Development of an extended-release formulation for apremilast and a level A in vitro-in vivo correlation study in beagle dogs

Meiqiong Tang \textsuperscript{a}, Ping Hu \textsuperscript{a,\*\textdagger}, Shigui Huang \textsuperscript{b}, Qiang Zheng \textsuperscript{a}, Hao Yu \textsuperscript{b}, Yun He \textsuperscript{a,\*}

\textsuperscript{a} College of Pharmaceutical Sciences and Innovative Drug Research Centre, Chongqing University, 55 South Daxuecheng Road, Chongqing, China

\textsuperscript{b} Liangjiang Medicine Ltd., Bldg.3 Innovation Productivity Center, Xiyong Free Trade Zone, Shapingba, Chongqing, China

Corresponding Authors: Ping Hu, Yun He

\textdagger Address of corresponding author:

College of Pharmaceutical Sciences and Innovative Drug Research Centre, Chongqing University, China

Post address: College of Pharmaceutical Sciences and Innovative Drug Research Centre, Chongqing University, 55 South Daxuecheng Road, Chongqing 401331, China

E-mail: ping.hu@cqu.edu.cn, yun.he@cqu.edu.cn
Summary

The primary objective of the present study was to develop extended-release matrix formulations of apremilast for the oral delivery and to study their in vitro and in vivo correlation. Five extended-release formulations containing hydroxypropylmethylcellulose (HPMC) as the retarding excipient with different release rate of apremilast were prepared. Dissolution tests of all the formulated tablets were performed in water, pH 4.0 and pH 6.8 buffer solutions. The in vitro release kinetics was studied and supported by Korsmeyer-Peppas’s 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., $C_{\text{max}}$, $t_{\text{max}}$, AUC. Higher $t_{\text{max}}$ and $t_{1/2}$, lower $C_{\text{max}}$ and $K_e$ 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.

Keywords

Drug release, Absorption, Deconvolution, Pharmacokinetics, In vitro-in vivo correlation

Chemical and Pharmaceutical Bulletin Advance Publication
1. Introduction

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 \(^1\). Apremilast was introduced to the clinic \(^2\) and recently approved for the treatment of psoriatic arthritis and plaque psoriasis in the USA and Europe \(^3\). Unlike TNF-\(\alpha\) inhibitors, which bind directly to TNF-\(\alpha\), apremilast causes a broad inhibition of multiple pro-inflammatory mediators such as interleukin IL-6, IL-10 and TNF-\(\alpha\) \(^4\) \(^5\), exerting therefore an overall anti-inflammatory effect \(^6\).

![Fig.1]

The marketed immediate-release (IR) formulation of apremilast was launched under the brand name of Otezla\(^\circledast\) (Celgene) \(^7\), which was designed to be orally administered at a dose of 10, 20 or 30 mg twice daily. Apremilast demonstrates rapid absorption (\(t_{\text{max}} \sim 2.5\) h), mean half-life of 6–9 h, CL/F of \(\sim 10\) L/h and absolute bioavailability of \(\sim 73\) % \(^8\). 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 \(^8\).

For the nature of chronic diseases, a long term treatment for psoriatic arthritis and plaque psoriasis with apremilast is generally recommended \(^5\). 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 improve the dosing, but also 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, extended-release 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 fitted to various mathematic models. 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) in vitro-in vivo correlations (IVIVC) were established employing model-dependent Wagner-Nelson and model-independent numerical deconvolution methods and were assessed by internal validation.
2. Experimental

2.1. 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 (Sichuan, 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).

2.2. Preparation of apremilast matrix tablets

Apremilast HPMC matrix tablets were prepared by formulations containing different amounts of HPMC. The active pharmaceutical ingredient (API) was grind by a pulverizer (QLM, Shenfei, Shenyang China). The size distributions of the API particles were measured by a particle size analyzer (Mastersizer, Malvern, Shanghai 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 (LGC100, 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 (GL145, compression punch: 17 mm x 7 mm, Guolongli automation, Beijing, China). The tablets were coated with thin-film coating material (Opadry 85F94245 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).

2.3. Hardness and friability of the prepared tablets

The uncoated and coated tablets form each formulation were tested for crushing strength using a hardness tester (YD-35, TiandaTianfa Technology, Tianjin, China). The friability of the tablets was tested by a friabiliator (FT-2000AE, Tiandatianfa Technology, Tianjin, China) at a speed of 25 rpm for 4 min. The values were determined and recorded as mean ± S.D (n= 12).

2.4. In vitro dissolution test

Dissolution profiles for each ER formulation were measured in the USP XXIII, apparatus 2 (paddle method) (RC8MD, Tiandatianfa Technology, Tianjin, China) 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. Zero-order ($Q = k_0t$), first-order ($\ln (100 - Q) = \ln Q_0 - k_1t$), Higuchi model ($Q = k_H t^{1/2}$), and Korsmeyer-Peppas ($Q = k t^n$) models were employed to fit the release profiles using regression analysis and the best equations were suggested, where $Q = M_t / M_\infty$, is the fraction of drug released after time \textquoteleft t\textquoteright, \textquoteleft k\textquoteright is the kinetic constant. For Korsmeyer-Peppas model, \textquoteleft n\textquoteright is the release exponent, which characterizes the different drug release mechanisms. A value of $n < 0.45$ indicates diffusion-controlled drug release; $0.45 < n < 0.89$ is a combination of both diffusion- and erosion-controlled release and $n > 0.89$ generally refers to the matrix erosion-controlled drug release\textsuperscript{27}.

2.5. HPLC conditions

The HPLC analysis system (Shimazu, Japan) consisted of a pump (Model LC-20AT), Shimazu DGU-20A5R interface, ultraviolet detector (Model SPD-20A), autosampler (Model SIL-20A) and a column oven (Model CTO-20A). A C\textsubscript{18} analytical column (ZORBAX 5 μm, 4.6 mm×150 mm, Agilent, USA) was used. The mobile phase consisted of acetonitrile 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.
2.6. Pharmacokinetic study

2.6.1. 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® 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.

2.6.2. 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. 5 µL of the reconstituted samples were injected and separated on a Capcell Pak C_{18} column (5 µm, 50 mm × 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 into a tandem quadrupole mass spectrometer with a positive ionization electrospray source (API 3000, Applied Biosystem, MA, US). 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.

2.6.3. 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 (t_{max}) and maximum plasma concentration of drug (C_{max}) were computed directly from the plasma concentration vs. time plot. All the data is reported as mean ± standard deviation.

2.7. In Vitro-In Vivo correlations (IVIVC)

2.7.1. 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 FDA guidance requires that an IVIVC model be created with products having at least two different release rates. 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.

2.7.2. Wagner-Nelson method

The Wagner-Nelson deconvolution method was applied to the in vivo data to determine the fraction of the drug absorbed. 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).

$$\frac{(X_A)_t}{(X_A)_{\infty}} = \frac{C_t + K \int_0^T Cdt}{K \int_0^\infty Cdt}$$

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. Ln (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
2.7.3. 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).

$$\int_0^t u(t)\,dt = \int_0^1 u(t)\,dt + \int_1^2 u(t)\,dt + \cdots + \int_{n-1}^n u(t)\,dt$$ (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).

$$\frac{1}{A_1} B_1 + \frac{1}{A_1} (B_2 - A_2 r_1) + \cdots + \frac{1}{A_i} (B_n - A_2 r_{n-1} - A_3 r_{n-2} - \cdots - A_n r_1)$$ (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).

2.7.4. 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 $C_{\text{max}}$ 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 to by multiplying by 100.

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

\[
\text{%PE } C_{\text{max}} = \left( \frac{C_{\text{max,abs}} - C_{\text{max, pred}}}{C_{\text{max,abs}}} \right) \times 100
\]

3. Results and discussion

3.1. Preparation and characterization of apremilast matrix tablets

Apremilast (60 mg) matrix tablets (F1-F5) 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).
3.2. *In vitro* dissolution studies of coated matrix tablets

The interaction between drug, water and polymer was the primary factor to control the drug release \(^{28}\). 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 active pharmaceutical ingredients (API) or reduction of the API particle sizes were widely applied to improve the dissolution. In this study, crystal forms A and B which possesses different nature of particle sizes from crystallization were used to test their difference in dissolution using water as the dissolution medium \(^{29}\). As Fig. 2 shows, the tablets composed of original apremilast crystal form A (D50 = 233.4 μm) shows a much slower release rate compared to original crystal form B (D50 = 35.2 μm) (Fig. 2A, API-1 and 2). The tablets composed of grinded crystal form A with a reduced particle (D50 = 32.6 μm) 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 (D50 = 4.9 and 5.2 μm, respectively), the release profiles remain almost identical to those of API 2 and 3 (Fig. 2A, API-4 and API-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 μm or even smaller, the release rates of the tablets were very close (Fig. 2B), which

---

**Chemical and Pharmaceutical Bulletin Advance Publication**
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 aceta
t 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 6h) 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 2h). 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\textsubscript{50%} and t\textsubscript{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\textsubscript{50%} and t\textsubscript{80%}, gradually decreased \textsuperscript{15,30}. 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).
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 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 Korsmeyer-Peppas 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.

3.3. 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 $2201.6 \pm 573.2$ and $2305.9 \pm 698.8$ ng/mL, the time of maximum plasma concentration ($t_{\text{max}}$) was $3.7 \pm 1.3$ and $4.3\pm1.0$ h, the area under the plasma concentration-time curve ($\text{AUC}_{\text{inf}}$) was $22801.3 \pm 6456.5$ 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).

<Fig. 4>

<Table 4>

3.4. In vitro-in vivo correlation

An IVIVC is generally defined as a predictive mathematical relationship between \textit{in vitro} dissolution and \textit{in vivo} exposure. It serves for several purposes such as optimizing formulations, confirming the quality control parameters of \textit{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 \textit{in vivo} data was analyzed as 1 compartment model, a level A \textit{in vitro-in vivo} correlation was assessed by using the Wagner-Nelson and numerical deconvolution methods in our study. The \textit{in vivo} absorption deconvolution and percentage drug dissolved versus percentage drug absorbed by two methods was plotted in Fig. 5 and Fig. 6, respectively. For Wagner-Nelson method, F2 showed good linear correlation coefficient (r\(^2\) = 0.991) from 0 to 9 hour (Fig. 5A and Table 5) while F4 showed good linear fit (r\(^2\) = 0.995)
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 \textit{in vitro–in vivo} relationship \cite{24, 33}. In fact, various reports provide scientific evidence for predictive non-linear correlations \cite{1, 34-37}. For example, first-order dissolution with delayed \textit{in vivo} absorption normally assumes a non-linear IVIVC \cite{35}. In our study, calculated \textit{in vivo} absorption of each formulation by numerical deconvolution was preferably fitted with a quadratic regression model to the \textit{in vitro} release data as IVIVC 1 and IVIVC 2 (Fig. 6A, B and 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 and 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 $C_{\text{max}}$ were 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. 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.

<Table 6>

4. Conclusions

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® 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.

Acknowledgments

This work was supported from Fundamental Research Funds for the Central Universities (10611201CDJXY460001) and Startup Funds for Young Scientists in Chongqing University (0236011104411).

Conflict of interest

The authors declare no conflict of interest.
References


8) Poole, R. M.; Ballantyne, A. D. *Drugs* 2014, 74, 825.


33) USP-XXIV *United States Pharmacopeial Convention, Inc.* Rockville, MD, USA., **2000**.


Fig 1. Chemical structure of (S)-N-(2-[[1-(3-ethoxy-4-methoxy-phenyl)-2-methane sulfonyl]ethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl) acetamide.
**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 50 rpm and in dissolution media at 37°C. Each value represents the mean ± standard deviation (n=12).
Fig 4. (A): Pharmacokinetic profile of apremilast in beagle dogs after oral administration of marketed immediate release tablet (IR, 30 mg), developed fast-extended release formulation (ER-F2, 60 mg) and slow-extended release formulation (ER-F4, 60 mg) at a single once-daily dose. Each value represents the mean ± standard deviation (n=6). (B): Summary of the $t_{\text{max}}$ and $C_{\text{max}}/D$ of three tested formulations.
Fig 5. (A and B) Linear regression plots of percent absorption versus percent dissolution of F2, F4 by Wagner-Nelson method. The plots of F2 and F4 were applied to linear fitting up to 9 h and 12 h, respectively; (C): deconvolution of F2 and F4 by Wagner-Nelson method.
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.
Fig 7. Observed (crossed circles and squares) and predicted (lines) apremilast plasma concentration (ng/mL) vs time profiles of extended release formulation F2 (A) and F4 (B) up to 24 h with three created IVIVC models.
## Table 1. Composition of apremilast matrix tablets (F1-F5)

<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>
<tr>
<td>Test</td>
<td>F1</td>
<td>F2</td>
<td>F3</td>
<td>F4</td>
<td>F5</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<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>
<tr>
<td>Formulations</td>
<td>Zero order</td>
<td>First order</td>
<td>Higuchi model</td>
<td>Peppas order</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
<td>-------------</td>
<td>---------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$k_0$ (%h$^{-1}$)</td>
<td>$r^2$</td>
<td>$k_o$ (%h$^{-1}$)</td>
<td>$r^2$</td>
<td>$k_{H}$ (%h$^{-1}$)</td>
</tr>
<tr>
<td>F1</td>
<td>7.53</td>
<td>0.628</td>
<td>31.90</td>
<td>0.705</td>
<td>37.37</td>
</tr>
<tr>
<td>F2</td>
<td>6.25</td>
<td>0.775</td>
<td>27.75</td>
<td>0.872</td>
<td>33.86</td>
</tr>
<tr>
<td>F3</td>
<td>5.81</td>
<td>0.799</td>
<td>25.11</td>
<td>0.890</td>
<td>31.25</td>
</tr>
<tr>
<td>F4</td>
<td>5.19</td>
<td>0.874</td>
<td>20.10</td>
<td>0.893</td>
<td>30.03</td>
</tr>
<tr>
<td>F5</td>
<td>4.57</td>
<td>0.902</td>
<td>16.88</td>
<td>0.933</td>
<td>27.03</td>
</tr>
</tbody>
</table>

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

---

**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 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>( \text{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>( \text{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>( \text{AUC}_{\text{INF,obs/D}} ) (h/mL)*</td>
<td>0.438 ± 0.155</td>
<td>0.380 ± 0.108</td>
<td>0.465 ± 0.132</td>
</tr>
<tr>
<td>( \text{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>( \text{C}_{\text{max/D}} ) (mL⁻¹)</td>
<td>0.062 ± 0.012</td>
<td>0.037 ± 0.010</td>
<td>0.038 ± 0.012</td>
</tr>
<tr>
<td>( \text{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>( \text{K}_{\text{e}} ) (h⁻¹)</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; \( \text{AUC}_{\text{INF,obs/D}} \): normalized by dose mean area under the plasma-concentration vs time curve; \( \text{C}_{\text{max}} \): peak plasma concentration; \( \text{C}_{\text{max/D}} \): normalized by dose peak plasma concentration; \( \text{t}_{\text{max}} \): time to peak plasma concentration; \( \text{K}_{\text{e}} \): elimination coefficient; Relative F: relative bioavailability.

Each value represents the mean ± S.D. (n=6).
Table 5. IVIVC fitting formula by Wagner-Nelson and numerical deconvolution method

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulation</th>
<th>Fitting equations</th>
<th>Model</th>
<th>r² value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Wagner-Nelson</strong></td>
<td>Formulation 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>$y = 0.67x - 0.03$</td>
<td>W-N 1</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>Formulation 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>$y = 0.71x - 0.03$</td>
<td>W-N 2</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>Formulation 2</td>
<td>$y = 2.522x^2 - 1.024x$</td>
<td>IVIVC 1</td>
<td>0.968</td>
</tr>
<tr>
<td><strong>Numerical deconvolution</strong></td>
<td>Formulation 4</td>
<td>$y = 2.759x^2 - 1.128x$</td>
<td>IVIVC 2</td>
<td>0.944</td>
</tr>
<tr>
<td></td>
<td>Formulation 2+4</td>
<td>$y = 2.567x^2 - 1.027x$</td>
<td>IVIVC 3</td>
<td>0.962</td>
</tr>
</tbody>
</table>

<sup>a</sup>: linear fitting up to 9 h; <sup>b</sup>: linear fitting up to 12 h; <sup>c</sup>: polynomial fitting (n= 2)
### Table 6. AUC and Cmax error between observed and predicted values.

<table>
<thead>
<tr>
<th>Model</th>
<th>Formulation</th>
<th>AUC&lt;sub&gt;0-24h&lt;/sub&gt; observed</th>
<th>AUC&lt;sub&gt;0-24h&lt;/sub&gt; predicted</th>
<th>Error (%)</th>
<th>MAP</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; observed</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; predicted</th>
<th>Error (%)</th>
<th>MAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVIVC 1</td>
<td>F-2</td>
<td>21228.4</td>
<td>21955.9</td>
<td>3.42</td>
<td>6.42</td>
<td>2033.3</td>
<td>1910.6</td>
<td>5.91</td>
<td>5.01</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>24582.8</td>
<td>22266.6</td>
<td>-9.42</td>
<td>2050.7</td>
<td>1964.1</td>
<td>4.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIVC 2</td>
<td>F-2</td>
<td>21228.4</td>
<td>21528.9</td>
<td>1.42</td>
<td>7.77</td>
<td>2033.3</td>
<td>1932.5</td>
<td>4.96</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>24582.8</td>
<td>21109.9</td>
<td>-14.12</td>
<td>2050.7</td>
<td>1978.4</td>
<td>3.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVIVC 3</td>
<td>F-2</td>
<td>21228.4</td>
<td>21596.9</td>
<td>1.71</td>
<td>6.67</td>
<td>2033.3</td>
<td>1879.7</td>
<td>7.42</td>
<td>6.58</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>24582.8</td>
<td>21723.8</td>
<td>-11.63</td>
<td>2050.7</td>
<td>1932.9</td>
<td>5.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Predicted by numerical convolution.