Physicochemical and Pharmacokinetic Characterization of Amorphous Solid Dispersion of Tranilast with Enhanced Solubility in Gastric Fluid and Improved Oral Bioavailability

Satomi ONOUE 1,*, Yoshiki KOJO 1, Yosuke AOKI 1, Yohei KAWABATA 1, Yukinori YAMAUCHI 2 and Shizuo YAMADA 1
1Department of Pharmacokinetics and Pharmacodynamics and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan
2Department of Pharmaceutical Physical Chemistry, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, Japan

Summary: In the present study, amorphous solid dispersion (ASD) formulations of tranilast (TL) with 8 hydrophilic polymers were prepared by a solvent evaporation method with the aim of improving dissolution behavior in gastric fluid and thereby enhancing oral bioavailability. The physicochemical properties were characterized with a focus on morphology, crystallinity, thermal behavior, dissolution, drug-polymer interaction, and stability. Of all TL formulations, ASD formulation with Eudragit EPO exhibited the highest improvement in dissolution behavior with a 3,000-fold increase in the first-order dissolution rate under acidic conditions (pH 1.2). Spectroscopic studies using infrared and near-infrared analyses revealed the drug-polymer interaction in the Eudragit EPO-based ASD formulation. On the basis of dissolution, crystallinity, and stability data, the maximum allowable drug load in the Eudragit EPO-based ASD formulation was deduced to be ca. 50%. Pharmacokinetic profiling of orally dosed TL formulations in rats was also carried out using UPLC/ESI-MS. After oral administration of the Eudragit EPO-based ASD formulation in rats, enhanced TL exposure was observed with an increase of oral bioavailability by 19-fold, and the variation of AUC was ca. 4 times lower than that with crystalline TL. With these data, the ASD approach could be a viable formulation strategy for enhancing the wettability and oral bioavailability of TL, resulting in improved therapeutic potential of TL for the treatment of inflammatory diseases.

Keywords: tranilast; amorphous; solid dispersion; dissolution; bioavailability

Introduction

Tranilast (TL) [N-(3,4-dimethoxy cinnamoyl) anthranilic acid] inhibits the release of inflammatory mediators from mast cells and basophils,1,2 so it has been clinically used for the treatment of inflammatory diseases such as bronchial asthma, atopic rhinitis, and atopic dermatitis.3 Recently, attention has been drawn to the therapeutic potential of TL for various chronic diseases, including hepatic fibrosis,4,5 pulmonary fibrosis,6,7 and cancer.8-10 In spite of the attractive biological functions of TL as an anti-allergic agent, its bioavailability after oral administration is low with high variability,11 possibly leading to limited clinical outcomes. The solubilities of TL in water and acidic medium (buffer solution of pH 1.2) were found to be 14.5 and 0.7 µg/mL, respectively,12 suggesting poor dissolution behavior of TL in the gastrointestinal tract, particularly the stomach. Generally, drug release is a crucial and limiting step for oral drug bioavailability, particularly for biopharmaceutical classification system (BCS) class II drugs with low gastrointestinal solubility and high permeability. Therefore, improvement of the dissolution characteristics in gastric fluid could be a promising approach to enhance the oral bioavailability and therapeutic potential of TL for clinical treatment of inflammatory diseases.
To improve the aqueous solubility of poorly soluble drugs, several types of approaches have been proposed, which include solubilization using good solvents or co-solvent mixtures, solid state manipulation, particle engineering, solubilization by lipids, and solubilization with complexing agents. In particular, dissolution and solubility enhancement can be achieved by dispersing the poorly soluble drug in a solid matrix carrier, either as a eutectic or phase-separated mixture, or as an amorphous solid dispersion (ASD). Previously, our group developed a crystalline solid dispersion (CSD) system of TL employing wet milling technologies. The CSD formulation of TL exhibited rapid dissolution behavior in water and improved oral bioavailability compared with those of crystalline TL. However, recent work demonstrated that ASD formulation of tacrolimus, an immunosuppressive agent, exceeded its CSD formulation in terms of both aqueous solubility and dissolution behavior. These observations prompted us to develop new ASD formulations of TL (ASD-TL) using various polymers and apply them to oral delivery systems, with the aim of enhancing the dissolution properties and oral bioavailability of TL with low variation.

In the present investigation, new ASD-TLS with eight hydrophilic polymers were prepared by a solvent evaporation method. Various ASD-TLS with drug loading of 10% were characterized by scanning electron microscopy (SEM) for morphology, polarized light microscopy (PLM) and powder X-ray diffraction (PXRD) for crystallinity, differential scanning calorimetry (DSC) for thermal behavior, and dissolution testing. Possible interaction between TL and the polymer was assessed by Fourier transform infrared (FT-IR) and near-infrared (NIR) spectral analyses. The formulation was optimized on the basis of results from dissolution and stability tests on the ASD-TL with various drug loads (10–90%). Pharmacokinetic (PK) profiling of TL after the oral administration of the optimized ASD-TL in rats was conducted using ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry (UPLC/ESI-MS).

### Materials and Methods

**Chemicals:** Crystalline TL was supplied from Kissei Pharmaceutical Co., Ltd. (Nagano, Japan). Amorphous TL was obtained by amorphization of crystalline TL using a solvent evaporation method. Briefly, 1 mg of crystalline TL was dissolved in 10 mL of 90% (v/v) 1,4-dioxane solution, and the solution was freeze-dried using an FD-81 freeze-dryer (Tokyo Rikakikai, Tokyo, Japan). Amorphization was checked by PXRD and DSC. Hydroxypropyl methylcellulose (HPMC) was provided by Shin-Etsu Chemical (Tokyo, Japan), and Kollidon VA64 and polyvinylpyrrolidone (PVP) were provided by BASF Japan (Tokyo, Japan). Eudragit EPO was supplied by Degussa Japan (Tokyo, Japan). Potassium bromide (KBr), 1,4-dioxane, hydrochloric acid (HCl), hydroxypropyl cellulose (HPC), and ammonium acetate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol (liquid chromatography grade) was purchased from Kanto Chemical (Tokyo, Japan). All other chemicals were purchased from commercial sources.

**ASD formulation of TL:**

**Preparation**

A solvent evaporation method was used for preparation of ASD formulation containing 10% (w/w) TL. Briefly, crystalline TL (30 mg) and hydrophilic polymer (270 mg) were dissolved in 17 mL of 90% (v/v) 1,4-dioxane solution. The solution was mixed well and frozen at −80 °C. The sample was freeze-dried using an FD-81 freeze-dryer. After drying, the sample was sieved with two different mesh sizes of sieves (1 and 2 mm).

**UPLC/ESI-MS analysis of TL**

The amount of TL in the obtained dry powder was determined by an internal standard method using a Waters Acquity UPLC system (Waters, Milford, MA, USA), which included a binary solvent manager, sample manager, column compartment, and SQD connected with MassLynx software. An Acquity UPLC BEH C18 column (particle size: 1.7 μm, column size: 2.1 mm × 50 mm; Waters) was used, and the column temperature was maintained at 50 °C. The standard (diclofenac) and samples were separated using a gradient mobile phase consisting of methanol (A) and 5 mM ammonium acetate (B) with a flow rate of 0.25 mL/min. The gradient conditions of the mobile phase were 0–0.5 min, 30% A; 0.5–3.5 min, 30–70% A; 3.5–4.0 min, 90% A; and 4.0–4.5 min, 30% A. Peaks for TL and the internal standard were detected at retention times of 2.56 and 3.45 min, respectively. Analysis was carried out using selected ion recording (SIR) for specific m/z 326 and 294 for TL [M−H]⁻ and diclofenac [M−H]⁻, respectively.

**Powder X-ray diffraction (PXRD):** A PXRD pattern was collected using D8 ADVANCE (Bruker AXS GmbH, Karlsruhe, Germany) with Cu Kα radiation generated at 40 mA and 35 kV. Data were obtained from 4° to 40° (2θ) at a step size of 0.014° and scanning speed of 4°/min.

**Thermal analysis:** DSC was performed using a DSC Q1000 (TA Instruments, New Castle, DE, USA). The DSC thermograms were collected in an aluminum closed-pan system using a sample weight of ca. 3 mg and a heating rate of 5 °C/min with a nitrogen purge at 70 mL/min. The temperature axis was calibrated with indium (ca. 5 mg, 99.99999% pure, onset at 156.6 °C).

**Scanning electron microscopy (SEM):** Representative SEM images of TL samples were taken using a scanning electron microscope, VE-7800 (Keyence Corporation, Osaka, Japan), without Au or Pt coating. For the SEM observations, each sample was fixed on an aluminum sample holder using double-sided carbon tape.

**Polarized light microscopy (PLM):** Representative PLM images of TL samples were taken using a CX41
microscope (Olympus Co. Ltd., Tokyo, Japan). TL samples were examined under various conditions including differential interference contrast and slightly uncrossed polaris, and using a red-wave compensator.

**Dissolution test:** Dissolution testing on TL and ASD formulations of TL (10%, w/w) with various polymers was carried out for 120 min in 900 mL of HCl solution (pH 1.2) with constant stirring of 50 rpm in a dissolution test apparatus NTR 6100A (Toyama Sangyo, Osaka, Japan) at 37°C. Each powder sample (450 µg of TL) was weighed in the dissolution vessel (final concentration of TL: 0.5 µg/mL). Dissolution testing on ASD formulations of TL (10–70%, w/w) with Eudragit EPO was conducted under a supersaturated condition. Briefly, each powder sample (3.15 mg of TL) was weighed in 900 mL of HCl solution (pH 1.2), and final concentration of TL in the dissolution vessel was 3.5 µg/mL, corresponding to ca. 5 times higher concentration than the equilibrium solubility of TL (0.7 µg/mL) at pH 1.2. Analyzed samples (0.6 mL) were collected at the indicated times (5, 10, 20, 40 and 60 min for dissolution testing under sink conditions; and 6, 10, 30, 60, 90 and 120 min for dissolution testing under supersaturated conditions) with a Toyama W-PAS-615 auto-sampler (Toyama Sangyo). The initial volume of dissolution medium was maintained by adding 0.6 mL of deionized water. The collected samples were diluted with an equal volume of methanol, and filtered through a 0.2-µm membrane filter (Millipore, Millipore, Billerica, MA, USA). The concentrations of TL were determined by Waters UPLC/ESI-MS as described in Materials and Methods (UPLC/ESI-MS analysis of TL).

**Spectroscopic analyses:**

*Fourier transform infrared (FT-IR) spectroscopy*

FT-IR analysis was conducted to evaluate drug-polymer interaction. Briefly, powder samples were prepared by mixing approximately 2 to 3 mg of TL and the physical mixture or ASD formulation with approximately 300 mg of KBr, and the mixture was pressed for preparation of the KBr disk. Spectra were recorded on IR Prestige-21 with IR solution software (Shimadzu, Kyoto, Japan), and 40 scans were performed with a resolution of 4 cm⁻¹.

*Near-infrared (NIR) spectroscopy*

NIR analysis was also carried out for further characterization of possible interaction between TL and polymer. Briefly, powder samples of TL and the physical mixture or ASD formulation were placed in cylindrical glass vials, and NIR spectroscopic analysis (32 scans) using a Bruker MPA system with a diffuse-reflectance integrating-sphere (Bruker Optics, Ettlingen, Germany) was performed in reflectance mode with a resolution of 2 nm over a range of 800 to 2,800 nm.

**Stability testing:** Stability study of the TL samples was carried out at 40 ± 2°C/75 ± 5% relative humidity (RH) or 60 ± 2°C for 4 weeks in a stability chamber (Labcare Pvt. Ltd., Mumbai, India). Samples after storage were subjected to the UPLC/ESI-MS, XRPD, DSC, and PLM experiments.

**Pharmacokinetic studies:**

*Animals*

Male Sprague–Dawley rats, weighing ca. 400 g (11 weeks of age; Japan SLC, Shizuoka, Japan), were housed two per cage in the laboratory with free access to food and water, and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). All procedures used in the present study were conducted in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka.

**Pharmacokinetic study**

After intravenous administration of TL (0.5 mg/kg) dissolved in DMSO, or oral administration of TL samples (10 mg TL/kg) suspended in 1 mL of distilled water, blood samples were obtained at a volume of 400 µL from the tail vein of unanesthetized rats at the indicated times (0.25, 0.5, 1, 3, 6, 12 and 24 h). Each blood sample (400 µL) was centrifuged at 10,000×g to prepare serum samples. The serum samples were kept frozen at below −20°C until they were analyzed. TL concentrations in serum were determined by UPLC/ESI-MS. In brief, 100 µL of methanol was added to 50 µL of serum sample, and the solution was centrifuged at 3,000 rpm for 10 min. The supernatant was filtered through the 0.2-µm filter, and then the filtrate was analyzed by UPLC/ESI-MS, as described in Materials and Methods (UPLC/ESI-MS analysis of TL). The pharmacokinetic parameters for TL were calculated by means of noncompartmental methods using the WinNonlin® program (Ver. 4.1, Pharsight Corporation, Mountain View, CA, USA).

**Statistical analysis:** For statistical comparisons, one-way analysis of variance (ANOVA) with pairwise comparison by Fisher’s least significant difference procedure was used. A p value of less than 0.05 was considered significant for all analyses.

**Results and Discussion**

**Physicochemical characterization of ASD formulations:** In the present study, ASD formulations of TL were prepared by a solvent evaporation method with the use of various polymers, including HPC(SSL), HPC(L), HPC(H), Eudragit EPO, HPMC, PVP(K30), PVP(K90), and Kollidon VA64 (Table 1). An ASD formulation can be defined as a distribution of active ingredients in amorphous form surrounded by inert carriers. To clarify amorphization of TL in the SD formulations, the physical state of each formulation was evaluated by PXRD and DSC (Fig. 1). As shown in Figure 1A, several intense peaks were observed in the PXRD pattern of crystalline TL, and they were indicative of the most stable anhydrous form (Form I). In contrast, all the ASD formulations of TL exhibited a halo diffraction pattern (Fig. 1A and Table 1), and typical peaks for Form I or other known crystalline forms were negligible. In addition
to PXRD analysis, the thermal behaviors of ASD formulations were studied for characterization of the physicochemical status of TL in these formulations. According to the DSC thermograms of several forms of TL (Fig. 1B and Table 1), crystalline TL produced a melting endotherm at 212°C, the thermal event of which was almost identical to that of Form I as reported previously.\textsuperscript{15} None of the prepared ASD formulations of TL displayed any thermal events in the examined temperature range. The lack of endothermal peaks of TL in ASD formulations suggested that TL in these formulations might exist in a high-energy amorphous state.

ASD formulation can be classified according to the molecular interaction of drug and carriers in solid solutions, solid suspensions, or a mixture of both.\textsuperscript{16} In particular, drug and carrier are totally miscible and soluble in amorphous solid solutions, developing a homogeneous molecular interaction between them. On the basis of formulation process and physicochemical data, the ASD formulations of TL that we prepared might be amorphous solid solutions.

For further characterization, SEM and PLM experiments were carried out for ASD formulations of TL (Fig. 2). In the SEM images, crystalline TL showed particles that were predominantly dispersed and irregularly shaped, with sizes ranging over about 10–100 µm (Fig. 2A-I), whereas the ASD formulation of TL had the appearance of typical, flaky freeze-dried material (Figs. 2A-II, 2A-III and 2A-IV).

Table 1. Physicochemical properties of TL formulations

<table>
<thead>
<tr>
<th>Molecular weight of polymers (Da)</th>
<th>PXPD</th>
<th>DSC (endotherm)</th>
<th>PLM</th>
<th>Initial dissolution rate (h\textsuperscript{-1}; mean ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalline TL</td>
<td>—</td>
<td>Form I 212°C</td>
<td>Intense birefringence</td>
<td>0.0042 ± 0.0020 (0.907)</td>
</tr>
<tr>
<td>Solid dispersion of TL with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxypropylcellulose (HPC-SSL)</td>
<td>15,000–30,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>6.8 ± 1.4 (0.982)</td>
</tr>
<tr>
<td>Hydroxypropylcellulose (HPC-L)</td>
<td>55,000–70,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>5.4 ± 1.5 (0.956)</td>
</tr>
<tr>
<td>Hydroxypropylcellulose (HPC-H)</td>
<td>250,000–400,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>0.80 ± 0.10 (0.996)</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose (HPMC)</td>
<td>ca. 86,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>4.1 ± 1.6 (0.999)</td>
</tr>
<tr>
<td>Polynylpyrrolidone (PVP K30)</td>
<td>ca. 40,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>3.1 ± 0.53 (0.917)</td>
</tr>
<tr>
<td>Polynylpyrrolidone (PVP K90)</td>
<td>ca. 360,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>2.7 ± 0.28 (0.972)</td>
</tr>
<tr>
<td>Polynylpyrrolidone-polyvinylacetate copolymer (KollidonVA64)</td>
<td>45,000–70,000</td>
<td>Halo Pattern</td>
<td>Negligible Weak birefringence</td>
<td>13 ± 2.7 (0.949)</td>
</tr>
<tr>
<td>Polymacrylates and polymethacrylates (Eudragit EPO)</td>
<td>ca. 150,000</td>
<td>Halo Pattern</td>
<td>Negligible No birefringence</td>
<td>16 ± 3.0 (0.911)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are correlation coefficient values.

Copyright © 2012 by the Japanese Society for the Study of Xenobiotics (JSSX)
compared with that of crystalline TL alone. Although TL in the new formulations was expected to be amorphous, PLM appearance revealed that TL molecules in most ASD formulations might be slightly recrystallized during the lyophilizing process as evidenced by weak birefringence (Table 1 and Fig. 2B). Interestingly, only ASD formulation of TL with Eudragit EPO (ASD-TL/EUD) showed no birefringence (Fig. 2B-IV). In general, detection sensitivity of PLM for crystallinity assessment is much higher than that of other analytical techniques such as PXRD and DSC. This could partially explain the data discrepancy. Only a very small amount of crystalline TL might be contained in most ASD formulations, whereas TL in the ASD-TL/EUD could be amorphized more completely. The enthalpy, entropy, and free energy of an amorphous drug can be much higher than those of its crystalline counterpart, so that the excess free energy of an amorphous TL may result in improvement in its dissolution behavior.

Dissolution behavior of ASD formulations: To clarify possible enhancement of dissolution behavior by ASD approaches, dissolution testing for ASD formulations was carried out in HCl solution (pH 1.2) to simulate gastric conditions (Fig. 3). Initial dissolution of TL from the powders was likely to follow first-order kinetics, and the corresponding first-order dissolution rates are given in Table 1. Poor dissolution behaviors were seen in crystalline TL at a first-order dissolution rate of 0.004 h⁻¹, and the physical mixture of TL and polymer also exhibited limited dissolution behavior in a similar manner as crystalline TL (data not shown). There was marked improvement in dissolution behavior in most ASD formulations of TL. Among all the ASD formulations tested, ASD formulation of TL with Eudragit EPO and Kollidon VA64 showed the highest concentration of dissolved TL compared with that of crystalline TL, the first-order dissolution rates of which were calculated to be ca. 3,000-fold higher than that of crystalline TL. These observations were consistent with previous reports, showing that the formulation of poorly soluble drugs as SD could lead to a marked improvement in the dissolution properties. Typical mechanisms for the improvement of dissolution characteristics of drugs by the ASD approach are reduction in particle size, the absence of crystallinity, and improved wettability. On the basis of the present findings, taken together with the preparation process, the presence of all the hydrophilic polymers tested might have a beneficial effect on wettability of TL, possibly by a homogeneous molecular interaction of drug and polymers.

As observed in the PXRD and DSC analyses, most TL could adopt an amorphous state in the ASD powders,
around the carboxylate moiety of TL samples. For further elucidation, second derivative NIR spectra of TL samples were superimposed (Fig. 4B). As observed in FT-IR spectral analysis, crystalline/amorphous TL and the physical mixture of TL and copolymer exhibited similar spectral patterns in the bands at 1,880–1,920 nm, representing the second overtones of the carboxylic acid C=O stretch.21 Interestingly, a clear transition was observed in the ASD-TL/EUD with an intense negative peak at 1,910 nm and a positive peak at 1,885 nm. These data provided further evidence for the interaction between TL and the copolymer. Eudragit EPO is a cationic copolymer based on dimethylaminoethyl methacrylate and neutral methacrylic esters, so the cationic functional group in the copolymer can electrostatically interact with a carboxylic acid of the TL molecule in the ASD formulation. The interaction with the polymer might be attributable to the improved dissolution behavior of TL and stabilization of the amorphous state. In addition to the solid-state physicochemical properties, the cationic copolymer can dissolve in solution of pH < 5; therefore, the copolymer tends to dissolve fast in gastric fluid. Dissolved copolymer might also interact with TL molecule in a medium, and the electrostatic interaction could lead to prevention of precipitation or re-crystallization.

Preferable loading amount of TL in a Eudragit EPO-based ASD formulation: Despite the development of extensive expertise with ASD formulations, they are not widely used in commercial products, mainly because there is a possibility that the amorphous state may undergo crystallization during processing or storage.16 In particular, drugs at higher concentration are often present in the crystalline form within ASD formulations or re-crystallize over time, resulting in unstable formulations with lower dissolution rates. In the present study, several ASD-TL/EUDs with various drug concentrations ranging from 10 to 90% were prepared for comparison. Preliminary PLM experiments demonstrated that partial re-crystallization occurred in the Eudragit EPO-based formulations of TL with drug concentrations of 80% or higher (data not shown). For further optimization, dissolution testing under supersaturated conditions (ca. 5 times higher than the equilibrium solubility of TL at pH 1.2) was carried out for ASD-TL/EUDs with various drug concentrations under 70% (Fig. 5). All the ASD-TL/EUDs exhibited rapid dissolution behavior, and complete supersaturation was achieved with the 2 h testing period. The ASD-TL/EUDs with a drug concentration of under 50% showed a tremendous increase in dissolution rate as evidenced by ca. 4–5-fold greater concentration of the dissolved TL than equilibrium solubility (Ceq) of TL at 5 min. Interestingly, reduced dissolution behavior was seen in the ASD-TL/EUDs with drug concentrations of over 60% in a drug load-dependent manner. The much higher supersaturated concentration and its duration have the potential to enhance the oral bioavailability of this poorly water-soluble drug.
Amorphous Solid Dispersion of Tranilast with Improved Solubility and Bioavailability

Temperature and humidity stresses sometimes affect the storage stability of amorphous drugs since they may increase drug mobility and promote drug crystallization. Since these physicochemical transitions may lead to a decreased solubility and dissolution rate of an ASD formulation of TL, a solid-state stability study was conducted for the ASD-TL/EUDs with a drug concentration under 50% (Table 2). The ASD-TL/EUD was stored at 60°C or 40°C/75% RH for 2 or 4 weeks, followed by PLM experiments to evaluate possible transition to a crystalline state. There were no significant changes in PLM observations of ASD formulations stored at 60°C, suggesting no re-crystallization during storage. These findings were consistent with the results from PXRD and DSC analyses on these formulations (data not shown). In contrast, weak birefringence was observed in ASD-TL/EUDs with a drug concentration of 50% stored at 40°C/75% RH for 4 weeks, although the physicochemical transition was negligible in both PXRD and DSC analyses. The very slight re-crystallization of TL might be caused only in an ASD formulation with a relatively high drug load. The Eudragit EPO used in the present study can absorb moisture, which may result in phase separation, crystal growth, or conversion from the amorphous to the crystalline state during long-term storage. Since slight re-crystallization was observed in the ASD-TL/EUD with a drug concentration of 50% after storage at 40°C/75% RH for 4 weeks, dissolution testing in acidic solution (pH 1.2) under supersaturated conditions was also carried out to clarify any possible reduction in dissolution behavior. Although the aged ASD-TL/EUDs with drug concentrations of 50% still exhibited rapid dissolution in acidic solution, the degree of supersaturation ($C/C_{eq}$) decreased to ca. 2.6 (data not shown). Thus, there was a 42% reduction in maximum supersaturation of TL after storage at 40°C/75% RH for 4 weeks, and this finding was consistent with the PLM observation on the aged ASD-TL/EUDs with a drug concentration of 50%. From the present results, moisture protection might be needed to prevent physicochemical transition of ASD formulations of TL.

Currently, TL is administered orally at a dose of 300 mg/day in Japan for the clinical treatment of several inflammatory diseases. In general, poorly water-soluble drugs have to be administered at a high dose; therefore, only a small amount of excipients can be added to the formulation in order to preclude difficulties relating to patient compliance. Thus, it is important to be able to produce SD formulations containing as much of the active pharmaceutical ingredient as possible. On the basis of the present findings on crystallinity, dissolution behavior, and solid-state stability, the maximum load with TL for Eudragit EPO was deduced to be as much as 50%, although moisture protection might be needed for long-term stability.

**Pharmacokinetic profiling of the Eudragit EPO-based ASD formulation:** The observations on the improved dissolution properties of ASD-TL prompted us to clarify the possible improvement in the oral bioavailability of TL, so the pharmacokinetic behaviors of crystalline TL and ASD-TL/EUD with a drug load of 50% were assessed in rats. The blood concentration–time profiles of TL in rats after oral administration of crystalline TL and ASD-TL/EUD (10 mg TL/kg) are shown in Figure 6, and relevant pharmacokinetic parameters including $C_{max}$, $T_{max}$, AUC$_{0-infty}$, and absolute bioavailability are listed in Table 3. Serum TL levels in the blood were found to be very low when crystalline TL was administered orally, and the $C_{max}$ and AUC$_{0-infty}$ values were 0.1 µg/mL and 0.8 µg·h/mL, respectively. The blood concentration of TL decreased gradually with apparent elimination kinetics of 0.17 h$^{-1}$. On the basis of the AUC$_{0-infty}$ value (3.29 µg·h/mL) of intravenously administered TL (0.5 mg/kg), absolute bioavailability of crystalline TL was calculated to be as low as 1.2%. In contrast, ASD-TL/EUD showed improved pharmacokinetic behavior compared with crystalline TL. Oral administration of ASD-TL/EUD resulted in rapid elevation of TL blood levels up to $C_{max}$ 4.6 µg/mL, and the AUC$_{0-infty}$ value was

---

**Table 2. Stability test on ASD formulation with Eudragit EPO (ASD-TL/EUD)**

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Loading amount</th>
<th>PLM observations on ASD-TL/EUD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No birefringence</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>N.C.</td>
</tr>
<tr>
<td>2 weeks 60°C</td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
<tr>
<td>2 weeks 40°C/75% RH</td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
<tr>
<td>4 weeks 60°C</td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
<tr>
<td>4 weeks 40°C/75% RH</td>
<td>N.C.</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

N.C.: not changed.

---

Copyright © 2012 by the Japanese Society for the Study of Xenobiotics (JSSX)
calculated to be 14.6 µg·h/mL. Absolute bioavailability of ASD-TL/EUD was calculated to be ca. 22%. Thus, there appeared to be ca. 19-fold enhancement in the oral bioavailability of TL with the use of the ASD approach. These findings were relatively consistent with the results from the dissolution test, demonstrating the accelerated dissolution behavior of ASD-TL/EUD. Interestingly, there was ca. 70% reduction of inter-individual variation in AUC from ASD-TL/EUD [coefficient of variation (CV): 13%] compared with that from crystalline TL (CV: 50%). Since solubility of TL is variable depending on the pH of the solvent medium, the individual variability in gastric condition or gastric dysfunctions may affect the drug release in gastric fluid and thereby cause inconsistent absorption. However, ASD formulations exhibited a marked increase in solubility of TL at acidic pH, and this might be attributable to improved and consistent absorption with low variation in bioavailability.

Previously, our group developed a CSD formulation of TL, in which fine crystalline particles of TL were dispersed in solid water-soluble matrices.11 There was marked improvement in the dissolution behavior of the CSD formulation compared with that of crystalline TL, and pharmacokinetic data on the CSD formulation were indicative of high systemic exposure with an increase of oral bioavailability by ca. 31-fold for crystalline TL. In this context, the ASD approach was slightly less effective for improving oral bioavailability than the CSD formulation strategy, although clinical application of the ASD formulation of TL might also provide better clinical outcomes than current TL-based medication for inflammatory or fibrotic diseases. In spite of its pharmaceutical usefulness, there would be difficulty in scaling-up for the manufacture of the CSD formulation, so the development of new optimized manufacturing techniques with high scalability have been attempted in academic and industrial research. In addition, the CSD approach employing wet-milling techniques might be unsuitable for pharmaceutical substances with low melting points since the generation of friction heat would result in partial amorphization during the wet-milling process. In contrast, potent scalable manufacturing processes to obtain ASD formulations have been developed, which include the melting method, the solvent-evaporation method, and the solvent-wetting method.16 These formulation techniques would be applicable to poorly water-soluble drugs with a low melting point as an alternative to the CSD approach. On the basis of physicochemical and pharmacokinetic properties, taken together with their manufacturability, ASD approaches can be a viable dosage option for improving the therapeutic potential of TL through enhancement of its wettability and oral bioavailability.

### Conclusion

In the present study, several ASD formulations of TL were prepared by a solvent evaporation method. There was significant improvement in the dissolution behavior of most ASD formulations, especially the ASD formulation with Eudragit EPO, which exhibited the highest dissolution behavior with better amorphization. Spectroscopic analyses on the ASD formulation suggested the interaction of TL with the copolymer, and the maximum drug load was deduced to be ca. 50% on the basis of the results from dissolution and stability studies. According to the pharmacokinetic profiles of ASD-TL/EUD, there was significant improvement in the systemic exposure of TL with increases in oral bioavailability by ca. 19-fold. From these observations of improved dissolution and pharmacokinetic behaviors, the ASD approach could be a promising formulation strategy for enhancing the bioavailability of TL, possibly leading to better clinical outcomes for the treatment of inflammatory and fibrotic diseases.

### Acknowledgments:

The authors are grateful to Kissei Pharmaceutical Co., Ltd. (Nagano, Japan), for kindly providing tranilast.

### References


