was 40°C. The peaks were monitored at an excitation wavelength of 284 nm and an emission wavelength of 343 nm, and the retention times were 13.3 and 21.7 min for R- and S-carcudilol, respectively. The coefficient of inter-day variation for the assay of R- and S-carvedilol was 13.1% and 12.7%, respectively, at a concentration of 500 nm. The detection limit for each enantiomer was 0.5 nm for the concentrations in the samples.

**Data Analysis** Values are expressed as the mean±S.E. The statistical significance of the difference between mean values was evaluated using an unpaired Student’s t-test. p<0.05 was considered to be statistically significant.

**RESULTS AND DISCUSSION**

The oxidation and glucuronidation of R- and S-carvedilol in three kinds of HLM were evaluated at the microsomal protein concentration of 1 mg/ml and at the racemic drug concentration of 1 µM. Figure 1 shows the percentages of carvedilol oxidized and glucuronidated following 15- and 25-min incubation, respectively. The percentage of S-carvedilol oxidized and glucuronidated in the pooled HLM (#18888) was 1.7- and 5.9-fold higher, respectively, than that of R-carvedilol. The oxidation and glucuronidation of carvedilol in two kinds of CYP2D6-deficient single donor HLM (HH31 and HH35) were also stereoselective for S-carvedilol. In general, the oxidation activity for S-carvedilol was approximately 2-fold greater than that for R-carvedilol, whereas the glucuronidation activity for S-carvedilol was approximately 4-fold greater than that for R-carvedilol (Fig. 1).

We then evaluated the effect of CYP-specific inhibitors, amiodarone, and desethylamiodarone on the oxidation of R- and S-carvedilol in three kinds of HLM (Table 1). According to previous reports, the concentration of CYP-specific inhibitors was determined as follows: 25 µM furafylline (inhibitor of CYP1A2), 10 µM sulfaphenazole (inhibitor of CYP2C9), 1 µM quinidine (inhibitor of CYP2D6), and 1 µM ketoconazole (inhibitor of CYP3A4). The concentrations of amiodarone (50 µM) and desethylamiodarone (25 µM) were determined according to the report of Ohyama et al. In the pooled HLM (#18888), the oxidation activity for R-carvedilol was significantly decreased by quinidine (to 49.7% of control), whereas that for S-carvedilol was decreased by furafylline (to 70.6% of control). The results confirmed that CYP2D6 and CYP1A2 in the pooled HLM contributed mainly to the oxidation of R- and S-carvedilol, respectively. In the presence of 50 µM amiodarone, the oxidation of R- and S-carvedilol in #18888 was inhibited marginally. The oxidation of both R- and S-carvedilol in #18888 was significantly inhibited by 25 µM desethylamiodarone (Table 1).

In two kinds of CYP2D6-deficient single donor HLM (HH31 and HH35), quinidine did not inhibit the oxidation of R- and S-carvedilol (Table 1). The oxidation of R- and S-carvedilol in HH31 was significantly inhibited by ketoconazole and furafylline, respectively, indicating that CYP3A4 and 1A2 in HH31 contributed mainly to the oxidation of R- and S-carvedilol, respectively. On the other hand, the oxidation of R- and S-carvedilol in HH35 was significantly inhibited by sulfaphenazole, indicating that CYP2C9 in HH35 catalyzed the oxidation of both R- and S-carvedilol. The oxida-
tion of R- and S-carvedilol in HH31 was inhibited marginally by amiodarone, whereas that in HH35 was inhibited significantly by amiodarone. In addition, desethylamiodarone significantly inhibited the oxidation of R- and S-carvedilol in both HH31 and HH35. These results indicated that amiodarone and/or desethylamiodarone inhibited the oxidation of both R- and S-carvedilol not only in the CYP2D6-sufficient HLM, but also in the CYP2D6-deficient HLM (Table 1).

We also evaluated the effect of UGT substrates, amiodarone, and desethylamiodarone, on the glucuronidation of R- and S-carvedilol in three kinds of HLM (Table 2). Bilirubin (the preferred substrate of UGT1A1), hyodeoxycholic acid (the preferred substrate of UGT2B4 and 2B7), or zidovudine (the preferred substrate of UGT2B7) was added to the reaction mixture at final concentrations of 250 μM, 250 μM, and 1 mM, respectively.14,15 The glucuronidation of R- and S-carvedilol in #18888, HH31, and also HH35 was inhibited significantly, but did not disappear completely even in the presence of high concentrations of the UGT substrates. In contrast, the glucuronidation of R-carvedilol in three kinds of HLM increased approximately 1.6-fold in the presence of 50 μM amiodarone, whereas that of S-carvedilol was increased only slightly by amiodarone. Similarly, the glucuronidation activity for R-carvedilol increased approximately 1.4-fold in the presence of 25 μM desethylamiodarone, whereas that for S-carvedilol was increased only slightly by desethylamiodarone (Table 2).

In summary, the oxidation and glucuronidation activities of HLM for S-carvedilol were approximately 2- and 4-fold greater, respectively, than those for R-carvedilol. In the presence of amiodarone (50 μM) and/or desethylamiodarone (25 μM), the oxidation activity for R- and S-carvedilol decreased significantly. In contrast, the glucuronidation activity for R-carvedilol was increased 1.6- and 1.4-fold by amiodarone and desethylamiodarone, respectively, whereas the glucuronidation of S-carvedilol was only slightly increased by amiodarone and desethylamiodarone. These results suggested that inhibition of S-carvedilol oxidation is responsible for the increased C/D ratio of S-carvedilol associated with coadministration of amiodarone in patients. On the other hand, the stimulative effect of amiodarone and/or desethylamiodarone on the glucuronidation of R-carvedilol may compensate for the inhibitory effect of those on R-carvedilol oxidation.

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