Pharmaceutical Approach to HIV Protease Inhibitor Atazanavir for Bioavailability Enhancement Based on Solid Dispersion System

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Atazanavir (ATV) is a low oral bioavailability (BA) compound and, clinically, is generally coadministered with ritonavir (RTV), which boosts the oral BA of ATV by inhibiting cytochrome P450 (CYP) 3A, and P-glycoprotein (Pgp) via the same metabolic pathway. However, depending on pharmacokinetic interaction, RTV-boosted ATV has great potential for other comedication. In this study we demonstrated the pharmaceutical approach to BA improvement of ATV without RTV in rats, based on the solid dispersion system using sodium laurel sulfate (SLS) as a carrier and Gelucire® 50/13 as an absorption enhancer. ATV solid dispersions in SLS were prepared by a conventional solvent method and, at ratios of ATV to SLS of 1:2 and 1:3, were demonstrated to form an amorphous state in powder X-ray diffraction (PXRD) analysis and exhibited 2.26- and 2.36-fold improvement in a dissolution test in comparison to bulk ATV, respectively. After oral administration to rats, ATV solid dispersion in SLS at a ratio of 1:2 showed a 3.5-fold increase in BA compared with bulk ATV. Moreover, the addition of Gelucire 50/13 to ATV solid dispersion, at a total ratio of Gelucire 50/13, ATV and SLS 1:1:2 gave 7.0- and 4.7-fold increase in Cmax and BA compared with bulk ATV, respectively, when the relative BA to RTV-boosted ATV reached 93%. The results in this study proved that a pharmaceutical approach could improve the bioavailability of ATV without pharmacokinetic interaction with RTV.

Key words atazanavir; solid dispersion; sodium laurel sulfate; gelucire 50/13; ritonavir; powder X-ray diffraction

The use of highly active antiretroviral therapy (HAART) has resulted in significant reductions in HIV-related mortality and morbidity and transformed HIV disease to a chronic syndrome.1,2) Despite improved clinical outcomes, in protease inhibitor (PI)-based HAART regimens, there are still many problems such as high pill burden, resistance to antiretrovirals and metabolic abnormalities (e.g., hyperlipidemia, hyperglycemia).3—5) Atazanavir (ATV) is an azapeptide compound and the seventh addition to the family of HIV PIs. ATV may have an advantage over other PIs because of once-daily dosing, distinct resistance profile, and a lack of insulin resistance and lipid increase.6,7) With these advantages, ATV has been successfully used in treatment-native and -experienced HIV patients.5) Similar to other PIs, however, ATV is poorly water-soluble and is known as a substrate of both hepatic metabolizing enzyme, cytochrome P450 (CYP) 3A, and intestinal drug efflux pump, P-glycoprotein (Pgp), thus resulting in low oral bioavailability (BA).9) Clinically, to resolve this disadvantage, ATV is generally used with low-dose ritonavir (RTV) in its HAART regimen. RTV is also classified as a PI and well known as a potent inhibitor of both CYP3A and Pgp. With this potent inhibition property, clinically, low-dose RTV has been demonstrated to boost the BA of ATV of another concomitant PI.10—12) On the other hand, HIV patients are typically treated with multiple other drugs in addition to their HAART regimen. Hence, based on pharmacokinetic interaction, RTV-boosted HAART has great potential for drug–drug interactions with CYP3A and Pgp.13,14) Moreover, it was also reported that RTV is not only a potent inhibitor but also a potent inducer of CYP3A and Pgp with chronic use.15,16) This contradictory characteristic of RTV would further complicate drug–drug interactions and facilitate the clinical difficulties of HIV patients whose HIV infection must be controlled by RTV-boosted HAART. Therefore, if possible, it is desirable to enhance the BA of ATV without RTV boost.

Although the enhancement of oral BA of poorly water-soluble drugs remains a challenging aspect of drug development, numerous pharmaceutical approaches has been investigated for enhancing dissolution properties and possibly BA. One of the most common is the solid dispersion method in which a drug is dispersed in a carrier to make it amorphous.17,18) In this study we demonstrated a pharmaceutical approach to the BA improvement of ATV without RTV in rats, based on a solid dispersion system using sodium laurel sulfate (SLS) as a carrier and Gelucire® 50/13 as an absorption enhancer.

MATERIALS AND METHODS

Materials ATV and RTV were extracted from commercially available capsules Reyataz® and Norvir®, respectively. SLS was supplied by Wako Pure Chemical Industries (Osaka, Japan) and Gelucire® 50/13 by Gattefosse (Saint-Priest, France). Hard gelatin capsules for animal use, size No. 9, were purchased from Qualicaps (Nara, Japan). Magnesium alumino metasilicates (Neusilín®, US2) were obtained from Fuji Chemical Industry (Toyama, Japan). Ethanol and all other reagents were of analytical grade and were used without further purification.

Animals All animal experiments were performed in accordance with the Guidelines for Animal Experimentation of Kyoto Pharmaceutical University. Male Wistar rats about 10 weeks old (250±20 g) were obtained from Nippon SLC Co., Ltd. (SLC, Hamamatsu, Japan). They had free access to food and water and were maintained in a temperature-controlled environment.
facility with a 12-h light/dark cycle for at least three days before use.

Preparation of Atazanavir Formulations The details of ATV formulations tested in this study are shown in Table 1. ATV solid dispersions in SLS at a ratio (w/w) of 1:1, 1:2 and 1:3 (SD1, SD2 and SD3) were prepared by the conventional solvent evaporation method as follows. ATV and the required amount of SLS were dissolved in a minimum volume of ethanol and this solvent was removed under vacuum at 70 °C and 45 rpm for 5 h. The resultant solid dispersion was kept in a refrigerator for 2 d to harden and placed in a vacuum oven at room temperature for another 2 d to remove any residual ethanol. The dispersions were then pulverized in a mortar and pestle, passed through a 750-µm sieve, and then stored in a desiccator at room temperature. The corresponding physical mixtures of ATV and SLS (Pmix1, Pmix2 and Pmix3) were obtained by blending both components through a 750-µm sieve. Physical mixtures of Gelucire 50/13 and ATV solid dispersion in SLS (Ge1/SD2 and Ge2/SD2) were also prepared by the following method. To obtain the solid state, Gelucire 50/13 was melted at 60 °C and Neusilin US2 (5% w/w) was added under stirring. After cooling to room temperature, solid Gelucire 50/13 was milled and passed through a 750-µm sieve, and then blended with the appropriate amount of ATV solid dispersion in SLS (SD2).

Powder X-Ray Diffraction (PXRD) PXRD analysis was performed with a linear X-ray diffraction system (RINT2500, Rigaku, Tokyo, Japan) in which CuKα rays (45 kV, 50 mA) were used as X-rays. The degree of diffraction was administered as hard gelatin capsules, size No. 9, at a dose of 7 mg/kg ATV. Bulk ATV alone and ATV coadministration with RTV (2 mg/kg) were also administered as negative and positive controls, respectively. To reduce the gastric pH and adjust to an environment that more closely mimics human physiology, all rats took the drug with 1% citric acid solution (1 ml/kg, 37 °C, pH 1.7). After administration, 0.25 ml aliquots of blood samples from the external left jugular vein were collected into heparinized centrifuge tubes at 0.5, 1, 2, 3, 4, 6, 8, and 12 h without constraint. Plasma samples were obtained by centrifuging the blood samples at 9000×g for 10 min, and immediately frozen at −80 °C until analysis by LC-MS.

Extraction Procedure and LC-MS Method The assay for ATV in plasma samples was performed by the previously reported LC-MS method. Briefly, 10 µl of nelfinavir (NFV, an internal standard, 100 µg/ml in methanol) and 100 µl of 2% ZnSO4 in 50% methanol solution was added to aliquots of 100 µl plasma sample in a 1.5 ml microcentrifuge tube and vortexed vigorously for 15 s. Diethyl ether (1 ml) was then added to the tube, vortexed for 30 s, and centrifuged at 12000×g for 5 min. The aqueous phase in test tubes was frozen in a cold bath at −10 °C and the ether phase was transferred to HPLC sample vials. The organic phase was evaporated to dryness at 70 °C in a water bath with air draught. The residues were reconstituted with 100 µl of mobile phase and then 30 µl was injected into the LC-MS system (Shimadzu), which consisted of the following components: a SIL-10A system controller, LC-10ADvp pump, SPD-10A UV detector, SIL-10ADvp automatic injector, CTO-10A column oven and an LC-MS-QP8000a mass spectrometer equipped with a CLASS-8000 work station. The analytical column for the separation of ATV was a Quicksorb ODS (2.1 mm i.d.×150 mm, 5 µm size, Chemco, Osaka, Japan), and the column temperature was maintained at 60 °C for all separations. Elution was carried out isocratically at a flow-rate of 0.2 ml/min with 90% acetonitrile containing 1% acetic acid. The mobile phase was degassed before use. Mass spectrometry was performed utilizing atmospheric pressure chemical ionization (APCI) in negative mode. The voltages of the APCI probe and the curved desolvolution line (CDL) were set at 5 kV and −30 V, respectively, and the flow rate of the nebulizing gas (N2) was set at 2.5 l/min. The temperatures of APCI probe and CDL were set at 400 and 250 °C, respectively. The voltages of defectors were set at −80 V. The peaks of ATV and NFV were detected as deprotonated ions at 705 and 568 m/z, respectively. ATV was quantified by calculating the peak area ratio of ATV against NFV.

Pharmacokinetic Analysis Noncompartmental pharma-

### Table 1. Compositions and Codes of ATV Formulations Tested in This Study

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Code</th>
<th>ATV</th>
<th>SLS</th>
<th>Gelucire 50/13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical mixture of ATV and SLS (1:1)</td>
<td>Pmix1</td>
<td>1</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Physical mixture of ATV and SLS (1:2)</td>
<td>Pmix2</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Physical mixture of ATV and SLS (1:3)</td>
<td>Pmix3</td>
<td>1</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Solid dispersion of ATV in SLS (1:1)</td>
<td>SD1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Solid dispersion of ATV in SLS (1:2)</td>
<td>SD2</td>
<td>1</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Solid dispersion of ATV in SLS (1:3)</td>
<td>SD3</td>
<td>1</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Physical mixture of Gelucire 50/13 and SD2 (1:1)</td>
<td>Ge1/SD2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Physical mixture of Gelucire 50/13 and SD2 (1:3)</td>
<td>Ge2/SD2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*The figures inside the parentheses represent the ratio (w/w) of each component.*
cokinetic analysis was applied to the plasma concentration–time data using a computer program, WinHARMONY.21) The terminal elimination rate constant (λz) was determined by the linear regression of at least three data points from the terminal portion of the plasma concentration–time plots. The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule up to the last measured plasma concentration (Cp(last)), and extrapolated to infinity using a correction term, namely Cp(last)/λz. The area under the first-moment curve to the last measured plasma concentration (AUMC) was also calculated using the linear trapezoidal rule and the addition of the concentration term after the last measured point (t(last)) to infinity, namely, t(last)Cp(last)/λz + Cp(last)/λz. The terminal elimination half-life (t1/2) was determined by dividing ln2 by λz. The mean residence time (MRT) was calculated by dividing AUMC by AUC. Total body clearance (Cl) was calculated by Di/AUC, where D represents the dose administered. Relative bioavailability (RBA) of tested formulations to the positive control, RTV-boosted ATV, was obtained by dividing AUC of each tested formulation and a positive control, and the AUC of each formulation, respectively.

Statistical Analysis All values are expressed as the mean±S.E. Statistical differences of the means were assumed to be significant when p<0.05 by one-way ANOVA followed by Dunnett’s multiple comparison.

RESULTS

Powder X-Ray Diffraction (PXRD) PXRD patterns obtained with ATV alone, SLS, Pmix3, SD1, SD2 and SD3 are shown in Fig. 1. The powder X-ray diffractogram of bulk ATV showed a distinctive peak at 19.06° 2θ and numerous peaks at 14—25° 2θ that indicated high crystallinity (Fig. 1a). A diffractogram of physical mixture of ATV and SLS (Pmix3) exhibited characteristic peaks derived from crystalline ATV and SLS (Fig. 1c). On the other hand, in case of solid dispersions (SD1, SD2 and SD3), the distinctive peak of crystalline ATV decreased as the ratio of SLS increased (Figs. 1d, e, f), and virtually disappeared at a ratio of 1 : 2 and 1 : 3 (SD2 and SD3). These observations suggest that ATV solid dispersions in SLS at a ratio of 1:2 and 1:3 could be dispersed homogeneously in an amorphous state.

In Vitro Dissolution Study The dissolution profiles of bulk ATV, solid dispersions and the corresponding physical mixtures are shown in Fig. 2 and the dissolution parameters obtained by moment analysis are shown in Table 2. The mean amount of dissolution (AD) of bulk ATV was 4.26±0.09 mg in this dissolution study. At a 1:1 ratio of ATV to SLS, the AD of the physical mixture and solid dispersion (Pmix1 and SD1) were 3.15±0.37 and 4.19±0.31 mg, respectively, and there was no improvement in comparison with bulk ATV, although MDT of Pmix1 and SD1 were significantly shorter than that of bulk ATV (13.93±1.99, 16.94±0.82 vs. 26.60±0.98 min, respectively). By increasing the ratio of SLS, however, the AD of the physical mixtures and solid dispersions were increased. Moreover, at the same ratio of SLS, solid dispersions were higher than corresponding physical mixtures. In the formulation of SD2 and SD3, especially, faster and complete dissolution was observed, when the relative % of AD to bulk ATV was 226.8 and 232.5, respectively.

In Vivo Oral Administration Study Figure 3 shows the mean plasma concentration profiles of ATV after oral administration of bulk ATV as a negative control, physical mixture (Pmix2), solid dispersions (SD1, SD2 and SD3) or RTV-boosted ATV as a positive control. The profiles of physical mixtures of Gelucire 50/13 and ATV solid dispersion in SLS (Ge1/SD2 and Ge2/SD2) are shown in Fig. 4. The pharmacokinetic parameters of ATV are summarized in Table 1. The Cmax and AUC0—inf of bulk ATV was 0.09±0.01 μg/ml and
DISCUSSION

The aim of this work was to improve the bioavailability of ATV by a pharmaceutical approach without RTV boost, a pharmacokinetic approach. In this study we focused on the solid dispersion system that has been used as a formulation for improving the solubility and bioavailability of poorly water-soluble drugs. Law et al.\(^{22,23}\) prepared RTV solid dispersions in polyethylene glycol 8000 at various ratios and demonstrated that 10% RTV solid dispersion gave a 22-fold increase in AUC compared with crystalline RTV after oral administration to dogs. There are many factors in the enhanced solubility of solid dispersions, such as decreased particle size of drug,\(^{24}\) specific form of drug,\(^{25}\) increasing drug wettability,\(^{26}\) and preventing drug aggregation by the carrier. Among them, the primary explanation is that transformation of the crystalline drug to the amorphous state could contribute to its dissolution enhancement.\(^{27-29}\) On the other hand, Lee et al.\(^{30}\) prepared solid dispersion microspheres of cyclosporine A using SLS and dextrin as carriers and reported that after oral administration to dogs, the AUC of cyclosporine A microspheres was 1.7-fold higher than that of cyclosporine A powder alone. SLS is well known as an ionic surfactant and has been used as an excipient for improving solubility and BA.\(^{31}\)

In this study using SLS as a carrier, we prepared an amorphous solid dispersion of ATV by the conventional solvent evaporation method (Fig. 1). As shown in Fig. 2, SD1 showed a rapid release of ATV within 30 min comparison to bulk ATV; however, there was no significant difference in AD value between SD1 and bulk ATV. In SD2 and SD3, these dissolution profiles were very similar and dramatically improved both dissolution rate and amount. Moreover, comparing with the corresponding physical mixtures, Pmix2 and Pmix3, further improvements were observed in amorphous

Table 3. Pharmacokinetic Parameters of ATV after Oral Administration Equivalent to 7 mg/kg ATV

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>C(_{\text{max}}) (µg/ml)</th>
<th>t(_{\text{max}}) (h)</th>
<th>t(_{\frac{1}{2}}) (h)</th>
<th>MRT (h)</th>
<th>CL(_{\text{tot}}) (l/h/kg)</th>
<th>V(_{\text{dss}}) (l/kg)</th>
<th>AUC(_{0—\infty}) (µg/ml·h)</th>
<th>RBA(^{a}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk ATV</td>
<td>0.09±0.01</td>
<td>3.6±0.6</td>
<td>2.54±0.44</td>
<td>5.68±0.35</td>
<td>11.14±1.59</td>
<td>63.92±9.90</td>
<td>0.70±0.13</td>
<td>19.6</td>
</tr>
<tr>
<td>Pmix2</td>
<td>0.35±0.02</td>
<td>2.4±0.2</td>
<td>2.79±0.36</td>
<td>5.38±0.47</td>
<td>4.02±0.42**</td>
<td>20.99±1.18**</td>
<td>1.83±0.20</td>
<td>51.1</td>
</tr>
<tr>
<td>SD1</td>
<td>0.33±0.05</td>
<td>2.6±0.5</td>
<td>2.54±0.14</td>
<td>4.88±0.23</td>
<td>4.56±0.33**</td>
<td>22.56±2.86**</td>
<td>1.56±0.10</td>
<td>43.6</td>
</tr>
<tr>
<td>SD2</td>
<td>0.59±0.02*</td>
<td>2.0±0.2*</td>
<td>2.18±0.17</td>
<td>3.96±0.21</td>
<td>2.84±0.03**</td>
<td>11.21±0.62**</td>
<td>2.47±0.02**</td>
<td>69.0</td>
</tr>
<tr>
<td>SD3</td>
<td>0.70±0.09**</td>
<td>2.1±0.3</td>
<td>2.68±0.21</td>
<td>4.43±0.36</td>
<td>3.32±0.30</td>
<td>14.16±1.41**</td>
<td>2.40±0.28**</td>
<td>67.0</td>
</tr>
<tr>
<td>SD2/Ge1</td>
<td>0.63±0.14**</td>
<td>2.6±0.4</td>
<td>3.63±1.37</td>
<td>6.33±1.42</td>
<td>2.19±0.21</td>
<td>13.34±2.72**</td>
<td>3.33±0.34**</td>
<td>93.0</td>
</tr>
<tr>
<td>SD2/Ge2</td>
<td>0.77±0.14**</td>
<td>1.8±0.2*</td>
<td>1.68±0.29</td>
<td>3.59±0.26</td>
<td>2.93±0.32**</td>
<td>10.45±1.20**</td>
<td>2.50±0.26**</td>
<td>69.8</td>
</tr>
<tr>
<td>RTV-boosted ATV</td>
<td>0.67±0.16**</td>
<td>2.8±0.2</td>
<td>1.41±0.34</td>
<td>4.43±0.38</td>
<td>2.26±0.50**</td>
<td>9.56±1.47**</td>
<td>3.58±0.57**</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^{a}\) Relative % of AD was obtained by comparison with that of the mean of bulk ATV. Each value represents the mean±S.E. of 4 experiments. *p<0.05 against the bulk ATV; **p<0.01 against the bulk ATV.
solid dispersion. These results suggested that the 1 : 1 ratio of ATV to SLS was relatively too low to form a complete amorphous state and the optimum ratio appeared to be 1 : 2. Moreover, dissolution improvements of ATV were caused by not only the surfactant effect of SLS itself but the transition of crystalline ATV to the amorphous state by SLS.

In an in vivo study, we coadministered ATV with RTV (RTV-boosted ATV) to rats, at clinical relevant dosages of 7 and 2 mg/kg, respectively, as a positive control, and bulk ATV alone at 7 mg/kg as a negative control. After the oral administrations of SD2 and SD3, their ATV plasma concentration profiles were very similar and the AUC0-∞ was 3.5- and 3.4-fold higher than that of bulk ATV, when their tmax were 2.0 and 2.1 h, respectively. These in vivo enhancements of AUC were well correlated with in vitro improvements. However, the RBAs of SD2 and SD3 were 69.0 and 67.0%, respectively, still insufficient in comparison to the positive control.

Therefore, in order to obtain the further improvement of the in vivo BA of ATV, we considered it would be needed to add an absorption enhancer to the SD2 formulation. Some of absorption enhancers, however, are in conflict with the aim of this study because their absorption enhancements depend on the inhibition of CYP and Pgp. Thus, we focused Gelucire 50/13 as an absorption enhancer, which would not affect CYP and Pgp. Gelucire 50/13, saturated polyglycolized glycerides, consists of mono-, di- and triglycerides and mono- and di-fatty acid esters of polyethylene glycol. The two numbers in its name correspond to its melting point and hydrophilic-lipophilic balance value, respectively. Gelucire 50/13 has been shown to increase the BA of orally administered drugs.32 In the original protocol, we expected that maximum improvements would be observed in ATV solid dispersion in both SLS and Gelucire 50/13 as carriers. Therefore, we prepared it by the solvent method and performed in vitro dissolution and in vivo oral administration studies. The results of both studies, however, unexpectedly showed that it was significantly less effective than ATV solid dispersion in only SLS, SD2 (data not show). We considered that these results were caused by the physicochemical properties of ATV itself. ATV solubility intensively depends on the pH value of the medium and ATV oral absorption also strongly depends on gastric pH. Accordingly, the dissolution amount of ATV in the stomach plays a dominant role in its absorption process.33,34 On the other hand, by the Biopharmaceutics Classification System, ATV is classified into a ClassII compound which has high permeability and low solubility. Moreover, when RTV-boosted ATV was directly administered in the duodenum instead of oral cavity to rats, the plasma concentration of ATV, however, was not detected (data not show). Consequently, we concluded that the most effective approach for BA enhancement of ATV could be the dissolution improvement in the stomach rather than the permeability improvement in the small intestine. The dissolution rate of Gelucire 50/13 at low pH, such as the environment in the stomach, is relatively lower than that of ATV. As a result, Gelucire 50/13 as a carrier of solid dispersion sets a limit on the release of ATV from its matrix and the following absorption. Consequently, we concluded that Gelucire 50/13 would be unsuitable as a carrier of ATV solid dispersion.

To make use of both the dissolution improvement of SLS and absorption enhancement of Gelucire 50/13, we tried another approach with Gelucire 50/13 adds to SD2 formulation simply by physically mixing (Ge1/SD2 and Ge2/SD2). After oral administration of Ge2/SD2 to rats, although the mean Cmax was highest in all administrations including the positive control, RBA did not show the further improvement with comparison to SD2, probably due to relatively high ratio of Gelucire 50/13 (40%). On the other hand, maximum improvement was observed in the Ge1/SD2 formulation, when the AUC was 4.7-fold higher than bulk ATV and the RBA was very close to the positive control (93%). Few studies have focused on the mechanism of Gelucire 50/13 in the absorption process of drugs and there is no concrete evidence. Gelucire 50/13 is capable of forming a sub-micron emulsion when it comes into contact with the physiological fluids in the small intestine.35 By forming an emulsion, Gelucire 50/13 may prevent solubilized ATV in the stomach from precipitating and re-crystallizing in a higher pH environment like the small intestine, thus resulting in the enhancement of BA, although there is no evidence in this study.

In conclusion, we prepared an amorphous solid dispersion of ATV in SLS by the conventional solvent method, which was used in vitro and in vivo. Moreover, in comparison to bulk ATV, the physical addition of Gelucire 50/13 as an absorbent to the ATV solid dispersion gave comparable improvement in rats to the clinical regimen, RTV-boosted ATV. The results in this study proved that a pharmaceutical approach could improve the bioavailability of ATV without pharmacokinetic interaction by RTV.

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