Original Research Paper

Evaluation of pharmacokinetics and pharmacodynamics relationships for Salvianolic Acid B micro-porous osmotic pump pellets in angina pectoris rabbit

Shu-Ling Kan\textsuperscript{a,1}, Jin Li\textsuperscript{b,1}, Jian-Ping Liu\textsuperscript{a,\ast}, Hong-Liang He\textsuperscript{a}, Wen-Jing Zhang\textsuperscript{a}

\textsuperscript{a}Department of Pharmaceutics, China Pharmaceutical University, Nanjing, PR China
\textsuperscript{b}Department of Pharmacy, Xuzhou Medical College, Xuzhou, PR China

\textbf{A B S T R A C T}

The work aims to investigate the in vitro release, pharmacokinetics (PK), pharmacodynamics (PD) and PK–PD relationships of Salvianolic Acid B micro-porous osmotic pump pellets (SalB-MPOPs) in angina pectoris New Zealand White (NZW) rabbits, compared with those of SalB immediate-release pellets (SalB-IRPs). The SalB plasma concentrations and Superoxide dismutase levels (PD index) were recorded continuously at predetermined time interval after administration, and the related parameters were calculated by using WinNonlin software. The release profile of MPOPs was more sustained than that of IRPs. PK results indicated that the mean $C_{\text{max}}$ was significantly lower, the SalB plasma concentrations were steadier, both area under concentration-time curve from 0 to 24 h ($\text{AUC}_{0-24\text{ h}}$) and from 0 to infinity ($\text{AUC}_{\infty}$) were presented larger, and both the peak concentration time ($T_{\text{max}}$) and mean residence time (MRT) were prolonged for MPOPs, as compared with those of IRPs. PD results suggested that peak drug effect ($E_{\text{max}}$) was lower and the equilibration rate constant ($k_{\text{eq}}$) between the central compartment and the effect compartment was higher of MPOPs vs. those of IRPs. PK–PD relationships demonstrated that the effect-concentration-time (ECT) course of MPOPs was clockwise hysteresis loop, and that of IRPs was counter-clockwise hysteresis loop. Collectively, those results demonstrated that MPOPs were potential formulations in treating angina pectoris induced by atherosclerosis.

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* Corresponding author. No.24, Tong Jia Xiang, Nanjing 210009, PR China. Tel.: +86 25 83271293, +86 15952038790 (mobile); fax: +86 25 83271293.
E-mail addresses: liujianpingjp@hotmail.com, kansldong@163.com (J.-P. Liu).
\textsuperscript{1} These authors contributed equally to this work.
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1. Introduction

Sustained-controlled release formulations have become an attractive dosage for many merits, such as cutting down side effect by decreasing the drug plasma concentration fluctuation, and reducing administration frequency to enhance the compliance. Researchers usually evaluate the formulations by PK parameters, such as \( C_{\text{max}} \), \( t_{1/2} \) and lower plasma drug concentration fluctuation [3]. PK parameters were always directly related to efficacy. However, in many cases, the relationship between plasma drug concentration and effect magnitude is non-linear, and the real effect magnitude couldn’t be estimated just by PK parameters [2]. PK–PD modeling can predict relevant parameters associated with onset, magnitude and time courses of dose-concentration-effect of a drug [3], thus verifying the rationality of the formulation design. Meanwhile, PK–PD modeling provides better understanding of drug efficacy and safety in drug development, its use could significantly reduce the cost in both early and late drug development. It is therefore vitally important to have a thorough understanding of the PK–PD relationships in designing controlled release system.

Salvianolic Acid B (SalB, Fig. 1), the highest-content aqueous bioactive components extracted from the root of Salvia miltiorrhiza Bunge, has many promising pharmacology effects such as antioxidant, anti-hepatic fibrosis, anti-aging and antitumor effects etc [4]. Its excellent antioxidant activity could reduce or even reverse the progression of atherosclerosis, so SalB has been widely used in the treatment of cardiovascular disease [5]. However, there is only less than 1% of initial drug plasma concentration in blood after 7 h intravenous injection due to the short half-lives and fast clearance [6]. Meanwhile, it was suggested that SalB, which belongs to biopharmaceutics classification system (BCS) III, had low oral bioavailability in vivo [7] probably because of its poor permeation [8]. To enhance the bioavailability, ameliorating the permeation and prolonging the duration were well considered in the past.

Efforts have been done mainly focusing on the two facts mentioned above [9–14], such as SalB controlled porosity osmotic pump tablets, FEGylated SalB liposomes and phospholipids complex (SalB-PC), etc. While in the oral solid formulations aspects, most of the works only addressed one of the shortcomings of the drug. Hence, in previous work [15], SalB-PC pellets have been prepared by us, which enhanced the permeability and oral bioavailability of the drug. Furthermore, once-a-day SalB-MPOPs were prepared successfully using coating technology by evaluating in vitro release profiles and in vivo absorption curves [16].

In the current research SalB-MPOPs were first prepared, the formulation was subsequently characterized by in vitro release and the parameters of PK and PD in angina pectoris NZW rabbits model induced by atherosclerosis, furthermore, the PK–PD relationship and action mechanisms of the SalB-MPOPs were investigated by PK–PD parameters and ECT course, compared with those of SalB-IRPs.

2. Materials and methods

2.1. Materials

SalB (98% pure) and SalB standard were purchased from Xi’an Honson Biotechnology Co., Ltd. (Xi’an, China) and the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), respectively. High-fat feed was purchased from Jiangsu synergistic biological engineering Ltd (Nanjing, China). SOD reagents box was provided by Nanjing Jian cheng Bioengineering Institute (Nanjing, China); calcium heparin was purchased from Changzhou Qianhong Biopharma Co., Ltd (Changzhou, Jiangsu, China). Oil red O was purchased from Sigma (Sigma Aldrich Chemical Company, Madrid, Spain), 5% gluconate solution was provided by JiangSu Dahongying pharmaceutical Co., Ltd. (Nanjing, Jiangsu, China). All other used reagents were analytical grade, but mobile phase were chromatographic grade and purchased from Chemical Reagent Co., Ltd. (Nanjing, China).

2.2. Preparation of pellets

SalB-IRPs of 800–1000 μm were composed of SalB-phospholipids complex (SalB-PC, MCC, Kollidon® CL-SF and Flowlac®100 at 30:40:15:15 (w/w/w/w)) in an extrusion-spheronization apparatus (JBZ-300, Shenyang, China) [15]. Osmotic pressure agent was incorporated into the SalB-IRPs composed materials and the 800–1000 μm SalB-MPOPs starter cores were prepared as described above in preparation of SalB-IRPs. Subsequently, SalB-MPOPs were prepared by coating the starter cores with aqueous dispersion containing Poly(vinyl alcohol)-poly(ethylene glycol)(PVA-PEG) as a pore-forming agent and Ethyl cellulose (EC) aqueous dispersion (brand name, Surelease®) using fluid-bed (HJQ-100, Shenyang, China) [16]. Both SalB-IRPs and SalB-MPOPs containing 25 mg SalB were sealed in hard gelatin capsules with a manual capsule filling machine (CapsulCN, Zhejiang, China).

2.3. In vitro release tests

2.3.1. Quantitative analysis of SalB

All of the determinations of SalB content were done by using HPLC and the system consisted of Shimadzu LC-20AB pump, a Shimadzu SIL-20AC autosampler, a Shimadzu SPD-M20A diode array detector (DAD) (Kyoto, Japan) and a Syngeri Hydro-RP C18 column (5 μm, 250 mm × 4.6 mm, Phenomenex,

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**Fig. 1 — The structure of Salvianolic Acid B.**
USA) protected by a C18 Securityguard column (5 μm, 10 mm × 4.6 mm, Kromasil, Sweden). The mobile phase was methanol-acetonitrile-formic acid-double deionized water (28:12:2:58, v/v/v/v) at flow rate of 1.0 ml/min. The detection wavelength was 286 nm. Column temperature was maintained at 35 °C during detection. For SalB preparations, the linear concentration range were 0.75–15.0 μg/ml (r = 0.9997), the recovery rates ranged from 98% to 102% with relative standard deviation (RSD) of less than 2%. And all RSD of the recovery rates ranged from 98% to 102% with relative standard deviation (RSD) of less than 2%. And all RSD of the intra-day and inter-day precision for SalB were below 2%.

### 2.3.2. Drug release test
Release tests were performed in rotating basket method USP 34 XXIII, apparatus II (ZRS-8G; Tianjin, China; basket method). The release medium was 900 ml of distilled water, capsules containing 25 mg of SalB were put into the rotating basket and immersed in the dissolution medium, the temperature was maintained at 37 ± 0.5 °C and rotation rate was 100 rpm. At predetermined time intervals, 5 ml of the dissolution solution was withdrawn and substituted with fresh dissolution medium. The samples were filtered through 0.22 μm filter and analyzed by HPLC for SalB as described above.

### 2.4. Pharmacokinetic studies

#### 2.4.1. Establishment of angina pectoris NZW rabbits
It showed [17,18] that rabbits have similar fat metabolism as humans, compared to other animals rabbits are prone to form atherosclerotic plaque by high-fat diet, which lead to angina pectoris. Atherosclerotic rabbit model was constructed to evaluate the efficacy of formulations. Thirty healthy male NZW rabbits (body weight 2.0 ± 0.9 kg) were purchased from Experimental Animal Center of China Pharmaceutical University (Nanjing, China). All experimental procedures were reviewed and approved by the institutional animal ethical committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. The animals were housed in a standard-condition room holding constant temperature and humidity (23 °C, RH55%) with a 12 h light/dark cycle. Rabbits were randomly divided into two groups, controlled group (n = 6) and model group (n = 24). The controlled group were fed normally, while the model group were operated by subcutaneous injection of homocysteine thiocactone (20 mg/ml in 5% glucose solution, daily 20–25 mg/kg), and raised on high-fat diet (10%cholesterol, 5%lard, 5% egg yolk powder, 0.5% sodium cholate, 79.5% ordinary feed) [19,20]. 8 weeks later, controlled group and 6 rabbits randomly from the model group were respectively injected into the HPLC system for analysis, and the HPLC conditions were identical to those in vitro.

#### 2.4.2. Animal experiment
The model animals were fasted for 12 h with free access to water before the experiment, and randomly divided into three groups: (1) controlled group; (2) SalB-IRPs group; (3) SalB-MPOPs group. The controlled group received a controlled capsule containing blank pellets without SalB (n = 6). SalB-IRPs and SalB-MPOPs at a dose equivalent to 250 mg/kg of SalB were filled into gelatin capsules with a manual capsule filling machine (CapsulCN, Zhejiang, China) and orally administered to the group (2) and group (3) rabbits (n = 6), respectively. Blood samples (1 ml) were collected from auricular vein with a heparinized tube at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48 h after dosing. Plasma specimens were separated by centrifugation at 3000 rpm for 10 min and stored at −20 °C until analysis.

#### 2.4.3. Plasma sample extraction and analysis
Prior to extraction, frozen plasma samples were thawed at ambient temperature. All the operations were protected from light. Rabbits plasma (200 μl) was mixed with 30 μl of hydrochloric acid (2.5 mol/l). After adding 1 ml of ethyl acetate, the acidified mixture was further acidified by vortexing vigorously for 3 min. The samples were centrifuged at 3000 rpm for 10 min, the organic layer was collected. The same extraction procedure was performed twice more and the organic phase was collected and evaporated to dryness under a nitrogen stream at 40 °C. The residue was reconstituted in 100 μl of 30% methanol with 0.01% formic acid. 20 μl of the supernatant was injected into the HPLC system for analysis, and the HPLC conditions were identical to those in vitro.

#### 2.4.4. Calibration curve
The SalB stock solution (50 μg/ml) was prepared in methanol. The stock solution was diluted at different times to get the working solutions, consisting of 0.50, 1.00, 2.00, 5.00, 10.00, 15.00 and 25.00 μg/ml SalB in rabbit plasma. 100 μl of these serial dilutions were individually transferred into Eppendorf tubes and the solvents were evaporated to dryness under nitrogen stream. 200 μl blank rabbit plasma was added into the above tubes and the SalB concentration were determined following the procedures mentioned in Section 2.4.3. A regression equation between concentration of SalB (C) and the corresponding peak area (A) was acquired.

#### 2.4.5. Method validation
Method validation including specificity, recovery, inter-day and intra-day precision was examined by HPLC.

### 2.5. Pharmacodynamics studies
SOD levels were detected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24 and 48 h after administration. Specifically, 2 ml blood was obtained from ear margin border vein of the three groups. After being static for few moments, the upper serum was removed and stored at −20 °C for analysis. When tested, the serum was melt and taken out 100 μl, SOD vitality was determined by Xanthine oxidase performed according to reagent box provided method.

#### 2.6. Data analysis and evaluation

##### 2.6.1. Curves plots between plasma drug concentration and time, SOD levels change and time
For the Sal-IRPs and Sal-MPOPs group, the curves were plotted between mean plasma concentration and time; SOD blood levels-rising and time.
SOD blood levels-rising effect at time t was plotted according to the formula:

\[(\Delta \text{SOD})_t = (\text{SOD})_t - (\text{SOD}_{\text{control}})_t\]  

(1)

Where \(\Delta \text{SOD}\) is the change of SOD plasma levels, \((\text{SOD})_t\) is SOD plasma levels of Sal-IRPs or Sal-MPOPs, \((\text{SOD}_{\text{control}})_t\) is SOD plasma levels of the control group at time t, respectively.

### 2.6.2. PD model fitting

The PD data were input in WinNonlin software to calculate \(E\) for fitting the experimental results, which showed that the optimal was \(E_{\text{max}}\)-model and the equation was as follows:

\[
E = \frac{E_{\text{max}} \times C_t}{C_{\tau} \times EC_{50}} 
\]

(2)

Where, \(E\) is ASOD, the change of SOD levels; \(E_{\text{max}}\) is the SalB maximum effect, \(C_t\) is the SalB plasma concentration in central compartment, \(EC_{50}\) was SalB effect compartment concentration when the effect value was half of maximal effect value.

### 2.6.3. PK parameters prediction and PK–PD combination model construction

Pharmacokinetic parameters, including \(C_{\text{max}}, T_{\text{max}}, t_{1/2},\) MRT, \(AUC_{0-\infty}\) and \(AUC_{0-\tau}\), were calculated by compartmental and non-compartmental analysis using the PKSolver (Version 1.0), and the most suitable compartment model was determined by the smallest Akaike information criterion (AIC) value \([21,22]\). Both the SalB-IRPs and SalB-MPOPs PK were fitted to one-compartment models.

### 2.6.4. PK–PD relationship

The central compartment drug concentration \((C_p)\) and effect-site drug concentration \((C_e)\) were calculated by WinNonlin software and expressed as following formulas respectively \([23]\):

\[
C_p = \frac{FX_0k_{40}}{V_0(k_4 - k_{10})} \times (e^{-k_{10}t} - e^{-k_4t}) = \frac{X_0k_4}{V_0(k_4 - k_{10})} \times (e^{-k_4t} - e^{-k_{10}t}) 
\]

(3)

\[
C_e = \frac{X_0k_{30}k_{40}}{V_0} \times \left[ \frac{e^{-k_4t}}{(k_3 - k_{10})(k_4 - k_3)k_{10}} + \frac{e^{-k_{10}t}}{(k_4 - k_3)(k_3 - k_{10})k_{10}} \right] 
\]

(4)

where, \(X_0\) is dosage, \(F\) is the oral bioavailability, \(V\) is the central chamber distribution volume, \(\tau\) is the quotient of \(VF\), \(k_4\) is the transport rate constant from central compartment to effect compartment, \(k_{10}\) is the effect compartment elimination rate constant. Other parameters are the same meaning with those previously mentioned.

Moreover, ECT courses were plotted.

### 2.7. Statistical analysis

All results were expressed as the mean ± standard deviation (SD) of six animals. Statistical analysis was performed using two-tailed student’s t-test (SPSS, version 10.0). Statistical differences were considered to be significant as \(P < 0.05\) or \(P < 0.01\).

### 3. Results and discussion

#### 3.1. SalB-MPOPs and SalB-IRPs release profiles

SalB-MPOPs were successfully prepared \([16]\). The in vitro release profiles of SalB-IRPs and SalB-MPOPs illustrated that SalB can be fast released from the IRPs but slowly from the MPOPs. Furthermore, the SalB-MPOPs was fitted to Zero order model following studying drug release from SalB-MPOPs with different dissolution kinetic model. SalB-MPOPs formulation was usually prepared by coating semi-permeable membrane (water insoluble materials) containing pore-forming agent (water soluble materials) \([24,25]\). In the current work, EC aqueous dispersion and PVA-PEG were taken as semi-permeable membrane and pore-forming agent, respectively. When the SalB-MPOPs contacted with aqueous environment, the pore-forming agent dissolved and formed drug release hole, where drug released at a low rate.

#### 3.2. Verification of model animals

As Fig. 2 depicted in ECG 2A1 and 2A2, the model group showed obvious depression of the ST segment and T wave inversion compared with the normal group, which confirmed the myocardial ischemia status.

The CDEI of rabbits indicated that the vessel wall was smooth in the normal group of 2B1, and intima-media thickness was not seen. While the model groups’ CDEI of 2B2 indicated that the vessel wall was roughness visibly, and the vascular intima-media thickness was significant. These phenomena confirmed that the athermanous plaque was formed.

Coronary arteriography is the gold standard of angina diagnosis clinically. Compared with the normal group of picture 2C1, the model group of picture 2C2 showed that the anterior descending coronary artery occluded completely at arrow 1, and the circumflex branch presented a bifurcate lesion at arrow 2. The results displayed that the angina pectoris model was constructed successfully.

The NZW rabbit’s aortic arch pathology slice picture illustrated in 2D1 that the vascular wall was smooth for normal NZW rabbits. While 2D2 displayed that abnormal protuberant existed in the inner vascular wall and the texture was loose for model rabbits, which were different to normal rabbits. The vascular wall of model rabbit was stained with oil red O, which further validated the lipid plaque’s formation. The induction method was rapid and feasible, and the model animals can be used for further study of PK and PD.

### 3.3. Pharmacokinetics

#### 3.3.1. Method validations

3.3.1.1. Specificity. Fig. 3 showed that SalB was well separated from the matrix impurities under the HPLC conditions, with retention times at 13.12 min, the resolution was greater than
1.5, tailing factor at 1.04 and the number of theoretical plates was greater than 5000.

3.3.1.2. Recovery. The absolute recovery of SalB was calculated by the percentage of the value measured in blank plasma samples to the standard solutions in the same concentration, and the value fluctuated from 80% to 86% at three concentrations tested as recorded in Table 1.

3.3.1.3. Range and linearity of the calibration curve. It showed that there was a good linearity between concentration of SalB (C) and the corresponding peak area (A). The linear range was 0.50–25 μg/ml, and the equation of calibration curve was $A = 131864 \times C + 224.5$, with $r = 0.9986$. Calculating at three times of the signal noise ratio, the detection limit of SalB was 10 ng/ml.

3.3.1.4. Inter-day and intra-day precision. The samples of 3.3.1.2 were also used to perform the precision tests. The inter-day precision was expressed as the relative standard deviation (RSD) of HPLC peak area from 5 repeats in a single run every other 2 h. The intra-day precisions were presented at RSD of that by consecutive 5 days.

As was shown in Table 2, all the RSDs were below 10% which were acceptable.

3.3.2. PK analysis

As plotted in Fig. 4 the mean SalB plasma concentration and time curves after single-dose administration in model animals and the related PK parameters were listed in Table 3.

As depicted in Fig. 4, the minimum plasma ($C_{min}$) was almost identical between SalB-MPOPs and SalB-IRPs, while $C_{max}$ of SalB-MPOPs was significant lower than that of SalB-IRPs, stating clearly that the fluctuation between $C_{max}$ and $C_{min}$ was greatly reduced in SalB-MPOPs group. Compared with SalB-IRPs, SalB-MPOPs had longer $T_{max}$, longer MRT, larger $AUC_{0-\infty}$ and $AUC_{0-\infty}$. These results indicated that the SalB absorption was at a slow rate, and the duration of SalB-MPOPs was longer than that of SalB-IRPs. The bioavailability of SalB-MPOPs might be higher than that of SalB-IRPs.

3.4. Effect-time course

SalB can enhance SOD vitality to protect muscle cell [26]. So SOD levels as continuously PD index were detected. Fig. 5 displayed the temporal profiles of $\Delta$SOD level after oral administration of SalB-IRPs and SalB-MPOPs in AP-NZW rabbits. In SalB-IRPs group the $(\Delta$SOD)$_{max}$ value was 104 U/ml at 8 h, then $\Delta$SOD value decreased quickly, while in SalB-MPOPs group $(\Delta$SOD)$_{max}$ was 56 U/ml at 10 h and the $\Delta$SOD value reduced gradually. This
demonstrated not only the peak efficacy time was delayed, but also the whole effect time was prolonged for SalB-MPOs group, compared with SalB-IRPs group. These might be mirrored by the corresponding PK parameters.

3.5. PK-PD relationships

The PK, PD data were input into the WinNonlin software, and the correlation parameters were shown in Table 4.

Table 1 – absolute recovery of SalB in plasma (mean ± SD, n = 3).

<table>
<thead>
<tr>
<th>C (µg/ml)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>80.34 ± 6.66</td>
<td>8.29</td>
</tr>
<tr>
<td>5.00</td>
<td>83.45 ± 3.94</td>
<td>4.72</td>
</tr>
<tr>
<td>25.00</td>
<td>86.36 ± 4.56</td>
<td>5.28</td>
</tr>
</tbody>
</table>

Fig. 3 – Typical HPLC chromatograms: (A) blank rabbit plasma sample; (B) rabbit plasma spiked with SalB; (C) rabbit plasma sample after oral administration of SalB pellets.
The results showed that the values of $E_{\text{max}}, EC_{50}$ and $t_{1/2eq}$ were remarkably higher of IR group than that of SalB-MPOPs group, while $K_{\text{ee}}$ value of SalB-MPOPs group was notably higher than that of SalB-IRPs group, which indicated that drug from SalB-MPOPs formulations was easier to reach balance between central compartment and effect compartment, and the lag time was short. In addition, significant differences were found for area under effect-time curve from 0 to 24 h ($AUE_{0-24}$) and from 0 to infinity ($AUE_{0-\infty}$) between SalB-IRPs and SalB-MPOPs formulations, which might be ascribed to different $AUC_{0-24}$ and $AUC_{0-\infty}$.

A direct correlation between the magnitude of SOD value ($AUE$) and system drug exposure ($AUC$) was speculated, and so was the relationship between the plasma drug concentration and effect [2]. Currently, researches on relationship between the plasma drug concentration and effect are extremely rare, which always hypothesizes a direct correlation [2].

### 3.6. ECT curves

In order to find the probable differences of ECT curves between SalB-IRPs and SalB-MPOPs, hysteresis loops were plotted using the present nonsteady-state and time-dependent data of central compartments concentration ($C_C$) or effect site concentration ($C_E$) at different time points.

In Fig. 6A, a counter-clockwise hysteresis loop displayed the relationship of the time course between SalB concentration in plasma or effect site and the SOD value following SalB-IRPs administration. From Fig. 6A, it can be seen that effect was lagged behind the blood concentration. These phenomena were probably because that SalB fast released from SalB-IRPs in vivo and the plasma concentrations increased rapidly accordingly, while time was needed for concentration to reach a balance in effect sites of cardiac tissue and vascular smooth muscle, so the effect lagged behind the blood concentration. In addition, $EC_{pT}$ and $EC_{pT}$ curves did not coincide completely, the dissociation of time courses of plasma concentration and effect might be attributed to that the plasma distribution was disequilibrium between center and effect compartments. The relationship between the concentration and effect was not correlated to the time course, which indicated that it did not go in one between the plasma center and the effect compartment.

In Fig. 6B, a clockwise hysteresis loop presented that the relationship of the time course between plasma or effect site SalB concentration and the SOD value following SalB-MPOPs' administration. It indicated that SalB plasma concentrations and effect were always synchronized, namely, during the whole time the concentration and effect were highly correlated. The loop for central compartment was almost overlapped with that for effect compartment, which indicated that plasma drug concentration and effect-site concentration balance were built more rapidly, and the center and the effect compartments were attributed to the same site. SalB released slowly from SalB-MPOPs formulation, and the plasma drug concentration curves were steady leading drug concentration to reach rapid equilibrium between plasma compartment and

### Table 2 – Measurement precision for SalB in spiked rabbit plasma (mean ± SD, n = 5).

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Inter-day</th>
<th>Intra-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>0.50</td>
<td>0.47 ± 0.03</td>
<td>6.38</td>
</tr>
<tr>
<td>5.00</td>
<td>5.03 ± 0.231</td>
<td>4.59</td>
</tr>
<tr>
<td>25.00</td>
<td>25.08 ± 2.042</td>
<td>8.14</td>
</tr>
</tbody>
</table>

### Table 3 – The pharmacokinetic parameters of SalB for SalB-IRPs and SalB-MPOPs after single oral administration in AP-NZW rabbits (mean ± SD, n = 6).

<table>
<thead>
<tr>
<th>Parameters (unit)</th>
<th>SalB-IRPs</th>
<th>SalB-MPOPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.35 ± 0.823</td>
<td>10.00 ± 0.415</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>8.45 ± 0.712</td>
<td>3.98 ± 0.092</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>3.02 ± 1.451</td>
<td>15.94 ± 0.512</td>
</tr>
<tr>
<td>$AUE_{0-24}$ (µg·h/ml)</td>
<td>31.92 ± 3.289</td>
<td>42.02 ± 4.534*</td>
</tr>
<tr>
<td>$AUE_{0-\infty}$ (µg·h/ml)</td>
<td>32.20 ± 3.865</td>
<td>43.43 ± 7.457*</td>
</tr>
</tbody>
</table>

*P < 0.01 versus SalB-IRPs.
Table 4 – PK–PD parameters of SalB-IRPs and SalB-MPOPs (mean ± SD, n = 6).

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Sal-IRPs</th>
<th>Sal-MPOPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>E_{max} (U/ml)</td>
<td>1037.36 ± 7.46</td>
<td>288.90 ± 17.21</td>
</tr>
<tr>
<td>EC_{50} (µg/ml)</td>
<td>31.59 ± 0.75</td>
<td>10.52 ± 0.67</td>
</tr>
<tr>
<td>K_{EC} (l/h)</td>
<td>0.093 ± 0.016</td>
<td>1.89 ± 0.15</td>
</tr>
<tr>
<td>t_{1/2eq} (h)</td>
<td>7.48 ± 1.08</td>
<td>0.37 ± 0.016</td>
</tr>
<tr>
<td>AUC_{0-∞} (h·U/ml)</td>
<td>970.75 ± 15.47</td>
<td>1268.25 ± 2.34</td>
</tr>
<tr>
<td>AUC_{0-∞} (h·U/ml)</td>
<td>104.33 ± 16.33</td>
<td>127.40 ± 2.51</td>
</tr>
</tbody>
</table>

\*p < 0.01 versus SalB-IRPs. The parameter t_{1/2eq} is the equilibration half time between the plasma and the effect compartment and it is equal to \(\ln 2/K_{EC}\).

**Fig. 6** — Temporal Plots of SOD versus SalB concentration after single oral administration of SalB-IRPs and SalB-MPOPs formulation to AP-NZW rabbits (250 mg/kg, n = 6). (A) SalB-IRPs and (B) SalB-MPOPs. The arrow indicates the time flow after oral administration. Cp: central compartment drug concentration, Ce: effect compartment drug concentration.

4. Conclusion

In this present work, the in vitro release, PK, PD and PK–PD relationships for SalB-MPOPs were reported, compared with those for SalB-IRPs. The provided analysis method of SalB in angina pectoris model rabbit plasma was feasible. The PK–PD relationships studies manifested that the central compartment and effect compartment of SalB-MPOPs were in the same site while SalB-IRPs’ were not. In summary, the present study established a nonsteady-state and time-dependent multiple PK–PD model of a single dose of SalB-IRPs and SalB-MPOPs in AP-NZW rabbits. The fast and short-lasting counter-clockwise hysteresis loop model well represents the relationship of the time course for SalB-IRPs, while the delayed and long-lasting clockwise hysteresis loop model well represents that for SalB-MPOPs. The SalB-MPOPs formulation was thought as a better drug delivery system than SalB-IRPs formulation.

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**References**


