Drug Interaction between Simvastatin and Cholestryramine in Vitro and in Vivo

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The interaction between simvastatin (SV), a prodrug lactone, HMG-CoA reductase inhibitor which converts to the active hydroxy acid form (SVH) in vivo, and cholestryramine (CT), an anionic exchange resin, was evaluated both in vitro and in vivo.

In an in vitro SV-stability study, it was shown that SV degraded gradually to SVH in an aqueous solution at pH 2 and 7. To evaluate the binding ability of SV or SVH to CT, the incubation of 5 μg/ml of SV or SVH solution with 200 mg of CT in various pH (2.0, 5.0 and 7.0) solutions was performed at 37 °C for 10 min. After incubation, the concentration of SV decreased by 59.02% (pH 2), 63.90% (pH 5) and 67.36% (pH 7), respectively, and an interaction between SV and CT was suggested. The values were much larger than those expected from the stability test of SV in the absence of CT. SVH was found to bind more strongly to CT. The binding ability of SVH to CT was 66.71% (pH 2), 87.44% (pH 5) and 92.11% (pH 7), respectively. Judging from these results, SV was considered to interact with CT by the following procedure: SV underwent hydrolysis to SVH in aqueous solution, then CT activated the hydrolysis by binding the formed SVH, resulting in a significant reduction in concentration of SV.

On the other hand, an in vivo animal experiment also demonstrated a significant reduction (about 50% with AUC) in the concentration of SVH in plasma following the coadministration of SV (500 mg/kg p.o.) and CT (600 mg/kg p.o.), compared with the administration of SV alone. This phenomenon suggested that a combination therapy using SV and CT might result in a smaller cholesterol-lowering effect of SV.

Key words simvastatin; cholestryramine; drug interaction; binding

HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors, which competitively inhibit the activity of HMG-CoA, the rate-limiting enzyme in the biosynthesis of cholesterol, are widely used for the treatment of hyperlipidemia. Such drugs clinically used in Japan are pravastatin (PV) and simvastatin (SV). The chemical structure of PV is an active form of hydroxy acid, while SV is a prodrug lactone form which is converted to hydroxy acid in vivo.

Physicians often attempt a combination therapy with HMG-CoA reductase inhibitor and other lipid-lowering agents, i.e., cholestryramine (CT), probucol and bezafibrate, to treat familial hypercholesterolemia, for which monotherapy with HMG-CoA reductase inhibitor is not sufficient.

As to the combination therapy with PV and CT, a negative ion exchange resin, CT is known to bind to PV and thereby to decrease the absorption of PV from the intestine. Pan et al. reported that CT decreased the bioavailability of PV by 40% to 50% when given together in human. 3 Therefore, extending the administration interval between PV and CT is encouraged to avoid this binding when they are administered together in the clinical field.

As to a combination therapy with SV and CT, such care is not necessary because SV is not in an acid form. However, Serajuddin et al. reported that SV lactone might undergo hydrolysis below pH 4 and above pH 6. 3 Therefore, SV may also interact with CT depending on the pH of the gastrointestinal tract when the CT is administered.

This paper describes the in vitro and in vivo interaction between SV and CT. In addition, the stability of SV was examined in detail.

MATERIALS AND METHODS

Materials SV was kindly donated by Banyu Pharmaceuticals Co., Ltd., Tokyo, Japan. CT (Questran, Bristol Myers Squibb Co., Ltd., Tokyo, Japan, 9 g packets containing 4 g anhydrous CT) was offered from Nagasaki University Hospital Pharmacy. The hydroxy acid form of SV (SVH) was synthesized by the hydrolysis of SV at our laboratory. All other chemicals were of analytical reagent grade.

Determination of SV-Stability The SV solutions were prepared in universal buffers (0.1 M citric acid/0.2 M disodium phosphate) adjusted to pH levels of 2, 5 or 7. Ten ml of 5 μg/ml of the SV solutions at pH 2, 5 and 7 were incubated for 180 min in a waterbath at 37 °C. An aliquot of each sample was removed to another tube at set times after incubation and was injected immediately directly into the HPLC system for determination of the amounts of unchanged SV and the SVH formed.

The stability of SV in acetonitrile was also determined in the same manner.

Determination of Binding Rates to the CT of SV and SVH This study was performed for SV and SVH. The concentrations of drugs used were calculated from the usual dose in humans on a weight basis so that the ratio between the drugs and resin would approximate that likely to be encountered in clinical practice. Ten ml aliquots of 5 μg/ml of SV or SVH solution and 200 mg of CT were placed in 50-ml glass centrifuge tubes, followed by incubation for 10 min in a waterbath at 37 °C. After centrifugation of the test tubes, the supernatant was

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removed carefully. Then, the sample was injected immediately into the HPLC system for determining the amounts of remaining drug in the buffer solutions.

Regarding SV, the same study was also performed in acetonitrile.

**The Effect of SV-CT Coadministration to Rats** The effect of CT on the pharmacokinetics of SV was examined after the oral administration of SV alone or with CT to rats. For the present study, male Wistar rats, 8 weeks old (180—200 g), were used. Prior to the experiment, the jugular vein was cannulated.4) The rats were fasted overnight and were not allowed to take water for 2 h after dosing. Each sample solution of SV and CT was prepared by suspending in 0.5% methyl cellulose. Tested doses were as follows: 500 mg/kg for SV and 600 mg/kg for CT. One group of rats was given SV alone via a stomach tube for oral administration, and the other group was also given CT via a stomach tube just after the administration of SV. Blood was collected from the jugular vein at set times after the administration, and it was centrifugated to remove the plasma. The plasma sample was used to determine the drug concentration.

**Plasma Sample Preparations for HPLC** The plasma concentration of SV was determined by HPLC. To 100 μl of plasma sample, 50 μl of internal standard solution (9-methylnanthracene, 10 μg/ml) and 1 ml of acetonitrile for deproteinization were added with vigorous mixing. After centrifugation, the supernatant was evaporated to dryness under reduced pressure at 40°C and dissolved in the mobile phase. The solution was injected into the HPLC system.

**HPLC System** The HPLC system consisted of a Shimadzu pump LC-6A equipped with an ultraviolet detector (SPD-6A, Shimadzu, Kyoto, Japan) set at 238 nm. Analyses were performed at room temperature on a Cosmosil 5C18-AR column (Nacalai Tesque, Kyoto, Japan). The mobile phase was water—acetonitrile—triethylamine—glacial acetic acid (750:250:1:1, v/v).2) The flow rate was 2 ml/min.

**Data Analysis** The area under the curve (AUC0−∞) was calculated using a standard linear trapezoidal integration with extrapolation to infinite time. The significance of difference was determined using Student’s t-tests (p < 0.05).

**RESULTS**

**SV Stability in Vitro** SV was slightly decreased to form SVH in aqueous solution at pH 2 and 7, but not at pH 5 (Fig. 1). At each time point, the total concentration of remaining SV and formed SVH was equal to the initial concentration of SV. The elimination rate at pH 2 was more rapid than that at pH 7, the rate constants being 3.06 × 10−3 min−1 at pH 2 and 1.30 × 10−3 min−1 at pH 7, respectively. Good stability of SV was found at pH 5, as reported previously.2)

In contrast, SV in acetonitrile was stable until 180 min.

**Determination of Binding Rates of SV and SVH** SV-concentration was significantly decreased, by 59.02, 63.90 and 67.36% after incubation with 200 mg of CT for 10 min at pH 2, 5 and 7, respectively (Fig. 2). However, it was unclear whether this decrease was due to the binding of SV lactone to CT, or to the degradation of SV to SVH.

The same study was also performed in acetonitrile, where SV lactone is stable, to confirm whether or not SV lactone is bound to CT. As a result, the binding of SV lactone to CT was not observed.

Regarding the binding between SVH and CT, SVH was able to bind to CT by 66.71, 87.44 and 92.11% at pH 2, 5 and 7 for 10 min, respectively.

**In Vivo Study** The effect of CT on SV pharmacokinetics was evaluated according to the plasma level of SVH because SVH was converted rapidly to SVH in rat plasma.5) In this study, no SV peak appeared on the chromatogram. Figure 3 shows the plasma concentration of SVH after the oral administration of 500 mg/kg SV with or without 600 mg/kg of CT to rats. The AUC0−∞ of SVH was significantly decreased, from 10.36 μg·h/ml in the control to 5.06 μg·h/ml, following coadministration with CT (p < 0.05).
DISCUSSION

CT is known to interact with many anionic drugs by binding these drugs.\(^6\) In an in vitro study, CT caused a significant decrease in SV-concentration when SV was incubated with CT in an aqueous solution at various pHs. This suggested that SV also interacted with CT, though SV was not in an acid form. However, SV might not react with CT directly, because the SV concentration did not change when it was incubated with CT in acetonitrile. CT seemed to bind to SVH, which was formed by the hydrolysis of SV in an aqueous solution. However, the amount of SVH formed by hydrolysis was small because the hydrolysis of SV to SVH occurred slowly in an aqueous solution. On the other hand, the concentration of SV decreased considerably after reacting with CT (Fig. 2). Accordingly, the explanation of this interaction could not be offered only by the binding of the formed SVH to CT. Judging from these facts, it is reasonable to consider that CT displaces the balance of interconversion reactions between SV and SVH in favor of SVH by binding the formed SVH, resulting in a significant reduction in the concentration of SV. The high binding ability of SVH to CT also may support this consideration.

Regarding the in vivo study, CT caused a significant reduction in the concentration of SVH in plasma when 500 mg/kg of SV and 600 mg/kg of CT were administered together to rats. This result indicated that the interaction observed in the in vitro experiments between SV and CT also occurred in the rat gastrointestinal. In addition, the decreasing ratio of $AUC_{0-\infty}$ was approximately 50% in the in vivo study, and this ratio corresponded well with that (about 60%) of the SV concentration after incubation with CT in the in vitro study, indicating that there is a good relationship between the in vivo and in vitro experiments. Considering the fact that the plasma level of SVH was significantly reduced by CT, the pharmacodynamics of SV might be affected by the coadministration of CT, though the pharmacodynamics of SV could not be evaluated in this study, as HMG-CoA reductase inhibitors, including SV, had no cholesterol-lowering effect on rats.\(^7\)

It may be preferred to extend the administration interval between SV and CT in a similar manner to that of PV when the combination therapy for these drugs is carried out in the clinical field. If the administration interval is extended, this treatment effect may increase even more, though previous investigators have reported that the combination therapy reduced the low density lipoprotein-cholesterol level by 50 to 60% in an experiment involving the concomitant administration of SV and CT in humans.\(^8-11\)

REFERENCES