Development of an Extended-Release Formulation for Apremilast and a Level A in Vitro–in Vivo Correlation Study in Beagle Dogs

Meiqiong Tang, Ping Hu, Shigui Huang, Qiang Zheng, Hao Yu, and Yun He

Apremilast (Fig. 1) is an oral small molecule inhibitor of type-4 cyclic nucleotide phosphodiesterase (PDE-4) which has a high therapeutic index and was developed by Celgene Company. Apremilast was introduced to the clinic and recently approved for the treatment of psoriatic arthritis and plaque psoriasis in the U.S.A. and Europe. Unlike tumor necrosis factor (TNF)-α inhibitors, which bind directly to TNF-α, apremilast causes a broad inhibition of multiple pro-inflammatory mediators such as interleukin (IL)-6, IL-10 and TNF-α, exerting therefore an overall anti-inflammatory effect.

The marketed immediate-release (IR) formulation of apremilast was launched under the brand name of Otezla® (Celgene), which was designed to be orally administered at a dose of 10, 20 or 30 mg twice daily. Apremilast demonstrates rapid absorption ($t_{\text{max}}$ ca. 2.5 h), mean half-life of 6–9 h, clearance calibrated by bioavailability (CL/F) of ca. 10 L/h and absolute bioavailability of ca. 73%. The recommended dosage of oral apremilast for most patients is 30 mg twice daily, taken with or without food. The dosage of apremilast should be titrated in order to reduce the risk of gastrointestinal adverse events.

For the nature of chronic diseases, a long term treatment for psoriatic arthritis and plaque psoriasis with apremilast is generally recommended. Tolerability or dose regimen issues possess limitations which may significantly impair patient compliance and therefore overall efficacy of treatment. Thus, an alternative drug delivery system is urgently needed to improve the poor tolerability and the need for frequent daily dosing during long-term regimen with IR formulations.

A better approach would be to develop extended-release (ER) formulations for once-daily administration of apremilast, which can not only to improve the dosing, but also to affect tolerability and efficacy. A more uniform plasma drug profile with fewer occasions when super- or subtherapeutic concentrations (peak and trough) of the plasma concentration that occur with multiple daily dosing with IR formulations would be ideal for the patients. By controlling the parameters of drug release, ER formulations offer the potential to optimize treatment and, decrease the occurrence of certain adverse effects at the same time.

In this study, apremilast extended-release matrix tablets were prepared by utilizing hydroxypropylmethylcellulose (HPMC) to form the matrix in order to achieve extended-release in dissolution media. Being a semi-synthetic hydrophilic matrix polymer, HPMC has been widely employed in the design of extended-release formulations due to its good compression, rapid hydration and gelling characteristics as well as its ease of use and very low toxicity. The in vitro release of apremilast from the developed formulations were studied and supported by Korsmeyer–Peppas equation as it presented highest values of correlation coefficients ($r^2$ up to 0.966). Among all formulated tablets, F2 (HPMC 25%) and F4 (HPMC 35%) were selected to perform an in vivo study in beagle dogs to obtain various pharmacokinetic parameters, i.e., peak plasma concentration ($C_{\text{max}}$), area under the plasma-concentration vs. time curve (AUC). Higher $t_{\text{max}}$ and $t_{1/2}$, lower $C_{\text{max}}$ and elimination coefficient ($K_{\text{e}}$) were observed for both extended formulations compared to marketed immediate-release products (Otezla®). Level A in vitro–in vivo correlations were created with the help of Wagner–Nelson and numeric deconvolution methods. Both formulations showed good in vitro–in vivo correlations whose accuracies were further verified by an internal validation.

Key words drug release; absorption; deconvolution; pharmacokinetics; in vitro–in vivo correlation

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studied and fitted to various mathematic models.\textsuperscript{18–20} The pharmacokinetics studies were performed in beagle dogs after oral administration of different formulations of ER matrix tablets and the marketed IR products. Level A (point to point) \textit{in vitro—\textit{in vivo} correlations (IVIVC)} were established employing model-dependent Wagner—Nelson\textsuperscript{21,22} and model-independent numerical deconvolution methods\textsuperscript{23} and were assessed by internal validation.\textsuperscript{24}

**Experimental**

**Materials** Apremilast was synthesized at Liangjiang Medicine Ltd. (Chongqing, China). HPMC K100LV was purchased from Dow Chemical (Shanghai, China). Lactose monohydrate was purchased from Meggle (Guangzhou, China). Magnesium stearate and silicon dioxide were purchased from Sunhere Pharmaceutical Excipients Ltd. (Anhui, China). Internal standard clopidogrel was purchased from the National Institute for the Control of Biological and Pharmaceutical Drugs (Shenyang, China). Formic acid and methanol (analytical grade) were purchased from VWR (Sichuan, China). Double distilled water was prepared with a water purification system (PCD-II, Chengdu, China).

**Preparation of Apremilast Matrix Tablets** Apremilast HPMC matrix tablets were prepared by formulations containing different amounts of HPMC. The active pharmaceutical ingredient (API) was ground by a pulverizer (QLM, Shenzhen, China). As shown in Table 1, the excipients and apremilast were sieved through a 30-mesh sieve and mixed in a 3D mixer (GH, Shunheji, Shanghai China) for 20 min. The mixed excipients and API were granulated using a dry granulator (LGCI00, Guolongli Automation, Beijing, China). Magnesium stearate and silicon dioxide were added as lubricant and mixed for another 5 min. All formulations containing 60 mg apremilast were compressed into keyboard shape tablets by a multi-punch tablet machine (GLI45, compression punch: 17×7 mm, Guolongli Automation). The tablets were coated with thin-film coating material (Opadry 8SF94245 containing polyvinyl alcohol, titanium oxide, polyethylene glycol, hydrated magnesium silicate and iron oxide red. Colorcon, Shanghai) until reaching a 2% weight gain compared to the uncoated tablets by a coating machine (BGB-5F, Xiaolun Pharmaceutical, Zhejiang, China).

**Hardness and Friability of the Prepared Tablets** The uncoated and coated tablets form each formulation were measured for crushing strength using a hardness tester (YD-35, Shanghai, China). The friability of the tablets was measured by a coating machine (BGB-5F, Xiaolun Pharmaceutical, Zhejiang, China).

**Table 1. Composition of Apremilast Matrix Tablets (F1–5)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1 (mg)</th>
<th>F2 (mg)</th>
<th>F3 (mg)</th>
<th>F4 (mg)</th>
<th>F5 (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apremilast</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Lactose</td>
<td>360</td>
<td>330</td>
<td>300</td>
<td>270</td>
<td>240</td>
</tr>
<tr>
<td>HPMC K100LV</td>
<td>120</td>
<td>150</td>
<td>180</td>
<td>210</td>
<td>240</td>
</tr>
<tr>
<td>Silicon dioxide</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
<td>600</td>
</tr>
</tbody>
</table>

**In Vitro Dissolution Test** Dissolution profiles for each ER formulation were measured in the United States Pharmacopeia (USP) XXIII, apparatus 2 (paddle method) (RC8MD, Tiantianfa Technology) set at a speed of 50 rpm, with 900 mL of distilled water, pH 4.0 and pH 6.8 50 mM phosphate buffers dissolution media, pre-warmed at 37.0°C. Five milliliters of dissolution samples were extracted at 1, 2, 3, 4, 6, 9, 12, 16, 20 and 24 h. The volume of dissolution media was kept constant by adding an equal volume of fresh dissolution media at the same temperature immediately after the sample collection. Sink condition was maintained during the sample extraction.\textsuperscript{25,26} After filtration through a membrane filter (0.45 μm) and dilution with distilled water, 50 μL of the samples were injected into the HPLC as described below. The result was calculated as percent drug release and graphed against time.

**HPLC Conditions** The HPLC analysis system (Shimadzu, Japan) consisted of a pump (Model LC-20AT), Shimazu DGU-20A interface, ultraviolet detector (Model SPD-20A), autosampler (Model SIL-20A) and a column oven (Model CTO-20A). A C18 analytical column (ZORBAX 5 μm, 4.6 mm×150 mm, Agilent, U.S.A.) was used. The mobile phase consisted of acetoniitride and 0.1% formic acid solution (isocratic wash off) at 40°C. The UV absorbance of the effluent (1.0 mL/min) was assayed at the wavelength of 230 nm.

**Pharmacokinetic Study**

**In Vivo Experiments** A pharmacokinetic crossover study was carried out in six healthy beagle dogs with apremilast ER tablets and marketed IR tablets. After being fasted overnight prior to dosing, each animal received the three tested formulations (ER formulation 2, ER formulation 4 with 60 mg of apremilast and commercially available IR formulation Otezla\textsuperscript{a} with 30 mg of apremilast) at different period. A wash-out period of one week was allowed between the different treatments. Five milliliters of blood was collected into heparin-vacutainer tubes from the forearm vein before administration (at 0 h) and at predetermined time intervals of 0.166, 0.333, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48 and 72 h after oral administration. The samples were separated as aliquots and stored at −80°C until further analysis. All experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University.

**Blood Sample Analysis Using LC-MS/MS** The concentrations of apremilast in beagle dog plasma were analyzed by an LC-MS/MS method. Briefly, to a 200 μL aliquot of plasma sample, 10 μL of acetonitrile containing clopidogrel (40 ng/mL) as the working internal standard, 50 μL of 5% formic acid solution in water and 1.3 mL of tert-butylmethylether were added and then vortexed for 3 min. After
centrifugation of the sample at 3000×g for 5 min, the organic layer was collected and dried by nitrogen gas and reconstituted with 100 µL of methanol. Five microliters of the reconstituted samples were injected and separated on a Capcell Pak C18 reversed phase column (5 µm, 50×2.0 mm, Shisheido, Japan) using gradient elution with acetonitrile and 0.1% formic acid solution at a flow rate of 0.4 mL/min. The column temperatures were 35°C. The eluent was introduced directly to a tandem quadrupole mass spectrometer with a positive ionization electrospray source (API 3000, Applied Biosystem, MA, U.S.A.). The collision energy was 30 eV for apremilast and clopidogrel. Precursor product ion transitions of m/z 478.3→178.4 and m/z 322.1→184.0 were monitored to quantify apremilast and clopidogrel, respectively. Linear calibration curves were obtained for the concentration range of 2–1000 ng/mL with the lower limit of quantification (LOQ) of apremilast was 2 ng/mL. Data acquisition and processing were accomplished using Analyst 1.4.2 software.

Pharmacokinetic Analysis
Pharmacokinetic parameters such as area under drug–concentration time curve (AUC) were calculated and analyzed with compartmental models using (DAS 3.2.7, Drug China, Shanghai, China). The time required to reach maximum plasma concentration (tmax) and maximum plasma concentration of drug (Cmax) were computed directly from the plasma concentration vs. time plot. All the data is reported as the mean±S.D.

In Vitro–in Vivo Correlations IVIVC

IVIVC Model
To create the IVIVC model, in-house in vitro data from ER formulation (60 mg) were correlated with the corresponding in vivo data. Two formulations with different release rates were chose for IVIVC establishment in that the U.S. Food and Drug Administration (FDA) guidance requires that an IVIVC model be created with products having at least two different release rates.2,3) To create the IVIVC model, in vivo data was deconvoluted using Wagner–Nelson method so that it could be properly matched with in vitro data from the same time point. Numerical deconvolution and convolution method was also employed to establish IVIVC and more importantly, to validate the accuracy of the established IVIVC models.

Wagner–Nelson Method
The Wagner–Nelson deconvolution method was applied to the in vivo data to determine the fraction of the drug absorbed.3,2) It assumes a one compartment pharmacokinetic model for the pharmacokinetic process of the drug. The Wagner–Nelson relationship can be described by the Eq. 1.

\[
\frac{(X_A)_{\text{t}}}{(X_A)_{\text{inf}}} = \frac{C_{(t)} + K \cdot \int_{0}^{T} Cdt}{K \cdot \int_{0}^{\infty} Cdt} \quad (1)
\]

where \(X_A\) is the fraction of absorption, \(C_{(t)}\) is the concentration of drug in the plasma at time \(t\), \(K\) is the rate constant of elimination, The rate of elimination \((K)\) was obtained by taking the negative slope of a time vs. In (concentration) plot for the final three time points of the plasma concentration of the IR formulation. \(\int_{0}^{T} Cdt\) is the calculated area beneath the plasma concentration curve from time zero to time \(T\). \(\int_{0}^{\infty} Cdt\) represents the AUC of time zero to infinite time, which was calculated by trapezoid method.

Deconvolution-Convolution
To convolute the in vitro dissolution data, a spreadsheet function was created that required an input of the predicted milligrams of drug dissolved at various time points. The convolution function consists of two parts, the rate function \(u(t)\) and the cumulative input function \(\int_{0}^{t} u(t) dt\). Generally, the convolution of the two functions, \(u(t)\) and \(\int_{0}^{t} u(t) dt\) is expressed as Eq. 2.

\[
\int_{0}^{t} u(t) dt = \int_{0}^{t} u(t) dt + \int_{t}^{2t} u(t) dt + \cdots + \int_{(n-1)t}^{nt} u(t) dt \quad (2)
\]

For IVIVC, \(\int_{0}^{t} u(t) dt\) is a “cumulative input function,” representing the total absorption in vivo. \(\int_{0}^{t} u(t) dt\) is, often expressed as Eq. 3.

\[
\frac{1}{A_k}B_k + \frac{1}{A_{k-1}}(B_k - A_{k-1}) + \cdots + \frac{1}{A_1}(B_k - A_{k-1} - A_{k-2} - \cdots - A_1) \quad (3)
\]

Where \(B\) is the blood concentration of ER tablets and \(A\) is the blood concentration of IR tablets as the unit impulse response, \(r\) is the absorption in unit interval. Both in vivo absorption fractions and the predicted blood concentrations of the ER formulations were calculated with the Eq. 3. The application of the IVIVC model allowed the prediction of the quantity absorbed (in nanograms) at each time point. Using this information the rate function \(u(t)\) was convoluted numerically at each time point. The rate and convolution calculations were carried out using Microsoft Office Excel® 2003 (Microsoft Corp., Redmond, WA, U.S.A.).

Prediction Error
The prediction error (%PE) calculation was used to quantitatively determine how well a given model can accurately predict a pharmacokinetic parameter of drug. The %PE of both Cmax and AUC are calculated using the same formula. In this formula the observed value is subtracted from the value predicted in the model, and the resulting value is then divided by the observed value. The value is then converted to a percentage by multiplying by 100.

\[
\% \text{PE} \ AUC = \left( \frac{\text{AUC}(\text{obs}) - \text{AUC}(\text{pred})}{\text{AUC}(\text{obs})} \right) \times 100
\]
\[
\% \text{PE} \ C_{\text{max}} = \left( \frac{\text{C}_{\text{max}}(\text{obs}) - \text{C}_{\text{max}}(\text{pred})}{\text{C}_{\text{max}}(\text{obs})} \right) \times 100
\]

Results and Discussion
Preparation and Characterization of Apremilast Matrix Tablets
Apremilast (60 mg) matrix tablets (F1–5) containing different amounts of the polymer HPMC K100LV were prepared according to the composition shown in Table 1. Hardness and friability as the physiochemical properties were measured and presented in Table 2. The hardness of the formulated products was between 9.7 and 11.8 kg. The friability was in the range of 0.2 and 0.5%. The tablets passed hardness and friability tests were coated with the coating materials until reaching 2% weight gain. The hardness of coated matrix tablets were tested and demonstrated identical results compared to the uncoated ones. Friability of coated tablets has been considerably reduced due to the protection of the coated thin film (data not shown).

In Vitro Dissolution Studies of Coated Matrix Tablets
The interaction between drug, water and polymer was the
Table 2. Hardness and Friability of Apremilast Matrix Tablets (Uncoated)

<table>
<thead>
<tr>
<th>Test</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (kg)</td>
<td>10.5±0.6</td>
<td>11.3±1.1</td>
<td>10.4±0.7</td>
<td>9.7±0.5</td>
<td>11.8±0.4</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.32±0.04</td>
<td>0.24±0.03</td>
<td>0.41±0.05</td>
<td>0.48±0.06</td>
<td>0.59±0.04</td>
</tr>
</tbody>
</table>

Fig. 2. Dissolution Profiles of Formulation 1 with Different APIs in Water

(A): API-1: crystal form A with $D_{50}=233.4\,\mu m$, API-2: crystal form B with $D_{50}=35.2\,\mu m$, API-3: crystal form A with $D_{50}=32.6\,\mu m$, API-4: crystal form A with $D_{50}=4.9\,\mu m$, API-5: crystal form B with $D_{50}=5.2\,\mu m$. (B): Summary of the time of 50% release of each formulation with different API.

Fig. 3. Dissolution Profiles of Apremilast from Formulation F1 to F5 in (A) pH 4.0 Acetate Buffer, (B) Distilled Water, and (C) pH 6.8 Phosphate Buffer

F1 to F5 represents the formulation containing 10% apremilast crystal form B and 20, 25, 30, 35, 40% HPMC, respectively. (D) Summary of the time of 50 and 80% release of formulation F1 to F5 in pH 6.8 PBS. The experiment was conducted in USP Apparatus 2 at 50rpm and in dissolution media at 37°C. Each value represents the mean±S.D. ($n=12$).
primary factor to control the drug release. As a BCS Class IV drug, apremilast possesses a very poor solubility in water, which presumably influences the dissolution of the drug in aqueous media. In order to achieve an acceptable dissolution profile, careful choice of crystal forms of the API or reduction of the API particle sizes were widely applied to improve the dissolution. In this study, crystal forms A and B which possess different nature of particle sizes from crystallization were used to test their difference in dissolution using water as the dissolution medium. As Fig. 2 shows, the tablets composed of original apremilast crystal form A \((D_{50}=233.4 \mu m)\) shows a much slower release rate compared to original crystal form B \((D_{50}=35.2 \mu m)\) (Fig. 2A, API-1, -2). The tablets composed of ground crystal form A with a reduced particle \((D_{50}=32.6 \mu m)\) shows a similar release profile comparing to that of crystal form B (Fig. 2A, API-3). Moreover, when the particle size of crystal form A and B was furtherly reduced to smaller distributions \((D_{50}=4.9, 5.2 \mu m, \text{respectively})\), the release profiles remain almost identical to those of API 2 and 3 (Fig. 2A, API-4, -5). The result indicated that the tablets composed of original crystal form A demonstrated a much slower release rate compared to that of original crystal form B as the API crystal with larger size takes longer time to be dissolved and diffused into dissolution media. However, once the particle size of crystal forms reached around 35 \(\mu m\) or even smaller, the release rates of the tablets were very close (Fig. 2B), which suggested that the release of apremilast from the prepared tablets could be greatly influenced by API particle sizes (or crystal forms), although such influence could be minimized by selection an appropriate crystal form of API or reduction the API particle sizes to a certain level.

The cumulative release profiles of apremilast from all formulations in acetate buffer pH 4.0, distilled water, and phosphate buffer pH 6.8 were tested and shown in Fig. 3. Generally all the formulations showed similar release profiles in these different dissolution media. A comparably high initial burst release (about 30\% in 2 h) of Formulation F1 was observed in all dissolution media as well as a faster release pattern (about 90\% in 6 h) of apremilast compared to formulation F2 to F5. This could be explained by the low amount of polymers present in the formulation F1. Formulations F2 to F5 showed extended-release dissolution profiles up to 16 h with low initial burst release (below 20\% in 2 h). Since the ER formulations will be mainly residing in the lower part of the gastrointestinal tract with a pH value around 6.8, the release data in pH 6.8 dissolution medium was processed for further analysis and IVIVC calculation. Time of 50 and 80\% release \(t_{50\%} \text{ and } t_{80\%}\) of formulations in pH 6.8 dissolution media were calculated and summarized in Fig. 3D, which showed that as the percentage amount of HPMC was increased from 20 to 40\%, the release rate, which can be typically represented by \(t_{50\%} \text{ and } t_{80\%}\), gradually decreased. The effect of coating for the matrix tablets were tested in pH 6.8 dissolution media. As expected, the dissolution profiles were almost identical (data not shown).

### Table 3. Correlation Coefficients and Rate Constant for Release Data of Apremilast Matrix Tablets after Fitting Zero Order, First Order, Higuchi and Peppas Models

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Peppas order</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(k_0 (%h^{-1}))</td>
<td>(r^2)</td>
<td>(k_1 (%h^{-1}))</td>
<td>(r^2)</td>
</tr>
<tr>
<td>F1</td>
<td>7.53</td>
<td>0.628</td>
<td>31.90</td>
<td>0.705</td>
</tr>
<tr>
<td>F2</td>
<td>6.25</td>
<td>0.775</td>
<td>27.75</td>
<td>0.872</td>
</tr>
<tr>
<td>F3</td>
<td>5.81</td>
<td>0.799</td>
<td>25.11</td>
<td>0.890</td>
</tr>
<tr>
<td>F4</td>
<td>5.19</td>
<td>0.874</td>
<td>20.10</td>
<td>0.893</td>
</tr>
<tr>
<td>F5</td>
<td>4.57</td>
<td>0.902</td>
<td>16.88</td>
<td>0.933</td>
</tr>
</tbody>
</table>

Zero-order \((Q=k_0 t)\), first-order \((\ln(100-Q)=\ln Q_0 - k_1 t)\), Higuchi models \((Q=k_2 t^{1/2})\), and Korsmeyer–Peppas \((Q=k t^n)\), where \(Q=M/M_\infty\).

![Fig. 4](image-url)  
(A) Pharmacokinetic Profile of Apremilast in Beagle Dogs after Oral Administration of Marketed Immediate Release Tablet (IR, 30mg), Developed Fast-Extended Release Formulation (ER-F2, 60mg) and Slow-Extended Release Formulation (ER-F4, 60mg) at a Single Once-Daily Dose; (B) Summary of the \(t_{max}\) and \(C_{max}/D\) of Three Tested Formulations

(A) Each value represents the mean±S.D. (n=6).
Table 4. Pharmacokinetic Parameters of Apremilast Delivered by Marketed Immediate Release Tablet and Developed Extended Release Formulation F2 and F4 after Oral Administration to Beagle Dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IR</th>
<th>ER-F2</th>
<th>ER-F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{\text{last}}$ (ng·h/mL)</td>
<td>13147.7±4649.6</td>
<td>22795.4±6454.3</td>
<td>27872.1±7921.8</td>
</tr>
<tr>
<td>$AUC_{\text{INF,obs}}$ (ng·h/mL)</td>
<td>13150.7±4653.3</td>
<td>22801.3±6456.5</td>
<td>27876.6±7923.1</td>
</tr>
<tr>
<td>$AUC_{\text{INF,obs/D}}$ (h/10ng·h/mL)</td>
<td>0.438±0.155</td>
<td>0.380±0.108</td>
<td>0.465±0.132</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>1865.2±356.7</td>
<td>2201.6±573.2</td>
<td>2305.9±698.8</td>
</tr>
<tr>
<td>$C_{\text{max/D}}$ (1/mL)</td>
<td>0.062±0.012</td>
<td>0.037±0.010</td>
<td>0.038±0.012</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>1.5±0.3</td>
<td>3.7±1.3</td>
<td>4.3±1.0</td>
</tr>
<tr>
<td>$K_e$ (1/h)</td>
<td>0.165±0.09</td>
<td>0.125±0.05</td>
<td>0.118±0.07</td>
</tr>
<tr>
<td>Relative $F$ (%)</td>
<td>100</td>
<td>86.7</td>
<td>105.9</td>
</tr>
</tbody>
</table>

$AUC$: area under the plasma-concentration vs. time curve; $AUC_{\text{INF,obs/D}}$: normalized by dose mean area under the plasma-concentration vs. time curve; $C_{\text{max}}$: peak plasma concentration; $C_{\text{max/D}}$: normalized by dose peak plasma concentration; $t_{\text{max}}$: time to peak plasma concentration; $K_e$: elimination coefficient; Relative $F$= relative bioavailability. Each value represents the mean±S.D. ($n=6$).

Fig. 5. (A, B) Linear Regression Plots of Percent Absorption versus Percent Dissolution of F2, F4 by Wagner–Nelson Method; (C) Deconvolution of F2 and F4 by Wagner–Nelson Method.

(A, B) The plots of F2 and F4 were applied to linear fitting up to 9 and 12h, respectively.

Fig. 6. IVIVC Correlation of Absorption and Dissolution Fractions by Numeric Deconvolution Method

(A) Linear and quadratic fit of the in vitro release and in vivo absorption fractions of extended formulation F2; (B) Linear and quadratic fit of the in vitro release and in vivo absorption fractions of extended formulation F4; (C) Linear and quadratic fit of the in vitro release and in vivo absorption fractions of combined F2 and F4 data; (D) Deconvolution of F2 and F4 by numeric deconvolution method.
The mechanism of apremilast release from all the coated tablets was investigated by applying the release profiles in pH 6.8 to fit four mathematic models: zero order, first order, Higuchi and Korsmeyer–Peppas (K–P) models. The calculated rate constant and correlation coefficients ($r^2$) values are shown in Table 3. The correlation coefficients of all the formulations showed better fitting of K–P release kinetics than those of zero order, first order and Higuchi models. Furthermore, the "$n$" values in K–P model suggest that except for that of F1 was slightly below 0.89, those values of other four formulations were above 0.89, which indicated the mechanism of drug release was dominated by polymer matrix erosion.

**In Vivo Study and Pharmacokinetic Parameters** Based on the in vitro release profiles of the five formulations, F2 and F4 were selected to represent relatively “fast” and “slow” formulations for in vivo pharmacokinetic study. The mean plasma concentration-time curves of apremilast in 6 beagle dogs after oral administration of formulation F2, F4 and the marketed IR tablets are shown in Fig. 4A. The pharmacokinetic parameters were calculated by non-compartment analysis and listed in Table 4. For the developed ER formulations F2 and F4, the maximum plasma concentration ($C_{\text{max}}$) was 2201.6±573.2 and 2305.9±698.8 ng/mL, the time of maximum plasma concentration ($t_{\text{max}}$) was 3.7±1.3 and 4.3±1.0 h, the area under the plasma concentration–time curve ($AUC_{\text{inf}}$) was 22801.3±6456.5 and 27876.6±7923.1 ng·h/mL, and the drug elimination coefficient during the terminal phase was 0.125±0.05 and 0.118±0.07 h$^{-1}$. Compared to the IR formulation, both ER formulations showed significantly lower $C_{\text{max}}$ by dose, reduced $K_e$, prolonged $t_{\text{max}}$, and relative bioavailabilities in the range of ±15% (Fig. 4B).

**In Vitro–in Vivo Correlation** An IVIVC is generally defined as a predictive mathematical relationship between in vitro dissolution and in vivo exposure. It serves for several purposes such as optimizing formulations, confirming the quality control parameters of in vitro release experiment and allowing minor changes of dosages after launching the market without providing new bioequivalence data.\(^{24,31,32}\)

At the early stage of modified release product development, an IVIVC study on animals can provide substantial information on the suitability of the formulations and the settings of dissolution experiment. As the in vivo data was analyzed as

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulation</th>
<th>Fitting equations</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Wagner–Nelson</td>
<td>Formulation 2(^a)</td>
<td>$y=0.67x−0.03$</td>
<td>W–N 1</td>
<td>0.991</td>
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<tr>
<td></td>
<td>Formulation 4(^b)</td>
<td>$y=0.71x−0.03$</td>
<td>W–N 2</td>
<td>0.995</td>
</tr>
<tr>
<td>Numerical deconvolution(c)</td>
<td>Formulation 2</td>
<td>$y=2.522e^{0.1128t}$</td>
<td>IVIVC 1</td>
<td>0.968</td>
</tr>
<tr>
<td></td>
<td>Formulation 4</td>
<td>$y=2.759e^{0.1128t}$</td>
<td>IVIVC 2</td>
<td>0.944</td>
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<tr>
<td></td>
<td>Formulation 2+4</td>
<td>$y=2.567e^{0.1128t}$</td>
<td>IVIVC 3</td>
<td>0.962</td>
</tr>
</tbody>
</table>

\(a\) Linear fitting up to 9 h. \(b\) Linear fitting up to 12 h. \(c\) Polynomial fitting ($n=2$).
1 compartment model, a level A in vitro–in vivo correlation was assessed by using the Wagner–Nelson and numerical deconvolution methods in our study. The in vivo absorption deconvolution and percentage drug dissolved versus percentage drug absorbed by two methods was plotted in Figs. 5, 6, respectively. For Wagner–Nelson method, F2 showed good linear correlation coefficient \( \left( r^2 = 0.991 \right) \) from 0 to 9h (Fig. 5A, Table 5) while F4 showed good linear fit \( \left( r^2 = 0.995 \right) \) until 12h (Fig. 5B, Table 5).

Although most of the work on IVIVC is based on linear models, both the FDA and the USP state that non-linear relationships are acceptable to describe the in vitro–in vivo relationship.\(^{24,33} \) In fact, various reports provide scientific evidence for predictive non-linear correlations.\(^{3,14,37} \) For example, first-order dissolution with delayed in vivo absorption normally assumes a non-linear IVIVC.\(^{35} \) In our study, calculated in vivo absorption of each formulation by numerical deconvolution was preferably fitted with a quadratic regression model to the in vitro release data as IVIVC 1 and IVIVC 2 (Figs. 6A, B, Table 5). This quadratic regression model suggested faster in vivo absorption fractions comparing to the in vitro dissolution fractions at the early time points, possibly due to the faster disintegration and release of the tablet in dogs with strong digestive capability. To further investigate the suitability of IVIVC model established from formulations with mixed release profiles, The IVIVC of combination of “fast” ER formulation F2 and “slow” ER formulation F4 was also established as IVIVC 3 (Fig. 6C, Table 5), whose correlation coefficient of \( r^2 \) (0.962) indicated a good correlation (Table 5).

The accuracy of the model was examined through an internal validation. This involved convoluting the dissolution data that corresponded to each formulation (Fast/Slow) by using the created IVIVC models (Fast/Slow/Fast and Slow as IVIVC 1/ IVIVC 2/IVIVC 3) and calculating the respective %PE for AUC and C\(_{\text{max}}\). The resulted predicted in vivo data was presented in Table 6. The FDA IVIVC guidance suggests that the mean absolute percent prediction error for the IVIVC should be less than 10% for both \( C_{\text{max}} \) and AUC and that each percent prediction error should not be greater than 15% for any one formulation. According to Table 6, the mean absolute percent prediction errors (MAPE) of AUC of three IVIVC models were 6.42, 7.77, 6.67% and MAPE for \( C_{\text{max}} \) was 5.01, 4.25, 6.58%. Since all of these values remain below the 10% threshold and no PE is above 15%, all three models passed internal validation.\(^{24} \) Particularly, IVIVC 1 showed the lowest total MAPE of AUC and \( C_{\text{max}} \) which indicates that it could be the most suitable model to fit the formulations in the range of test (Fig. 7).

**Conclusion**

In conclusion, apremilast matrix extended-release tablets were prepared by easy manufacture process of dry granulation and compression technology. The developed formulations showed extended in vitro drug release and in vivo pharmacokinetic profiles by comparing their pharmacokinetic parameters with the commercially available immediate-release product Otezla\(^{6} \) in beagle dogs under fasting conditions.

Furthermore, two tested extended-release formulations allowed establishing strong relationship between in vitro and in vivo data in beagle dogs. The quantitative correlation study could be regarded as a first step to predict the extent of in vivo absorption from dissolution data of formulations with various release rates.

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**Conflict of Interest**

The authors declare no conflict of interest.

**References**