Tanshinone IIA – loaded pellets developed for angina chronotherapy: Deconvolution-based formulation design and optimization, pharmacokinetic and pharmacodynamic evaluation

Hong-Xiang Yan, Jin Li, Zheng-Hua Li, Wen-Li Zhang, Jian-Ping Liu

Abstract

This paper put forward a deconvolution-based method for designing and optimizing tanshinone IIA sustained-release pellets (TA-SRPs) with improved efficacy in the treatment of variant angina. TA-SRPs were prepared by coating TA ternary solid dispersion immediate-release pellets (TA-tSD-IRPs) with the blends of polyvinyl acetate (PVAc) and polyvinyl alcohol–polyethylene glycol (PVA–PEG) using fluidized bed technology. The plasma concentration–time curve of TA-tSD-IRPs following oral administration as a weight function was investigated in New Zealand white rabbits. The predicted/expected plasma concentration–time curve of TA-SRPs as a response function was simulated according to the circadian rhythm of variant angina during 24 h based on chronotherapy theory. The desired drug release profile of TA-SRPs was obtained via the point-area deconvolution procedure using the weight function and response function, and used for formulation optimization of TA-SRPs. The coating formulation of TA-SRPs was optimized as 70:30 (w/w) PVAc/PVA–PEG with 5% (w/w) coating weight due to the in vitro drug release profile of these TA-SRPs was similar to the desired release profile (similarity factor $f_2 = 64.90$). Pharmacokinetic studies of these optimized TA-SRPs validated that their actual plasma concentration–time curve possessed a basically consistent trend with the predicted plasma concentration–time curve and the absolute percent errors (%PE) of concentrations in 8–12 h were less than 10%. Pharmacodynamic studies further demonstrated that these TA-SRPs had stable and improved efficacy with almost simultaneous drug concentration–efficacy. In conclusion, deconvolution could be employed in the development of TA-SRPs for angina chronotherapy with simultaneous drug efficacy and reduced design blindness and complexity.

1. Introduction

Tanshinone IIA (TA, Fig. 1), one of the major liposoluble bioactive constituents isolated from the roots of Chinese herb Salviae miltiorrhiza Bunge (Danshen), exhibits a variety of cardiovascular activities, including prevention and treatment of angina pectoris (Shang et al., 2012; Gao et al., 2012). TA has poor water-solubility (2.8 mg mL$^{-1}$) (Li et al., 2008), short half-life (1–2 h) (Zhang et al., 2013), substantial intestinal first pass metabolism (Hao et al., 2007) and low oral bioavailability (Li et al., 2005). Recently, a number of new drug delivery systems have been employed to resolve these issues, e.g. solid dispersion (Hao et al.,...
2006), solid lipid nanoparticles (Zhang et al., 2008), solid inclusion complex (Fan et al., 2005), intravenous lipid emulsion (Chu et al., 2003; Gaynor et al., 2008; Vaughan and Dennis, 1978; Yeh et al., 2002; Sunesen et al., 2005). In the current study, we made an attempt to design and optimize chronotherapeutic TA-SRPs with appropriate and favorable time in a day, such as 6 pm. In this case, high plasma concentrations could be reached in 8–12 h (between 02:00 and 06:00) with time to peak concentration around 10 h (at 04:00) so as to prevent the sudden angina attacks in the sleeping hours early in the morning.

Deconvolution (Langenbucher and Mysicka, 1985; Qi et al., 2003; Gaynor et al., 2008; Vaughan and Dennis, 1978; Yeh et al., 2001), recommended by FDA (Administration et al., 1997), has been widely applied in the pharmaceutical research involved with in vivo/in vitro correlation of dosage forms (Sirisuth and Eddington, 2002; Sunesen et al., 2005). In the current study, we made an attempt to design and optimize chronotherapeutic TA-SRPs with a deconvolution-based method. Herein, numerical deconvolution was utilized from a new perspective to reduce the blindness and complexity throughout the chronotherapeutic formulation development process. Weight function was the plasma concentration–time curve of TA-SD-IRPs following oral administration in normal New Zealand white (NZW) rabbits. Response function was the predicted/expected plasma concentration–time curve of TA-SRPs, which was simulated according to the incidence of variant angina during 24 h based on angina chronotherapy theory. The desired drug release profile of TA-SRPs was determined by deconvolution using the weight function and the response function, and subsequently used for guiding the formulation optimization of TA-SRPs. For the formulation optimization, two coating polymers, polyvinyl alcohol–polyethylene glycol (PVA–PEG) graft copolymer (Kollicoat® IR), with different permeability were used in combination to adjust drug release from TA-SRPs. The pharmacokinetic and pharmacodynamic studies in rabbits were performed to verify whether TA-SRPs optimized based on deconvolution had a suitable plasma drug concentration time course with better drug efficacy.

2. Materials and methods

2.1. Materials

TA (98.63%) was purchased from Xi’an Honson Biotechnology Co., Ltd. (Shanxi, China). TA standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sugar spheres (0.75–0.85 mm) were supplied by JRS Pharma (Rosenberg, Germany). PVP-K29/32 was from China Division, ISP Chemical Company (Shanghai, China). Poloxamer 188 (Pluronic® F68), polyvinyl acetate dispersion (Kollicoat® SR30D) and polyvinyl alcohol–polyethylene glycol graft copolymer (Kollicoat® IR) were obtained from BASF Chemical Company (Ludwigshafen, Germany). Talc (1200 mesh) was received from Merck-Schuchardt (Hohenbrunn, Germany). 1,2-propylene glycol was delivered from Shanghai Chemical Agent Co., Ltd. (Shanghai, China). Gelatin capsules were from Suzhou Capsugel Ltd. (Suzhou, China). High-fat fodder was provided by Jiangsu Xietong Biological Engineering Co., Ltd. (Nanjing, China). Nitric oxide (NO) reagent box was purchased from Beyotime Institute of Biotechnology (Shanghai, China). Medical 5% glucose solution was from Jiangsu Dahongying-Hengshun Pharmaceutical Co., Ltd. (Nanjing, China). All the reagents were of analytical grade except methanol, which was of chromatographic grade.

2.2. Animals

Healthy male NZW rabbits (body weight 2.0 ± 0.9 kg) were purchased from Experimental Animal Center of China Pharmaceutical University (Nanjing, China). The rabbits were housed in a temperature and humidity controlled room (23 °C, 55% air humidity) with free access to water and standard rabbit chow for at least 5 days to adapt to the new environment prior to the experiments. All the experiments were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University.

2.3. Preparation of pellets

2.3.1. Preparation of TA-SD-IRPs

TA-SD-IRPs were prepared by a single-step fluid-bed coating technique. A hydrophilic polymer PVP and a surfactant poloxamer 188 were used as dispersing carriers of drug substance TA. Firstly, TA, PVP and poloxamer 188 at a definite ratio (1:4:1, w/w) were dissolved in a mixed solvent of ethyl acetate–anhydrous ethanol (5:1, v/v) to form a clear solution. Then the solution under continuous stirring was sprayed onto the sugar cores (5 g) from a bottom nozzle of 0.5 mm in diameter) in a fluid-bed granulator and coater (JHQ-100; Shenyang, China). This equipment was attached to a peristaltic pump (HL-2; Shanghai, China). The preparation of TA-SD-IRPs was protected from light exposure and carried out under the condition of coating temperature of 35–37 °C, spray rate of 1.0–1.2 mL min⁻¹, atomization pressure of 1.5–1.6 bar and air blow rate of 100–150 mL min⁻¹. After drug/carriers layering, the pellets were further fluidized for 15 min at 30–35 °C to minimize the solvent residue. The resulting pellets were placed in a container for subsequent studies.

Fig. 1. Chemical structure of tanshinone IIA.
2.3.2. Preparation of TA-SRPs

The fluid-bed granulator and coater (nozzle diameter 0.8 mm) was loaded with 5 g of TA-tSD-IRPs for each run. Opadry® II was sprayed onto these pellets to produce the layered coating of TA-SRPs. The lower permeable PVAc and higher permeable PVA-PEG were used as main coating materials. 1,2-propylene glycol (plasticizer, 2.5% w/w, based on total polymer mass) was added into PVAc suspension and stirred overnight. The tcalc (antiadherent, 25% w/w, based on total polymer mass) was then dispersed in the polymer suspension. Subsequently, PVA-PEG was added to form a suspension containing 15% w/w total solid content. The coating suspension was sieved through a 80-mesh screen and then sprayed onto the previous pellets under continuous stirring. The coating parameters were as follows: temperature 38–40 °C, spray rate 0.6–0.8 mL min⁻¹, atomization pressure 0.8–1.2 bar and air blow rate 120–180 mL min⁻¹. Subsequent to the coating, the pellets were further fluidized for 10 min in the coating chamber and cured for 24 h at 60 °C in an oven.

A series of TA sustained-release coated pellets were prepared with different PVAc/PVA-PEG ratios (90:10, 85:15, 70:30 w/w) and coating weights (3%, 5%, 10% w/w, representing coating thickness). The coating weight was calculated by equation $F = (W_a - W_b)/W_b \times 100\%$, where $W_a$ and $W_b$ are the accurate weights of pellets before and after coating, respectively.

2.4. In vitro release studies

2.4.1. Quantitative analysis of TA

20 μL of release medium containing TA was injected into high pressure liquid chromatography (HPLC, Shimadzu LC-20A, Kyoto, Japan). The system was made up of a Shimadzu SIL-20AC autosampler, a Shimadzu LC-20AB pump and a Shimadzu SPD-M20A diode array detection set at 268 nm. The separation was completed at 30 °C on a Synergi Hydro-RP C18 column (5 μm, 250 mm × 4.6 mm, Phenomenex, USA) protected by a C18 SecurityGuard column (5 μm, 10 mm × 4.6 mm, Kromasil, Sweden). The mobile phase was methanol/water (90:10, v/v) at a flow rate of 1.0 mL min⁻¹. The standard curve was found to be linear in the 0.05–5.00 μg mL⁻¹ range: $A = 205,386 \times C + 1779$, $r = 0.9997$ (where $C$ is the concentration of TA and $A$ is the corresponding peak area, $n = 3$). The recovery rates of low, middle and high concentration for TA were all in the range of 98–102% and the RSD were less than 2%. The RSD of intra-day and inter-day precision for TA were below 2%.

2.4.2. Drug release tests

TA-SRPs equivalent to 2.5 mg TA were sealed in hard gelatin capsules by a manual capsule-filling machine (CapsulCN, Zhejiang, China). Then the release experiments were carried out in USP34 Apparatus I (rotating basket method; ZRS-8G, Tianjin, China) at a rotation rate of 100 ± 1 rpm. The release medium was 900 mL of distilled water maintained at 37 ± 0.5 °C and 0.5% (w/v) sodium dodecyl sulfate (SDS) was added for perfect sink conditions of TA. At pre-determined time points of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 h, 5 mL of samples were withdrawn and replaced with an equivalent-volume of fresh medium. The samples were filtered through 0.22 μm filter and then quantified for TA by HPLC as described above. All the release tests were implemented in triplicate ($n = 3$) and the cumulative release percents and standard deviations were calculated.

2.4.3. Investigation of drug release stabilities

The drug release stability studies of TA-SRPs were conducted in a ZRS-8G release tester (Tianjin, China) in triplicate ($n = 3$) to investigate the influence of different pH condition, release method and rotation rate on drug release of TA-SRPs. The release mediums with different pH (900 mL) used were distilled water containing 0.5% SDS, 0.1 M HCl and pH 6.8 phosphate buffer solution (PBS), separately and were all maintained at 37 ± 0.5 °C. Two release methods were rotating basket method and paddle method. The rotation rates were investigated at 50, 100, 150 rpm, respectively. All the sampling method and analysis were same as the descriptions in Section 2.4.2.

2.4.4. Analysis of release data

The similarity factor ($f_2$) is a measurement of the similarity of the release profiles (similarity factor $f_2 > 50$, difference < 10%) (Jantratid et al., 2009). Equation of $f_2$ is shown as follows:

$$f_2 = 50 \log \left\{ \left[ 1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right]^{-0.5} \times 100 \right\}$$

(1)

where $n$ is the number of time points, $R_i$ is the release value of the reference at time $t$, and $T_i$ is the release value of the test at time $t$.

2.5. Pharmacokinetic studies

2.5.1. Animal experiments

The male NZW rabbits (body weight 2.0 ± 0.9 kg) were fasted for 12 h but supplied with water ad libitum before the experiments. Capsules of TA-tSD-IRPs or TA-SRPs were orally administered (30 mg kg⁻¹ of TA) to six rabbits ($n = 6$). At pre-determined time points of 0 (pre-treatment), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 and 48 h post-dosing. 1.5 mL of blood samples were collected from marginal ear veins of the rabbits and put into heparinized tubes to avoid clotting. Then the plasma samples were separated successfully by centrifugation at 3000 rpm for 10 min and stored at −20 °C until analysis.

2.5.2. Plasma sample processing and TA determination

Processing of the thawed plasma samples were performed under subdued light at room temperature. A single-step protein precipitation procedure was adopted to extract TA from the rabbit plasma. To begin with, 200 μL of plasma sample and 400 μL of ethyl acetate were pipetted into a 10 mL centrifuge tube and vortex-mixed for 3 min to precipitate protein fully. Afterwards, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was transferred into a clean centrifuge tube and evaporated to dryness under a N2 stream at 40 °C in a water bath. Then the residue was redissolved in 200 μL of methanol and centrifuged at 12,000 rpm for 10 min to isolate the supernatant. 20 μL of the supernatant was injected into HPLC for quantitative analysis of TA. The chromatographic condition was same as described in Section 2.4.1. The linearity of the method was achieved in the 0.005–0.5 μg mL⁻¹ concentration range: $A = 175264C + 324.5$, $r = 0.9976$ (where $C$ is the concentration of TA and $A$ is the corresponding peak area, $n = 3$). The RSD of method recoveries, extraction recoveries, intra-day and inter-day variabilities were all less than 10%, which indicated the rationality of this bioanalytical method for TA determination.

2.5.3. Pharmacokinetic analysis

Pharmacokinetic parameters such as time to maximum concentration ($t_{\text{max}}$), maximum plasma concentration ($C_{\text{max}}$), mean residence time ($MRT$), area under the plasma concentration–time curve from 0 to t h and 0 to infinity ($AUC_{0-t}$ and $AUC_{0-\infty}$) were calculated by non-compartmental analysis using WinNonlin program (version 1.5, Scientific Consulting, Inc., Cary, NC, USA). Data were expressed as mean values ± standard deviations (Mean ± SD).
2.6. Pharmacodynamic studies

2.6.1. NZW rabbit model of angina

Eighteen male NZW rabbits (body weight 2.0 ± 0.9 kg) were administered daily with dl-homocysteine thiocionate (20 mg mL⁻¹ in 5% glucose solution, 20–25 mg kg⁻¹) by subcutaneous injection, and fed by high-fat fodder (79.5% ordinary feed, 10% cholesterol, 5% lard, 5% egg yolk powder, 0.5% sodium cholate, w/w) (Kan et al., 2014). Eight weeks later, the NZW rabbits with angina induced by atherosclerosis were obtained and could be used for the evaluation of the drug efficacy of pellets.

2.6.2. Animal experiments and NO analysis

Eighteen NZW rabbits with angina were fasted for 12 h but supplied with water ad libitum before the experiments, and randomly divided into three groups (n = 6): controlled group, TA-tSD-IRPs group and TA-SRPs group. The controlled group was orally administered with the controlled capsules containing blank pellets without TA. The TA-tSD-IRPs group and TA-SRPs group were orally administered with respective drug pellets capsules (30 mg kg⁻¹ of TA). At pre-determined time points of 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h after administration, 2 mL of blood samples were collected from marginal ear veins of these rabbits. After standing for a period of time in the test tubes, the upper serum was obtained for NO analysis. NO concentration as the pharmacodynamic index was detected by nitrate reduction method described on NO reagent box. The average concentrations and standard deviations were calculated.

3. Results and discussion

3.1. Deconvolution method

Deconvolution method can be used to directly obtain in vivo dynamic information of drug from the pharmacokinetic data. Input rate function, weight function and response function in vivo are involved in this method. It is based on the convolution integral, which is defined as

\[ R(t) = \int_0^t I(\theta) \cdot W(t - \theta) d\theta \]

For a sustained release preparation, \( R(t) \) is the plasma drug concentration at time \( t \) following administration of a sustained release dosage unit. \( W(t) \) is the unit impulse response, which is the plasma drug concentration function following administration of the corresponding oral solution or immediate-release preparation. \( I(t) \) represents the in vivo release rate of the sustained release preparation at time \( t \). Deconvolution involves estimating the in vivo drug release rate \( I(t) \) using the observed in vivo \( R(t) \) data and observed in vivo reference data \( W(t) \).

Providing the observed time points are \( t_1, t_2, t_3 \ldots t_n \), the corresponding response values are \( R_1, R_2, R_3 \ldots R_n \) and the input values are \( I_1, I_2, I_3 \ldots I_n \), where \( I_i \) represents average in vivo drug input rate within each time interval \( t_{i-1} \sim t_i \), respectively. A working expression (3) can be derived from formula (2).

\[ R_i = \sum_{k=1}^{i} I_k \cdot AUC_{t_i, t_{i-1}} \]

\[ = I_1 \cdot AUC_{t_1, t_0} + I_2 \cdot AUC_{t_2, t_1} + \cdots + I_{i} \cdot AUC_{t_{i}, t_{i-1}} \]

(3)

where \( AUC \) is area under the plasma drug concentration–time curve of oral solution or immediate-release preparation (weight function) within time interval \( t_i - t_{i-1} \), which can be calculated by the trapezoid algorithm.

Then \( I_i \) can be calculated in turn by formula (4), which is the transformation of formula (3).

\[ I_i = \frac{R_i - I_1 \cdot AUC_{t_1, t_0} - I_2 \cdot AUC_{t_2, t_1} - \cdots - I_{i-1} \cdot AUC_{t_{i-1}, t_{i-2}}}{AUC_{t_i, t_{i-1}}} \]

(4)

An integration of \( I_i \) yields the cumulative drug release amount \( C_A \) at each time point \( t_i \).

\[ C_A = \sum_{k=1}^{n} I_k \cdot (t_k - t_{k-1}) \]

(5)

3.2. Design of TA-SRPs based on deconvolution

3.2.1. Evaluation of weight function

Weight function was the plasma concentration–time curve of TA-tSD-IRPs in NZW rabbits after oral administration (shown in Fig. 2). The pharmacokinetic data were processed following a non-compartmental model method and the important pharmacokinetic parameters (\( T_{\text{max}}^{\text{w}}, C_{\text{max}}^{\text{w}}, MRT^{\text{w}}, AUC_{0-\infty}^{\text{w}}, \text{and } AUC_{0-t}^{\text{w}} \)) were presented in Table 1. Compared to the compartment model, the non-compartmental model analysis is easier to automate, and has least intervention decisions made by the user. It is especially suitable to be used in predicting the systemic drug concentrations by convolution or estimating the time-course of absorption by deconvolution (Gillespie, 1991). Hence, all the pharmacokinetic data obtained from animal experiments in this study were analyzed following a non-compartmental model.

3.2.2. Simulation of response function

Response function was the predicted/expected plasma concentration–time curve of TA-SRPs, which was simulated and fitted according to the incidence of angina pectoris. Fig. 3 illustrated the statistical relative percentage of variant angina attacks (AA) during 24 h (starting from 18:00). Given the expected plasma concentration at the time point of the maximum AA frequency (04:00, \( C_{\text{max}}^{\text{w}} \)) equaled to \( C_{\text{max}} \), of weight function, a series of plasma concentration values at the corresponding time points (\( C_{0.5h}, C_{1h}, C_{2h}, C_{3h}, C_{4h}, C_{5h}, C_{6h}, C_{12h}, C_{16h}, C_{24h} \)) were calculated accordingly, which were proportional to the percentage of AA. Assuming \( C_{48h} \) of this calculated curve equaled to \( C_{48h}^{\text{w}} \) of weight function, area under this plasma concentration–time curve (\( AUC_{0-48h} \)) was analyzed using WinNonlin program and subsequently compared with \( AUC_{0-48h}^{\text{w}} \) of weight function. All the concentration values were adjusted proportionally together, and the predicted/expected

![Fig. 2. The plasma concentration–time curve of TA-tSD-IRPs in rabbits after oral administration in a dose of 30 mg kg⁻¹ (n = 6) and the mean predicted plasma concentration–time curve of TA-SRPs.](image-url)
plasma concentration–time curve was simulated successfully till \( \text{AUC}\text{w}_{0–24} \) within 80–120% of \( \text{AUC}\text{w}_{0–24}^0 \) for its bioequivalence.

The final mean predicted plasma concentration–time curve of TA-SRPs as a response function was depicted in Fig. 2. Following a non-compartmental model analysis, the pharmacokinetic parameters \( (T_{\text{max}}, C_{\text{max}}, \text{MRT}, \text{AUC}_{0–1}, \text{AUC}_{0–\infty}) \) (mean values) were presented in Table 1. Among them, \( C_{\text{max}} \) was about 0.56 times of \( C_{\text{max}}^0 \) value. Meanwhile, \( \text{AUC}_{0–1} \) value was about 100.22% of \( \text{AUC}_{0–1}^0 \) value, which indicated the bioequivalence of the design for TA-SRPs. Obviously, the plasma drug concentration time course of these newly designed chronotherapeutic TA-SRPs were synchronized with the circadian rhythm of variant angina during 24 h (Fig. 4).

### Table 1

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters (TA-tSD-IRPs) ( (n = 6) )</th>
<th>Pharmacokinetic parameters (TA-SRPs)</th>
<th>Predicted (mean)</th>
<th>Observed ( (n = 6) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>4.00 ± 0.025</td>
<td>10.00</td>
<td>10.00 ± 0.315</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng mL(^{-1}))</td>
<td>81.51 ± 17.170</td>
<td>46.00</td>
<td>42.51 ± 10.330</td>
</tr>
<tr>
<td>( \text{MRT} ) (h)</td>
<td>9.88 ± 0.462</td>
<td>16.37</td>
<td>16.94 ± 0.679</td>
</tr>
<tr>
<td>( \text{AUC}_{0–1} ) (ng h mL(^{-1}))</td>
<td>949.16 ± 135.391</td>
<td>951.27</td>
<td>948.47 ± 115.234</td>
</tr>
<tr>
<td>( \text{AUC}_{0–\infty} ) (ng h mL(^{-1}))</td>
<td>958.73 ± 143.245</td>
<td>961.98</td>
<td>986.66 ± 127.982</td>
</tr>
</tbody>
</table>

3.2.3. Calculation of input rate function

Input rate function was the in vivo drug release rates of TA-SRPs, which was obtained via the point-area deconvolution procedure using the weight function and response function. Input values \( I_i \) were calculated in turn by formula (4), where total response \( R_i \) is the predicted plasma concentration of TA-SRPs at time point \( t_i \), \( \text{AUC} \) is the area under the curve of weight function within time interval \( t_{i–1} \sim t_i \). \( I_i \) represents average in vivo drug release rate of TA-SRPs within time interval \( t_{i–1} \sim t_i \).

#### 3.2.4. Desired drug release profile of TA-SRPs

The in vivo cumulative release amount \( \text{CA}_i \) of TA-SRPs at time point \( t_i \) could be obtained by formula (5), which is equal to the sum of TA release amount within each time interval \( t_{i–1} \sim t_i \) during \( t_{i–1} \sim t_i \) where the release amount within each time interval is equal to average in vivo drug release rate \( I_i \) multiplied by time interval \( t_i – t_{i–1} \). In vivo cumulative drug release percent \( CR_i (\%) \) of TA-SRPs at each time point \( t_i \) is equal to the ratio of the cumulative release amount \( \text{CA}_i \) at time point \( t_i \) to the total cumulative release amount \( \text{CA}_{\text{total}} \). Then the desired cumulative drug release profile of TA-SRPs was obtained. It was important to note that the calculated negative input values \( I_i \) should be replaced with zero in order to minimize the instability in the calculation (Langenbucher and Mysicka, 1985). This resulted in a stepwise and relatively smooth appearance of the desired drug release profile, as shown in Fig. 5.

3.3. Formulation optimization of TA-SRPs

On one hand, pellets as a multiparticulate drug delivery system can overcome the poor and variable gastrointestinal tract drug absorption and possess the ability to reduce or eliminate the influence of food on bioavailability (Chopra et al., 2013). On the other hand, coating film produced by blends of PVAc and PVA–PEG has many characteristics, including a drug release independent of pH and ionic strength and a high resistance to mechanical stress (Liu et al., 2012; Mies and S., 2004; Meyer and K., 2004). Therefore, similar release patterns in vivo with in vitro were readily achieved for TA-SRPs coated with PVAc/PVA–PEG due to little effect of the condition of gastrointestinal tract on drug release (Gaynor et al., 2008). Under this premise, the in vivo desired drug release profile of TA-SRPs determined by deconvolution could be used for guiding the formulation optimization of TA-SRPs. Here, all the in vitro drug...
release profiles of TA-SRPs varying PVAc/PVA–PEG blend ratios and coating thickness were compared with the desired drug release profile to screen the optimal coating formulation. The release profile of the optimal formulation was similar to the desired drug release profile (similarity factor \( f_2 \geq 50 \)). Eq. (1) was used for the calculation of \( f_2 \), where \( n \) is the number of time points, \( R_t \) and \( T_t \) are the desired and observed cumulative drug release percent at time \( t \), respectively (Zolnik and Burgess, 2008).

3.3.1. Ratio of PVAc/PVA–PEG

The variation of the blend ratio of coating components is an efficient way to modify drug release patterns for the formulation (Ensslin et al., 2009). In vitro release behaviors of TA-SRPs coated with different ratios (90:10, 85:15, 70:30 w/w) of PVAc/PVA–PEG at the same coating weight of 5% (w/w) were evaluated and compared with the desired drug release profile by \( f_2 \) analysis. As shown in Fig. 6, as the relative amounts of PVA–PEG (a soluble film coating polymer) increased, the release of TA from pellets became faster and more complete. The drug release of TA-SRPs with 70:30 ratio of PVAc/PVA–PEG was close to 100% in 24 h while pellets with 90:10 and 85:15 ratios of PVAc/PVA–PEG released only approximate 50% of the total drug. Moreover, the drug release profile for 70:30 ratio was similar to the desired release profile \((f_2 = 64.90)\), whereas the drug release profiles for 90:10 and 85:15 ratios distinctly differed from the desired one \((f_2 = 25.10 \text{ and } f_2 = 29.30, \text{ respectively})\). Hence, 70:30 ratio of PVAc/PVA–PEG was chosen as the optimal film coating composition and used for the further investigation.

3.3.2. Coating weight (thickness)

In addition to the blend ratio variation of PVAc/PVA–PEG, the coating film thickness also plays a vital role in drug release (Ensslin et al., 2009). In vitro release behaviors of TA-SRPs coated with 70:30 (w/w) ratio of PVAc/PVA–PEG were investigated at different coating weights (3%, 5%, 10% w/w) and compared with the desired drug release profile by \( f_2 \) analysis (Fig. 7). The formulation of 3% coating weight was eliminated due to a less satisfactory similarity \((f_2 = 46.60)\), which was attributed to a slightly faster release during 12 h. Although the drug release profiles for 5% and 10% coating weights were both similar to the desired release profile \((f_2 = 64.90 \text{ and } f_2 = 59.49, \text{ respectively})\), the former was selected as the optimal formulation allowing for a more complete drug release, the time and costs consumption for preparation.

It was worth noting that the disparity in the release pattern of TA-SRPs with different coating weights \((f_2 = 46.60, 64.90 \text{ and } 59.49, \text{ respectively})\) was much smaller than that of different blend ratios \((f_2 = 25.10, 29.30 \text{ and } 64.90, \text{ respectively})\), which indicated that the ratio variation of coating polymers PVAc/PVA–PEG was dominant for the controlled drug release of TA-SRPs.

3.4. Investigation of drug release stabilities

By using the TA-SRPs prepared with the optimal coating formulation (70:30 of PVAc/PVA–PEG with 5% coating film weight) and operation process, the influence of different pH condition, release method and rotation rate on drug release was investigated and compared by \( f_2 \) analysis according to Eq. (1).

3.4.1. Release medium

The different release condition in vitro was used to simulate the different gastrointestinal tract environment in vivo. Due to pH changes in the gastrointestinal tract, the release study of the optimized TA-SRPs in three kinds of release mediums with different pH was necessary to investigate the effect of different pH condition on drug release of TA-SRPs. As depicted in Fig. 8a, the drug release behaviors in 0.1 M HCl and pH 6.8 PBS were similar to that in distilled water containing SDS (0.5%) with \( f_2 \) of 93.41 and 68.67. This result indicated that TA-SRPs could keep a stable drug release in different release mediums. The release extent and rate were independent of pH variation.

3.4.2. Release method

The drug release behaviors of the optimized TA-SRPs by using rotating basket method and paddle method were compared in this release study. As shown in Fig. 8b, two release profiles were extremely alike with \( f_2 \) of 84.78. It indicated that these TA-SRPs could keep a stable and similar drug release regardless of the choice of the release method.

3.4.3. Rotation rate

Three release profiles of the optimized TA-SRPs obtained at a rotation rate of 50, 100 and 150 rpm were presented in Fig. 8c. As it could be seen in this figure, with the significant increase of rotation rate from 50 to 150 rpm, the drug release at each time point increased slightly especially from 4 h. With 100 rpm as a reference, \( f_2 \)-test values for 50 rpm and 150 rpm were 70.18 and 70.72, respectively. With 50 rpm as a reference, \( f_2 \)-test value for 150 rpm was 55.10. These results showed the differences between three release profiles were all less than 10%, which indicated that the rotation rate had no remarkable influence on the drug release of TA-SRPs.
3.5. Pharmacokinetic studies

In Section 3.3, the coating formulation of TA-SRPs was optimized as follows: 70:30 of PVAc/PVA–PEG with 5% coating film weight. However, pharmacokinetic studies were needed to validate whether the actual plasma drug concentration time course of this formulation was in accordance with the predicted one.

The actual plasma concentration–time curve of the optimized TA-SRPs (70:30 of PVAc/PVA–PEG and 5% coating weight) and the simulated mean predicted plasma concentration–time curve of TA-SRPs were plotted together in Fig. 9. Apparently, plasma drug concentrations of two curves possessed a basically consistent trend over the entire period of time. The percent prediction error (%PE) of TA concentration at each time point was calculated by equation

\[ \%PE = \frac{\text{observed value}}{\text{predicted value}} \times 100\% \]

(Qi et al., 2003). The absolute %PE values from 4 to 16 h did not exceed 15% and the absolute %PE values from 8 to 12 h were less than 10%. Such low %PE values suggested that the actual observed plasma concentration values were indeed very close to the predicted concentration data calculated and fitted according to AA frequency. These results demonstrated that these optimized pellets succeeded in producing a sustained release effect in vivo with relatively higher plasma concentrations in 8–12 h. In addition, the observed pharmacokinetic parameters (\(T_{max}\), \(C_{max}\), MRT, \(AUC_{0–1}\), and \(AUC_{0–\infty}\)) of the optimized TA-SRPs were given and compared with the predicted values in Table 1. These almost equivalent numerical values further confirmed that in vivo pharmacokinetic process of these TA-SRPs after oral administration was in accordance with the predicted one.

3.6. Pharmacodynamic studies

In addition to the pharmacokinetic studies, pharmacodynamic studies were also necessary to estimate the in vivo real drug efficacy of these optimized TA-SRPs.

3.6.1. Pharmacodynamic index

TA can stimulate NO production in vascular endothelial cells (Huang et al., 2007). NO is a powerful vasodilator and plays a significant role in the treatment of the cardiovascular diseases. As an important regulator of the cardiovascular system, NO can dilate coronary artery, increase coronary blood flow, increase myocardial hypoxia tolerance, protect vascular endothelial cells and prevent myocardial ischemia. In this study, NO concentration variation in the serum was used as the pharmacodynamic index to investigate the drug efficacy of TA pellets. The equation is

\[ (\Delta N)_{t} = (N_{t}) - (N_{control})_{t}, \]

where \((\Delta N)_{t}\) is serum NO concentration variation at time \(t\), \((N_{t})\) is NO concentration after administration of TA-tSD-IRPs or TA-SRPs at time \(t\), \((N_{control})_{t}\) is NO concentration of the controlled group at time \(t\). The \(\Delta N\) concentration–time curves of the TA-tSD-IRPs and the optimized TA-SRPs after oral administration were presented in Fig. 10. TA-tSD-IRPs could generate a fast and high drug efficacy in vivo, but they lost efficacy quickly. In contrast, TA-SRPs produced drug efficacy slowly, and maintained the stable efficacy for a long time, which could reduce the drug side effects effectively.

3.6.2. Relationship between drug concentration and efficacy

The drug efficacy-concentration curves of the TA-tSD-IRPs and the optimized TA-SRPs were plotted together in Fig. 11. It could be found that \(\Delta N\) concentration versus TA plasma concentration following administration of TA-tSD-IRPs or TA-SRPs at time \(t\), \((N_{control})_{t}\) is NO concentration of the controlled group at time \(t\). The \(\Delta N\) concentration–time curves of the TA-tSD-IRPs and the optimized TA-SRPs after oral administration were presented in Fig. 11. TA-tSD-IRPs showed a clockwise hysteresis loop, the arrows indicating the direction of time. With the increase of drug concentration from about 46 to 80 ng mL\(^{-1}\), the drug efficacy of TA-SRPs was increased accordingly, while time to maximal efficacy was earlier than time to peak drug concentration. This result indicated that their drug efficacy did not always have a positive relationship with the plasma concentration.
concentration. In addition, with the decrease of drug concentration, the drug efficacy of TA-SRPs significantly decreased, compared with the drug efficacy of same drug concentration in the rising phase of drug concentration. These results manifested that after administration of TA-tSD-IRPs, although a high drug efficacy could be achieved quickly, the NZW rabbits with angina also produced drug tolerance for TA rapidly in vivo. In contrast, ∆NO concentration versus TA plasma concentration following administration of TA-SRPs showed a counter-clockwise hysteresis loop with almost simultaneous drug concentration-eficacy (Fig. 11b). Time to maximal efficacy was equal to time to peak drug concentration. And the higher drug efficacy could be obtained in the declining phase of drug concentration, compared with that in the rising phase. These were because TA-SRPs released TA slowly in vivo and then TA could continuously stimulate NO secretion in vascular endothelial cells, not generating drug tolerance. Therefore, TA-SRPs had better efficacy in treating variant angina, compared with TA-tSD-IRPs.

4. Conclusion

In this research, the formulation design and optimization of TA-SRPs was carried out based on deconvolution and variant angina chronotherapy theory. The pharmacokinetic and pharmacodynamic studies in NZW rabbits confirmed these optimized TA-SRPs had a suitable plasma drug concentration time course with better drug efficacy. In comparison to the common design method, this pattern could markedly reduce blindness and complexity in the development of chronotherapeutic modified-release preparations. As a promising approach, it has a great potential to be adopted in the design and optimization of other drugs or dosage forms in the further.

Acknowledgements

This study was financially supported by National Natural Science Foundation of China (No. 81473151) and the Priority Academic Program Development of Jiangsu Higher Education Institutions. Thanks to JRS, ISP, Colorcon and BASF corporations for providing the excipients and sugar spheres.

References


