Inhibitory effects of amiodarone on simvastatin metabolism in human liver microsomes

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Objective To investigate the effects of amiodarone (AMD) on simvastatin (SV) in human liver microsomes and the possible underlying mechanisms. Methods Time-, NADPH- and concentration-dependent inhibitions were tested in HLM. The logarithm of relative inhibition values was plotted versus preincubation time (0, 5, 10, 15, 20 min) for a series concentration of AMD used (0, 2, 5, 25, 50 μmol/L), and the slopes determined by linear regression. These slope values represent the observed inactivation rate constants (k_{inact}). A double-reciprocal plot was then constructed using the reciprocal of the k_{inact} (y-axis) and the reciprocal of the associated inhibitor concentration (x-axis) to estimate the values of k_{mic} and K_{i}, which were two principal kinetic constants that were specific for mechanism-based inhibition (MBI), drug-drug interactions (DDI) potential was predicted based on in vitro data and by using the in vitro-in vivo extrapolation. Results The time-, concentration- and NADPH-dependent characteristics confirmed that when SV was the substrate of CYP3A4, the inhibition of AMD to CYP3A4 is MBI. K and k_{mic} value were calculated to be 5.1 μmol/L and 0.018 min^{-1}. The CL of SV was reduced 2.96-5.63 fold when it was administrated with AMD. Conclusion Based on the results, AMD would inhibit SV metabolism via the mechanism-based manner, which would lead to DDI when they are taken together. Careful clinical observation is recommended when AMD and SV have to be simultaneously prescribed. (J Geriatr Cardiol 2009; 6:115-118)

Key words amiodarone; simvastatin; CYP3A4; drug-drug interaction; mechanism-based inhibition

Introduction

Simvastatin (SV) is widely used in the treatment of hypercholesterolemia and prevention of cardiovascular events.1 Amiodarone (AMD) is a potent antiarrhythmic and antiangular drug. CYP3A is the major enzyme subfamily responsible for the metabolisms of both SV and AMD by human liver microsomes.2, 3 Some cases of statin-related toxicity have also been reported.4, 5 The incidence is heightened, when statins are used in combination with AMD, which is connected to their pharmacokinetics. However, some recent in vivo data suggest that AMD can increase the AUC of SV and simvastatin acid.6 In this in vitro study, we investigated potential pharmacokinetic interactions between AMD and the CYP3A4 substrate SV.

Methods

Chemicals

SV was kindly provided by Bio-pharm Co., Ltd of Dalian Medical University, AMD hydrochloride was kindly provided by Shandong Pingyuan Pharmaceutical Factory. D-glucose-6-phosphate (G-6-P), G-6-P dehydrogenase and NADPH were purchased from Sigma-Aldrich (St. Louis, Miss., USA). Testosterone was from Acros Organics (Morris Plains, N. J., USA). Ketoconazole was from ICN Biomedicals (Aurora, Ohio, USA). Human livers were obtained from one Chinese autopsy sample from Dalian Medical University. All other reagents were of the highest purity commercially available or HPLC grade.

Human liver microsomes

Liver specimens were stored in liquid nitrogen until preparation of microsomes. Pooled human liver microsomes (HLMs) were prepared from liver tissue and stored at -80°C as described previously. Protein concentrations of the microsomes were determined by the method of Lowry using bovine serum albumin as a standard.7 Determination of P450 content was performed according to Omura and Sato.8

Preincubation-dependent inhibition using human liver microsomes

To investigate the effect of preincubation on AMD inhibition of SV in vitro, various concentrations of AMD (0, 25, 10, 25, 50 μmol/L, final concentrations) were added to incubation vials containing the necessary cofactors (a reduced NADPH-regenerating system: 10mmol/L G-6-P, 1U/ml G-6-P dehydrogenase, 4 mmol/L MgCl2, 100 mmol/L potassium phosphate buffer(pH 7.4)) and human liver microsomal protein. Reaction mixtures were constructed such that one
set of vials had enzyme and AMD present during the preincubation of 0 and 20 min at 37°C, while a separate set were constructed with enzyme alone during the preincubation. Aliquots of each preincubation reaction were then transferred in a 1:10 dilution to separate incubation vials containing fresh cofactors and SV (final concentration 1 μmol/L), thus allowing the detection of any effect on enzymatic activity as a consequence of preincubation with inhibitor. Preincubation reactions were initiated with the addition of human liver microsomal protein (final concentration 0.5 mg/ml) and were preincubated (without substrate) for 0 and 20 min in a rotating 37°C water bath. All incubations were performed in duplicate. Reactions were allowed to incubate for an additional 10 min as previously described and then terminated by the addition of cold acetonitrile with internal standard (100 μl 15 μmol/L Testosterone solution) and kept on ice. These experiments were compared with duplicate assays in which various concentrations of AMD (as described above) were added to the second incubation vial instead of the preincubation vial. Incubation vials were centrifuged at 14, 300rpm for 10 min at 4°C, and the supernatant was transferred for analysis. HPLC was performed using a Kromasil column (ODS, 150 mm x 4.6 mm, 5 μm) with a linear gradient of acetonitrile and water (50% acetonitrile decreased to 20% acetonitrile in 10 min and increased back to 50% acetonitrile from 12 min to 20 min). The flow rate of 1 ml/min and elute were monitored at 237 nm. All incubations were performed in duplicate.

**Time-dependent incubations using human liver Microsomes**

To investigate the possible time-dependent inactivation of CYP3A4 activity, we used a two-step incubation scheme to assay AMD inhibition of SV. During the preincubation period, mixtures containing AMD (0, 2, 5, 25, 50 μmol/L final concentrations) and HLM (0.05mg/ml final concentration) were assembled as described above and incubated in a 37°C water bath (0, 5, 10, 15 and 20 min). Aliquots of each reaction were then transferred in a 1:10 dilution to separate incubation tubes containing fresh cofactors and SV (1 μmol/L final concentration). Each concentration tested was performed in duplicate. Reactions were allowed to incubate for an additional 10 min as described above, then by addition of cold acetonitrile and kept on ice. The internal standard (15 μmol/L testosterone solution) was added to each reaction vial. All remaining sample processing and HPLC analysis were then completed as described above.

**Data Analysis**

For all HPLC analysis, peak heights of the metabolite were expressed as a ratio to the internal standard (testosterone) peak height for each concentration of inhibitor. These peak height ratios represent the remaining CYP3A4 activity in the HLM and were expressed as a percentage of the time-matched control samples without inhibitor. This procedure takes into account the anticipated time-related loss of enzyme activity under the incubation conditions.

The calculation of the kinetic constants, which describe mechanism-based enzyme inactivators, was performed as described. Briefly, the relative inhibition of SV was determined by comparing the means of peak height ratios of time-matched samples with AMD versus control samples without AMD. The logarithm of relative inhibition values were plotted versus preincubation time for each concentration of AMD used, and the slopes determined by linear regression. These slope values represent the observed inactivation rate constants, which were used to calculate the half-life of the inactivation reaction. A Kitz-Wilson plot was then constructed using the calculated half-life values (y-axis) and the reciprocal of the associated inhibitor concentration (x-axis). The apparent K and k values were determined from the reciprocal of the y-axis intercept and the negative reciprocal of the x-axis intercept of the Kitz-Wilson plot, respectively. To predict SV’s change of AUC administered with AMD, the following equation (Eq. 1) was used.

\[
\frac{AUC_{p0}'}{AUC_{po}} = \frac{CL_{int}'}{CL_{int}} + \frac{k_{deg} + \frac{[I]k_{max}}{[I] - K_i}}{k_{deg}}
\]

Where AUC is the area under the concentration-time curve of SV, CL is substrate’s intrinsic clearance, AUCp0 and CL are the same meaning accordingly administered with inhibitor. [I] is the inhibitor concentration at the enzyme active site, K is the inactivator concentration required for half-maximal inactivation, kmax is the maximal rate constant of inactivation, kdeg is the degradation rate constant.

**Results**

A mechanism-based component was evident for AMD, as the product formation was significantly reduced (i.e., more potent inhibition) when the inhibitor was preincubated with HLM (Fig. 1). But the IC50 value still higher than 50 μmol/L with the absence or presence of the inhibitor.

The formation of main product of SV was inhibited by
increasing the preincubation time of AMD with HLMs in a concentration-dependent manner (Fig 2).

The Kitz Wilson plot of mean data points (Fig 3) indicated a K_i value of 5.11M, and a k_{inact} value of 0.018min^{-1}.

![Kitz Wilson plot of mean data points](image)

**Figure 2** Effects of preincubation time and AMD concentration on inhibition of product formation from SV.

![Kitz-Wilson plot](image)

**Figure 3** Kitz-Wilson plot of inactivation half-life (y-axis) versus reciprocal of AMD concentration (x-axis).

The average AMD concentration is 0.71-1.79 µmol/L. The plasma binding ratio of AMD was reported to be 96% and liver to plasma ratio was about 50 in rat. AMD’s unbound drug concentration in the human liver was calculated to be 1.41-3.58 µmol/L. The value of k_{inact} is assumed to be 0.00128min^{-1}. The values of AUC of SV incubated with AMD were calculated to be 3.96-6.63. That is, the fold of AUC of SV with the absence of AMD increased 2.96-5.63.

**Discussion**

When a certain drug acts as a mechanism-based inhibitor of CYPs, and irreversibly inactivates the isoforms, there will be time intervals before the liver synthesizes new enzymes to restore original activities. Consequently, the potential of inducing DDI will increase. Time- and NADPH-dependent inhibitions were observed, which concluded AMD as a mechanism-based inhibitor to CYP3A4.

These contain structures of a tertiary amine and a furan ring, which is reported to bind with CYP covalently after metabolism by the CYP may make AMD to be mechanism-based inactivators of CYP3A4. Verapamil has been reported to inactivate SV catalyzed by CYP3A4 with IC_{50} value of 23-26 µmol/L and k_{inact} value of 0.15±0.04 min^{-1}, K_i value of 2.9±0.6 µmol/L. The k_{inact} and K_i value of AMD for CYP3A4 activities obtained in this study were compared with those of verapamil. Therefore, it was suggested that AMD would be weaker mechanism-based inactivators of CYP3A4 than verapamil. But the terminal elimination half lives of AMD after long term oral treatment are approximately 40days or longer. Therefore, the inactivation of AMD might cause severe drug interactions.

When we discuss drug interaction via the inhibition of CYP activities, the unbound concentration of the inhibitor around the CYP enzyme in liver. In the study of James F, AMD’s concentration in liver was higher than that in plasma. To predict the extent of in vivo using the unbound concentration of AMD in liver will be more accurate. But in Becquemont L’s study, AMD’s concentration in liver was not shown. According our study, the prediction of extent was higher than the in vivo result. Firstly, the therapy duration is different. AMD was administered for 3 days in in vivo study, but in Asami K’s study the duration was beyond 6 months. The short duration may not evoke the inhibitor’s maximum effect. Secondly, total dose of AMD in in vivo study was about 1.2g, which is lower than Asami K’s study (total dose was 40.26-251.2g). It was reported the tissue AMD concentrations appeared to be related more closely to the total dose than to its plasma concentration. Thirdly, liver to plasma ratio used in prediction was of rats, which may be different from human beings’ Therefore, these may result in an underestimation of the magnitude of the interaction with SV.

**Myopathy, which can rarely present as rhabdomyolysis, is an uncommon but serious side-effect of statins treatment and occurs in 0.1% of patients treated with standard doses of SV.** There are some case reports about significant inhibition of CYP3A4-mediated metabolism of SV in vivo during chronic therapy. In these cases, the SV’s concentration was 40-80mg daily and AMD’s concentration was 200-600mg daily. Most side effects of statins are dose related. It was reported that Asians achieve similar benefits as Westerners when they were administered SV 20mg daily. So it is more safe using SV 20mg daily than 40mg daily at the same dosage of AMD. But the severe interaction may occur due to the interindividual difference and the prolongation of the treatment duration. It was demonstrated in human microsomes that mechanism-based inhibition of SV by AMD is the main cause of the drug-drug interaction. Pravastatin as a hydrophilic statin, which isn’t metabolized by CYP3A4 rarely occur drug interaction with AMD. Hydrophilic statins are recommended in order to avoid a drug interaction if AMD and a statin have to be simultaneously prescribed.

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References


